THE SYNTHESIS OF 3-FLUOROASPARTIC ACID*

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SUMMARY

A new synthesis of 3-fluoroaspartic acid is based on the reaction of dibenzyl difluoromaleate (**3**) with dibenzylamine. Reduction of this product (**4**) with sodium cyanoborohydride gave dibenzyl 2-dibenzylamino-3-fluorosuccinate (**5**), and thence hydrogenolysis of the benzyl groups afforded fluoroaspartic acid (**6**). Stereochemistry of the products and of the intermediates is discussed.

INTRODUCTION

Since 1957, I have been trying, on and off, to synthesize 3-fluoroaspartic acid. Motivation of my attempts was a possibility that the substitution of fluorine for hydrogen could impart to the amino acid antimetabolic properties, and thus make it interfere with the synthesis of proteins in cancer cells. Antimetabolic properties of fluorinated compounds are well documented. Thus fluorocitric acid deactivates the enzyme aconitase and prevents degradation of citric acid in the Krebs' cycle [1], and 5-fluorouracil, incorporated in ribonucleic acids, inhibits synthesis of amino acids [2]. It was especially the use of the latter compound as a cancerostatic that encouraged me in my efforts. If fluoroaspartic acid were metabolized analogously to aspartic acid, it would form 5-fluorouracil, and in this way generate a cancerostatic *in situ*.

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* Dedicated to Emeritus Professor W.K.R. Musgrave on the occasion of his 70th birthday.

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For whatever reasons, several attempts were made by other chemists to synthesize 3-fluoroaspartic acid. Connors et al. did not get too far in their acetamidomalonate synthesis using ethyl chlorofluoroacetate [3]. My experiments starting with diethyl fluorooxalacetate or with ethyl chlorofluoroacetate and diethyl acetamidomalonate failed in the penultimate step giving fluorine-free products [4]. These negative results were confirmed by Lettre et al. [5]. Meanwhile, Bose et al. attempted Gabriel synthesis using dimethyl and diethyl 2-bromo-3-fluorosuccinate but found that elimination of hydrogen bromide and especially hydrogen fluoride took place instead of nucleophilic displacement of bromine [6]. Many of Tolman's experiments did not prove successful either [7]. Doubts were expressed whether or not 3-fluoroaspartic acid can exist at all because of a strong possibility of elimination of hydrogen fluoride [8].

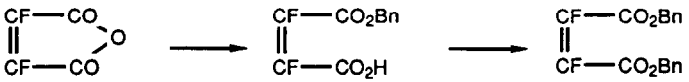
It was not until 1978 that several successful syntheses of 3-fluoroaspartic acid emerged. Duschinsky reported a ten step synthesis of threo-3-fluoroaspartic acid [9] and later on published it in a laconic form in Experientia [10]. In the same journal Matsumoto et al. described a very simple synthesis of erythro-3-fluoroaspartic acid based on replacement by fluorine of one diazotized amino group in 2,3-diaminosuccinic acid [11]. Kollonitsch et al. prepared both diastereomers by fluorodehydroxylation of both 2-amino-3-hydroxysuccinic acids [12]. Pandit et al. obtained both stereoisomers, but mainly the erythro-form, by two independent syntheses [13].

The structure of the erythro-compound was confirmed by x-ray crystallography [14]. Abeles et al. synthesized both diastereomeric 3-fluoroaspartic acids by a modification of Kollonitsch's method [12] and confirmed the structure of the threo isomer by x-ray crystallography [15]. Both erythro- and threo-3-fluoroaspartic acids were also synthesized by Beguin et al. as a part of a study of complexation of amino acids by crown ethers as a means of determining absolute configuration [16].

In 1982, Duschinsky subjected a product of the reaction of dimethyl acetamidomalonate with methyl chlorofluoroacetate to milder hydrolytic conditions than I was using, and succeeded in preparing both diastereomers of 3-fluoroaspartic acid albeit in low yields [17].

RESULTS

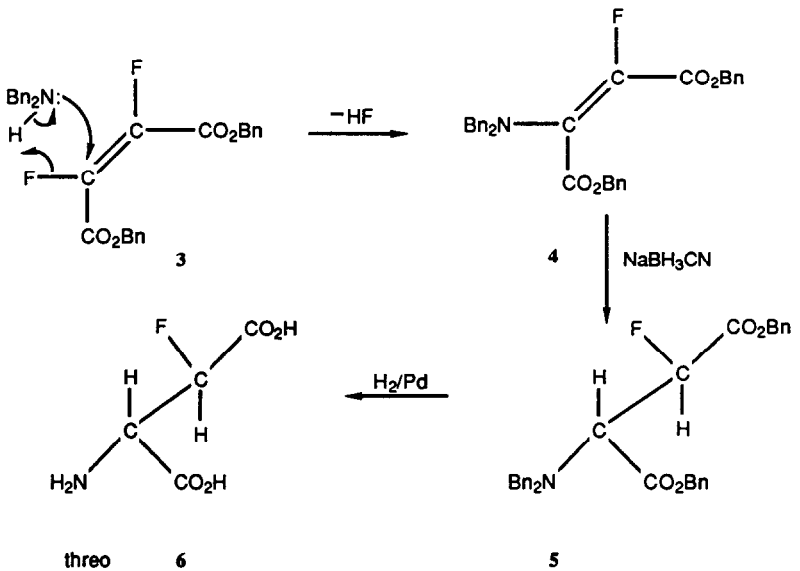
In 1977, I started a synthesis of 3-fluoroaspartic acid according to the scheme shown below. Difluoromaleic anhydride (**1**) was converted to monobenzyl ester (**2**) and this to dibenzyl difluoromaleate (**3**) (Direct esterification of difluoromaleic acid by heating with two equivalents of benzyl alcohol resulted in a spontaneous exothermic reaction and decomposition) (Scheme I).



1 Bn = $PhCH_2$ **2** **3**

Scheme I

Treatment of **3** with dibenzylamine gave dibenzyl 2-dibenzylamino-3-fluoromaleate (**4**) via the addition/elimination process shown in Scheme II.



threo **6** **5**

Scheme II

Formation of **4** took place most probably by a concerted mechanism. No intermediate addition product of dibenzylamine to dibenzyl difluoromaleate was detected. NMR spectra of a mixture of these two compounds taken at intervals during the reaction showed only disappearing signals of the starting material and increasing signals of the products.

The cis-position of the two carboxylic groups in **4** was proven by ^{13}C NMR. The $^{3}J_{CF}$ coupling constant (27 Hz) is consistent with that of fluoromaleic acid (20 Hz), and contrasts with that of fluorofumaric acid (~1 Hz).

Catalytic hydrogenation of **4** over palladium was expected to saturate the double bond and hydrogenolyze the benzyl groups to give erythro-3-fluoroaspartic acid. Instead, fluorine was hydrogenolyzed even under very mild conditions giving dibenzylamine hydrofluoride. Later studies confirmed that vinylic fluorine - in contrast to fluorine on saturated carbon - is hydrogenolyzed unexpectedly readily [18].

Since catalytic hydrogenation was unfeasible, other methods were tried to reduce the double bond without removing fluorine. Hydrides and complex hydrides were unsuccessful. Even sodium cyanoborohydride in methanol and acetic acid applied successfully to a similar fluorinated enamine [13] failed. Only when the enamine **4** was first converted to its hydrochloride with gaseous hydrogen chloride according to Borch [19] did the reduction work giving predominantly dibenzyl threo-2-dibenzylamino-3-fluorosuccinate (**5**). Hydrogenolysis over palladium afforded threo-3-fluoroaspartic acid (**6**), sometimes accompanied by a smaller amount of the erythro diastereomer.

The assignment of the configuration of the 3-fluoroaspartic acid is based on its genesis and on the comparison of its melting point, and especially its ^{1}H and ^{19}F NMR with the data published in the literature. Since these constants differ widely depending on the method of preparation, authors, solvents, pH, and even the instruments used, the assignment would not be unambiguous. Table I shows the constants of the 3-fluoroaspartic acid described in this paper and those of both diasteromers of 3-fluoroaspartic acid as published. The final proof of the configuration of the 3-fluoroaspartic acid was accomplished by direct comparison of the NMR spectra with those of Pandit's erythro-fluoroaspartic acid whose configuration was established by x-ray crystallography. Both ^{1}H and ^{19}F NMR spectra of a sample prepared from a mixture of both diastereomers prove unequivocally that the acid described in this work has threo-configuration.

TABLE 1

Melting points and ^{1}H and ^{19}F NMR of 3-Fluoroaspartic acids

CONFIGURATION	M.P.	^{1}H NMR ppm						^{19}F NMR ppm			REF
	°C	-CH(NH_2)-	J_{HF}	J_{HH}	-CHF-	J_{HF}	J_{HH}	ϕ	J_{gem}	J_{vic}	
ASSUMED ERYTHRO	164-166°dec	4.88dd	28.8	1.8	5.62dd	46.2	1.8				11
THREO	157-158°dec[a]	4.95dd[b]	26	2	5.92dd[b]	44	2				12
ASSUMED ERYTHRO	144-145°dec[c]	5.07dd[b]	29	2	5.85dd[b]	47	2	-202.5dd	47	29	12
ERYTHRO[c]	174°dec	4.27dd	27	2.5	5.11dd	50	2.5	-193dd	48	28	13
ERYTHRO	175°dec	4.62dd	31	2	5.53dd	47	2				17
THREO	165°,167°dec										10
	169-171°dec										
	161°,162°dec	4.55dd	27	3	5.59dd	45	3				17
ERYTHRO	174°dec	4.55dd[e]	29	2.4	5.30dd[e]	49	2.4				15
		4.98dd[f]	29	2	5.74dd[f]	48	2				
THREO[g]	175°dec	4.46dd[e]	29	2.4	5.50dd[e]	45	2.4				15
		4.89dd[f]	29	2	5.87dd[f]	44	2				
ERYTHRO								-198.2dd[h]	47.6	29.1	16
THREO								-199.3dd[i]	44.5	26.5	16
THREO	171-173°dec	4.54dd	29.0	2	5.50	44.8	2	-202.6	44.9	29.0	This work
THREO[j]		**4.69**	**29.04**	**2.29**	**5.70**	**44.86**	**2.32**	**201.06**	**45.24**	**29.66**	**This work**
ERYTHRO[j]		**4.69**	**29.04**	**2.29**	**5.55**	**47.65**	**2.09**	**199.9**	**46.20**	**30.10**	**13**

a Found 177° by Stern [15]
b HCl salt
c
d Proved by x-ray [14]
e Found 170° by Duschinsky [17]
f 270 MHz
f 80 MHz
g Proved by x-ray [15]
h Varied from -201.6 to -192.3 at pH from 1 to 6 [16]
i Varied from -199.0 to -194.3 at pH from 1 to 6 [16]
j NMR readings of a mixture of 13's erythro and this work's threo isomer

EXPERIMENTAL SECTION

Commercial reagents were used throughout unless stated otherwise. Melting points were taken on Thomas-Hoover Unimelt apparatus. Infrared spectra were recorded on Pye-Unicam 1025 IR spectrophotometer, ^{1}H and ^{19}F NMR spectra on Varian EM 390 (90 MHz), Bruker WP200 (200 MHz) and WP270 (270 MHz) spectrometers in carbon tetrachloride or deuteriochloroform solutions containing TMS and HFB or TFA as internal standards. Fluorine shifts are negative upfield from fluorotrichloromethane.

Difluoromaleic Acid and Difluoromaleic Anhydride (1)

According to the literature [20] fluoranil was oxidized with 40% peroxyacetic acid to difluoromaleic acid in 46% yield. Mp 214-216° dec., ^{19}F -132.3(s) ppm. Lit. [21] mp 219-220° dec; [20] mp 219-220°, ^{19}NMR -125.6 (s) ppm. Distillation of 7.5 g (0.049 mol) of difluoromaleic acid from 7.5 g (0.053 mol) of phosphorus pentoxide gave 4.4 g (67%) of difluoromaleic anhydride **1**, bp 119-124°/710 mm, ^{19}F NMR -139.5 (s) ppm. Lit. [20] bp 39°/20 mm; [21] mp 20°, bp 128°.

Monobenzyl Ester of Difluoromaleic Acid (2) (Benzyl Hydrogen Difluoromaleate) (nc)

To 3.00 g (0.0224 mol) of difluoromaleic anhydride **1** was added 6 ml of benzene. Temperature rose to 27° and a precipitate was formed which partly dissolved on stirring. A solution of 2.42 g (0.0224 mol) of benzyl alcohol in 5 ml of benzene was added all at once and the precipitate slowly dissolved. After stirring the mixture overnight the solvent was evaporated at 50° at 20 mm. The white crystalline residue of **2** (5.31 g, 97.8%) had mp 71.5-76° (79-81° after recrystallization from carbon tetrachloride or a mixture of pentane and dichloromethane 2:1).

Analysis: Found: 54.86% C, 3.56% H. Calcd. for $C_{11}H_8F_2O_4$ (242.21): 54.55% C, 3.34% H.

^{1}H NMR: δ 7.25 (m, 5H), 5.26 (s, 2H), 9.75 (s, CO_2H) ppm; ^{19}F NMR: -134.8 (d, J=5), -123.1 (m) ppm.

Dibenzyl Difluoromaleate (3) (nc)

After the conventional methods for the conversion of monobenzyl ester of difluoromaleic acid to the dibenzyl ester such as heating with benzyl alcohol, benzyl alcohol and trifluoromethanesulfonic acid, trifluoroacetic anhydride, or treatment with dicyclohexylcarbodiimide failed, the following procedure proved successful [22]:

To a solution of 3.90 g (0.016 mol) of **2** in 30 ml of hexamethylphosphorus triamide (HMPA) was added 1.15 g (0.0083 mol) of finely powdered anhydrous potassium carbonate (predried at 140°), and the mixture was magnetically stirred for 30 minutes at room temperature. Benzyl bromide (2.86 g, 0.0168 mol) was added all at once and the mixture was stirred in a lightly stoppered flask at room temperature for 10 hours. Only a small amount of a solid remained undissolved (pH 6). The light brown suspension was diluted with 75 ml of water, 30 ml of benzene was added, the benzene layer was separated, the aqueous layer was extracted with two 25 ml portions of benzene, the benzene solutions were dried over anhydrous magnesium sulfate and evaporated at 50° at 17 mm. The residue - a light brown oil (6.8 g, 83%) was chromatographed over 200g of silicagel (Mallinckrodt CC7). After elution of 0.41 g of benzyl bromide with 600 ml of hexane, elution with 775 ml of benzene gave, after evaporation at 50° at 40 mm, 3.01 g (56.6%) of **3**.

Analysis: Found: 65.48% C, 4.65% H. Calcd. for $C_{18}H_{14}F_2O_4$ (332.29): 65.06% C, 4.25% H.

^1H NMR: δ 7.12 (m, 5), 5.02 (s, H) ppm.

^{19}F NMR: -138.5 (s) ppm.

Dibenzyl 2-Dibenzylamino-3-fluoromaleate (4) (nc)

A solution of 3.00 g (0.009 mol) of dibenzyl difluoromaleate **3** in 5 ml of carbon tetrachloride was treated with 1.8 g (0.009 mol) of dibenzylamine and 0.91 g (0.009 mol) of triethylamine, and the mixture was stirred magnetically at room temperature (30°). Soon crystals of triethylamine hydrofluoride started depositing. The progress of the reaction was monitored by NMR. After 5 days 92% of the ester has reacted. After two more days, the semicrystalline mixture was shaken up with two 10 ml portions of water, the aqueous layer was separated, extracted with 10 ml of carbon tetrachloride, the combined organic solutions were dried with anhydrous magnesium sulfate and evaporated at 75° at 17 mm. The orange thick oil (4.515 g) showed R_F 0.62 on silica gel TLC in benzene elution and contained 8% of unreacted dibenzylamine as determined by NMR. Yield of the pure compound was 90%. Chromatography of 1.00 g of the oil over 30 g of silica gel (Mallinckrodt, CC7) gave 0.811 g of chromatographically pure and sterically uniform compound **4** on elution with 165 ml of benzene.

Analysis: Found: 75.81% C, 5.70% H, 3.59% F, 2.70% N.

Calcd. for $C_{32}H_{28}FNO_4$ (509.55): 75.42% C, 5.54% H, 3.73% F, 2.75%. N.

IR (film): 3080 (w), 3045 (w), 2960 (w), 1745 (s), 1710 (s), 1610 (s), 1540 (s), 1502 (m), 1460 (s), 1390 (s), 1325 (s), 1310 (s), 1265 (s), 1210 (s), 1155 (sh), 1127 (s), 1084 (m), 1033 (s), 1008 (m), 965 (w), 910 (w), 795 (w), 757 (s), 702 (s) cm^{-1}.

^{1}H NMR: δ 4.22 (s, 2H), 5.06 (s, 1H), 5.14 (s, 1H), 7.1-7.35 (m, 5H) ppm.

^{19}F NMR: -161.9 (CCl_4), -162.8 ($CDCl_3$) (s) ppm.

Dibenzyl 2-Dibenzylamino-3-fluorosuccinate (5) (nc)

In a 100 ml flask, hydrogen chloride (0.39 g, 0.0107 mol) was introduced into a solution of 1.25 g (0.00245 mol) of the enamine **4** in 12.5 ml of anhydrous tetrahydrofuran. The flask was immersed in an ice bath, and a solution of 0.33 g (0.00525 mol) of sodium cyanobohydride in 7.5 ml of methanol (precooled in an ice bath) was added all at once. An immediate reaction took place with strong foaming. The mixture was stirred at room temperature for 3 hours. Thereafter, 10 ml of water was added to dissolve the salts. The clear yellow solution was alkalized with a solution of 2 g of sodium carbonate in 40 ml of water to pH 10, extracted with four 20 ml portions of ether, the ether layers were combined and washed with two 5 ml portions of water, dried with anhydrous magnesium sulfate, and the ester was evaporated at 32° at 80 mm to give 1.12 g (89.3%) of **5**, a mixture of diastereomers in the ratio of 85% : 15%.

^{19}F NMR: -199.6 (85%) ppm, dd, J_{HF} gem 48.1 Hz, J_{HF}vic 22.9 Hz.
-197.2 (15%) ppm, J_{HF} gem 45.7, dd, J_{HF}vic 30.7 Hz.

3-Fluoroaspartic Acid (6)

A solution of 1.12 g (0.00219 mol) of **5** in 17 ml of ethanol was hydrogenated over 0.44 g of 10% palladium on activated carbon at room temperature and atmospheric pressure. After 2 hours, the theoretic volume of hydrogen was absorbed. After additional 20 hours the mixture was filtered, the catalyst was washed with ethanol, and the ethanolic solution containing toluene was evaporated to dryness at 36° at 40 mm. The partly crystalline residue (0.64 g) contained, according to ^{19}F NMR, impure 3-fluoroaspartic acids. Digestion of the catalyst for 45 minutes at 30-40° with 25 ml of distilled water gave, after filtration and evaporation of the filtrate at 40-42° at 32 mm, 0.11 g (33.3%) of pure threo-3-fluoroaspartic acid (R,S;S,R); mp 171-173° dec. (unchanged after recrystallization from water).

^{1}H NMR: δ 4.54 ppm (-$CHNH_2$) (dd, J_{HF}vic 29.1 Hz, J_{HH} 2.0 Hz); 5.55 ppm, -(CHF-), (dd, J_{HF}gem 44.8 Hz, J_{HH} 2.0 Hz).

^{19}F NMR: - 202.6 ppm (dd, J_{HF}gem 44.9 Hz, J_{HF}vic 29.0 Hz).
Literature values of melting points and ^{1}H and ^{19}F NMR are listed in Table I.

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