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SYNTHESIS OF 2- AND 3-FLUOROTYROSINE WITH DILUTE FLUORINE GAS

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SUMMARY

Differences in reactivity and selectivity of fluorine gas towards L-tyrosine and the O,N-diacetylated derivative of L-tyrosine methyl ester have been exploited for the synthesis of 2- and 3-fluorotyrosine. Both 2- and 3-fluorotyrosine were identified by ^{1}H, ^{19}F and ^{13}C NMR spectroscopy and high resolution mass spectrometry. The short synthesis time and high reaction yields allow this procedure to be used for the incorporation of the short lived positron emitting radionuclide ^{18}F into the aromatic ring of L-tyrosine.

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INTRODUCTION

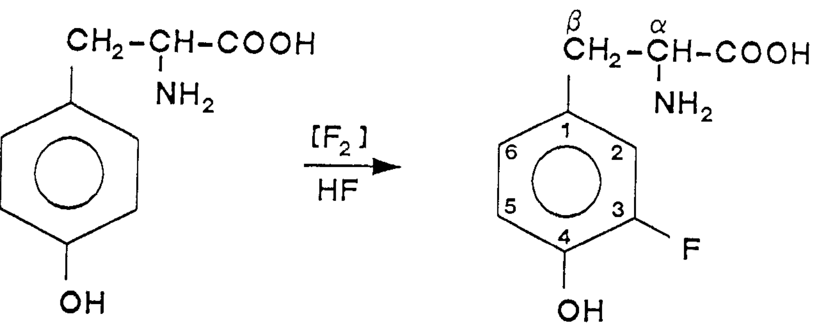
Tyrosine is a nonessential aromatic amino acid derived in animal cells by the hydroxylation of phenylalanine. It is a precursor of thyroxine and of the neurotransmitter hormones adrenaline and noradrenaline. It is also a precursor of the central neurotransmitter dopamine. In addition, it is a constituent of many proteins. If a suitable gamma emitting label can be found for tyrosine, it should be possible to use standard techniques of nuclear medicine to study hormone, neurotransmitter or protein synthesis. Carbon-11 ($T_{1/2}$ = 20 min) and nitrogen-13 ($T_{1/2}$ = 10 min) would be obvious labels but their relatively short half life would preclude their use in studies lasting beyond a couple of hours.

Fluorine-18 ($T_{1/2}$ = 110 min) has been used successfully to label 3, 4-dihydroxyphenylalanine [1] and the resulting 6-[^{18}F]fluorodopa has been used to visualize and quantitate the cerebral metabolism of dopamine in living human brain [2]. To date, ^{18}F has been introduced into the aromatic ring of tyrosine by the Schiemann reaction [3], a time-consuming multistep radiochemical synthesis that yields very small amounts (1-3%) of 3-fluorotyrosine. We now describe a method for a high yield synthesis of 3-fluorotyrosine by direct fluorination of tyrosine. We also report a method, using dilute fluorine gas, for the synthesis of 2- and 3-fluorotyrosine. The synthesis and identification of 2-fluorotyrosine has not been reported in the literature. The synthesis of 2-fluorotyrosine by direct fluorination requires modification of the reactivity and orientation of tyrosine towards electrophilic substitution. This has been achieved by the derivatization of the phenolic group. Because our methods produce high yields and short synthesis times, the procedures can also be applied for the incorporation of ^{18}F into tyrosine.

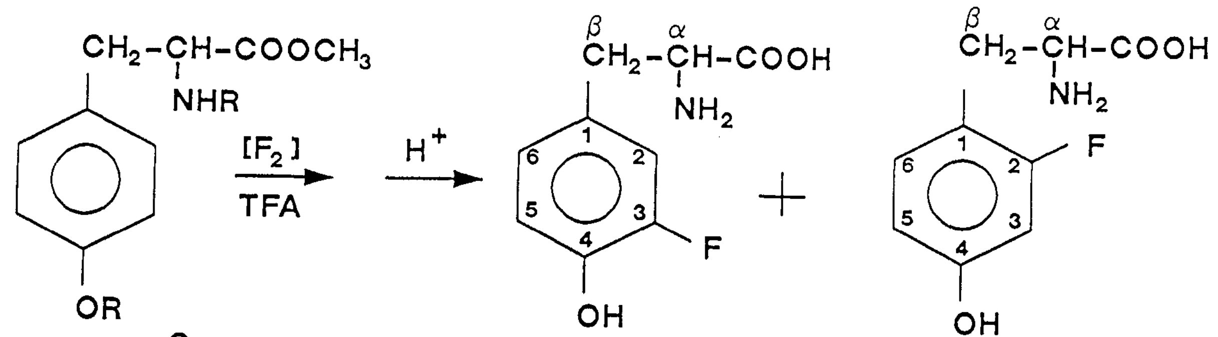
RESULTS AND DISCUSSION

Identification of products: HPLC analysis of the reaction mixture (from Scheme I) gave two UV peaks, a major one at 25 min and a second one at 33 min. A comparison of the retention time as well as spiking the sample with authentic tyrosine enabled us to assign the earlier peak to tyrosine. The sample eluting at 33 min had the same retention time and co-chromatographed with authentic 3-fluorotyrosine. The 33 min sample and 3-fluorotyrosine showed similar U.V. characteristics (λ max = 274 nm) and their ^{19}F NMR spectra consisted of multiplets at -136.6 ppm. (Fig. 1). ^{1}H NMR spectrum of the sample revealed one less proton in the aromatic region when compared with the spectrum of L-tyrosine. The ^{1}H chemical shifts and coupling constants were also similar to those of authentic 3-fluorotyrosine (Table 1). Carbon-13 chemical shifts of both compounds agreed with the values predicted from fluorine substituent shifts [4] (Table 2). In addition, the low and high resolution fast atom bombardment mass spectra of the sample were identical to those of 3-fluorotyrosine and showed protonated molecular ion at M/z = 200 [$(m+H)^+$]. The chemical yield, using F-18 labelled fluorine gas, was 46% with respect to ^{18}F-fluorine.

2-Fluorotyrosine was isolated along with 3-fluorotyrosine from reaction Scheme II. HPLC analysis of the reaction mixture showed major peaks for tyrosine, 3-fluorotyrosine and another peak at 34 min. We have ascribed the latter peak to 2-fluorotyrosine on the basis of the following observations. The high resolution mass spectrum of the combined peaks was identical to that of 3-fluorotyrosine. A radiochromatogram of the reaction mixture showed the ratio of F-18 activity in the peaks eluting at 33 and 34 min to be 62:38. A ^{19}F NMR spectrum of the combined peaks gave multiplets at -136.6 ppm (3-fluorotyrosine) and at -115.8 ppm (Fig. 1). The distribution of the



SCHEME I



SCHEME II

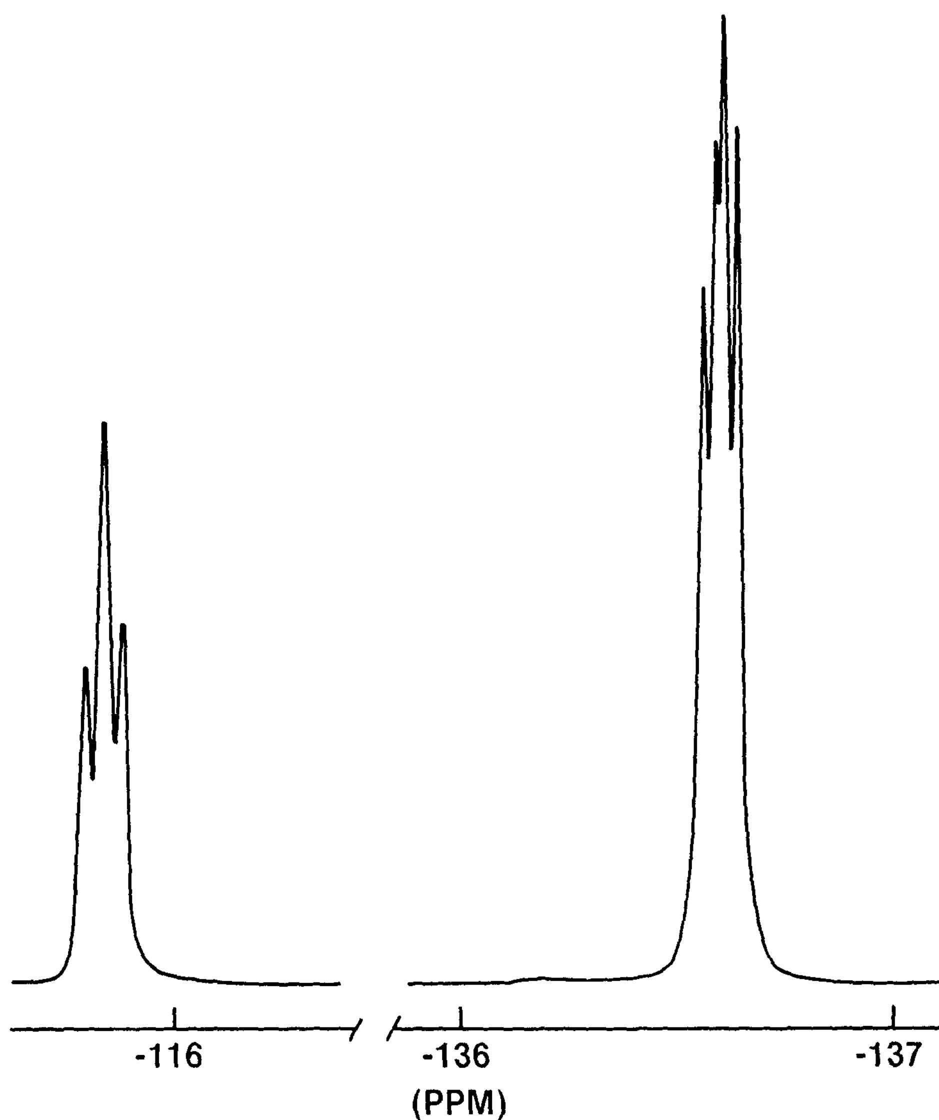


Fig. 1. ^{19}F spectrum of 3-fluorotyrosine (right) and 2-fluorotyrosine (left)

areas under these multiplets (60:40) agreed with the distribution of F-18 on the radiochromatogram. A carbon-13 spectrum of the mixture gave signals well resolved from those arising from 3-fluorotyrosine. Their

TABLE 1

^{1}H chemical shifts and coupling constants of 3-fluorotyrosine.

Chemical Shifts (δ) ppm			Coupling Constants (Hz)	
	3-F tyrosine	33 min sample		
H-α	4.10	4.03	$^3J_{\alpha\beta}$	5.7
H-β	3.05	2.97	$^3J_{\alpha\beta'}$	7.5
H-β'	2.94	2.87	$^2J_{\beta\beta'}$	-14.8
H-2	6.86	6.79	$^4J_{H_2H_6}$	1.9
H-5	6.77	6.68	$^3J_{H_5H_6}$	8.2
H-6	6.75	6.70	$^3J_{H_2F}$	12.0
			$^3J_{H_5F}$	8.2

chemical shifts agreed with the values predicted for 2-fluorotyrosine (Table 2). The combined chemical yield of 3- and 2-fluorotyrosine using fluorine-18 fluorine gas was 26%.

Synthesis of 3-fluorotyrosine using the pyrolysis followed by acid hydrolysis of 2-methoxy-5 (2',2'-dicarbethoxy-2'-acetamidoethyl)-phenyl diazonium tetrafluoroborate [3] gives 3-fluoro-DL-tyrosine which is of limited use for biological and pharmaceutical investigations because the L-enantiomer is needed for these studies. It has been shown that electrophilic fluorination of the aromatic ring using F_2 [5], XeF_2 [6], and acetyl hypofluorite [7] does not affect the chiral centre on the side

TABLE 2

^{13}C chemical shifts (a) (ppm) and ^{13}C - ^{19}F coupling constants (b) (Hz) of 3- and 2-fluorotyrosine.

a) Chemical Shifts

	3-Fluorotyrosine		2-Fluorotyrosine
COOH	172.3	COOH	---
C_α	55.1	C_α	54.6
C_β	35.7	C_β	30.6
C_1	127.6 (127.6)*	C_1	113.5 (112.5)*
C_2	118.2 (117.8)	C_2	162.9 (167.1)
C_3	152.5 (152.3)	C_3	104.4 (102.9)
C_4	144.0 (142.1)	C_4	158.2 (157.2)
C_5	119.4 (118.0)	C_5	113.1 (112.7)
C_6	127.0 (127.6)	C_6	133.6 (132.9)

b) Coupling Constants

	3-Fluorotyrosine		2-Fluorotyrosine
$^3J_{1,F}$	5.7	$^2J_{1,F}$	16.1
$^2J_{2,F}$	18.4	$^1J_{2,F}$	243.8
$^1J_{3,F}$	240.7	$^2J_{3,F}$	25.0
$^2J_{4,F}$	12.8	$^3J_{4,F}$	12.3
$^3J_{5,F}$	---	$^4J_{5,F}$	2.3
$^4J_{6,F}$	2.6	$^3J_{6,F}$	5.7

*Calculated values using fluorine substituent parameters are shown in brackets.

chain. We, therefore, believe that the fluorotyrosine obtained from both reaction schemes is the L-enantiomer.

We [1] have shown that direct fluorination of catecholamines in anhydrous hydrogen fluoride produces monofluorinated products in high yields. Our results show that the fluorination of tyrosine in anhydrous

hydrogen fluoride is highly regiospecific. Similar results were obtained when the reaction was carried out in trifluoroacetic acid instead of hydrogen fluoride, even though the yield of 3-fluorotyrosine was reduced to 23%. An electrophilic substitution similar to the ionic halogenation reactions of aromatic compounds can be offered as a possible reaction mechanism. Formation of 3-fluorotyrosine (Scheme I) is consistent with this mechanism because the ortho and para directing nature of the -OH group in tyrosine would yield predominantly 3-fluorotyrosine. Misaki [8] has reported similar results when phenol and p-cresol were used as the substrate for direct fluorination.

Protection of the phenolic group by a less activating group towards electrophilic substitution, such as the acetyl group, should favor the formation of 2-fluorotyrosine. Indeed,the direct fluorination of O,N-diacetyltyrosine methyl ester in trifluoroacetic acid solution (Scheme II) produced both 2- and 3-fluorotyrosine. The ratio of 2- to 3-fluorotyrosine was the same even when the reaction was carried out in solvents such as CH_3CN or $CHCl_3:CFCl_3$ (1:1). However, the yields of 2- and 3-fluorotyrosine, when these solvents were used, were very low (1-3%).

When HF, rather than TFA, was the solvent, 98% of the monofluorinated product was 3-fluorotyrosine. This may be due to hydrolysis of the acetyl groups by HF, after which the fluorination reaction becomes similar to Scheme I. Use of acetyl hypofluorite as the fluorinating agent did not improve the regiospecificity of the reaction depicted in Scheme II. We are unable to assess how much steric hindrance from the side chain contributes to the orientation effect. If the fluorination proceeds predominantly by a 'polar substitution mechanism' [9], then the protection of the phenolic group by a more electronegative substituent should be more effective for the formation of 2-fluorotyrosine.

Reaction Scheme I provides an efficient and rapid (120 min) method for the production of ^{18}F labelled 3-fluorotyrosine. Our results show that 70 mCi of ^{18}F [F_2] is sufficient to produce 5 - 6 mCi of 3-[^{18}F]fluorotyrosine. We have demonstrated that the reactions in Scheme II can be used to make 2-fluorotyrosine. Separation of 2- and 3-fluorotyrosine requires improved HPLC conditions. Our preliminary results indicate that millicurie quantities of 2-[^{18}F]fluorotyrosine can be obtained by careful fractionation of the eluate.

EXPERIMENTAL

L-Tyrosine (Fisher Scientific), m-fluoro-DL-tyrosine (Sigma), hydrogen fluoride (Matheson), and trifluoroacetic acid (BDH) were obtained commercially and used without further purification.

NMR Spectroscopy

The ^{1}H NMR spectra were recorded at 500.13 MHz on a Bruker AM-500 spectrometer. When D_2O:DCl was used as solvent, the chemical shifts were reported with respect to HDO (4.6 ppm). When $CDCl_3$ was used as solvent, the residual chloroform signal (7.24 ppm) relative to TMS was used as an internal reference. ^{19}F spectra were recorded at 235.36 MHz on a Bruker WM-250 spectrometer. Samples were dissolved in D_2O:DCl and the chemical shifts were reported in ppm relative to the external $CFCl_3$ (0 ppm). Carbon-13 spectra were recorded on a Bruker AM-500 spectrometer at 125.76 MHz over a 30.0 KHz sweep width in 32 K data points. The ^{13}C chemical shifts were reported relative to external TMS. The ^{13}C chemical shifts of the aromatic carbons in 2- and 3-fluorotyrosine were calculated using fluorine substituent shifts [4] in ortho and meta positions of L-tyrosine.

Mass Spectrometry

High resolution mass spectra were obtained with a double focusing VG ZAB-E mass spectrometer under positive ion fast atom bombardment conditions. Trifluoroacetic acid (20%) in glycerol was used as the matrix and xenon was the bombarding species (8 KeV). Accurate masses and elemental composition of the protonated molecular ions were determined under high resolution conditions (resolution 6000), using glycerol cluster ions as the reference.

HPLC analyses

HPLC analyses were done using two semi-preparative reverse phase columns (Waters μ-Bondapak C_{18}, 7.8 mm x 300 mm) with 0.1% acetic acid as mobile phase at 2 ml/min. The eluate was monitored for UV absorption at 280 nm. In experiments where fluorine-18 was used to determine the yield of the reaction with respect to fluorine, the eluate was also passed through a sodium iodide detector. For identification purposes, the eluates were co-chromatographed and their retention times were compared with those of authentic L-tyrosine and 3-fluoro-DL-tyrosine, (25 and 33 minutes respectively).

Preparation of L-methyl-N-acetyl-(4-acetoxyphenyl)alanine (1)

A stream of HCl gas was bubbled through a cold ($0^{o}C$) suspension of L-tyrosine (3.9 g) in methanol for 1-2 min. The resultant clear solution was stirred overnight. The solvent was evaporated, the residue was redissolved in methanol and evaporated again. The residue was then dried overnight to give L-tyrosine methyl ester hydrochloride (4.5 g, 88% yield). The crude tyrosine methyl ester HCl was stirred, with cooling, in a mixture of pyridine-acetic anhydride (1:1, 25 ml) for 10 min. The mixture was poured into 1 M sulphuric acid (100 ml) and the product was

extracted with ethyl acetate (3 x 60 ml) to yield a white solid (6.0 g). This was recrystallized from ethyl acetate, filtered and washed with hexane-ethyl acetate (1:2) to give 5.2 g of white solid, mp 98-100^{0}C. The ^{1}H NMR spectrum of (1) in $CDCl_3$ comprised signals at 1.90 (S, 3H), 2.20 (S, 3H), 3.02 (2H, ABX Pattern J_{AB} = -13.70 Hz, J_{AX} = J_{BX} = 5.70 Hz), 3.65 (S, 3H), 4.79 (m, 1H J_{CH-NH} = 7.3 Hz), 6.13 (broad doublet 1H) and 6.93-7.03 (4H AA$'$ BB$'$). A high resolution mass spectrum of the compound gave a major peak at M/z 280 $[m+H]^+$.

3-Fluorotyrosine: Reaction Scheme I

L-Tyrosine (100 mg) was placed in a Kel-F vessel and hydrogen fluoride was condensed into it under vacuum at -196^{0}C. The mixture was degassed and equilibrated at -65^{0}C. Dilute fluorine gas (0.5% in neon) was bubbled through the solution at approximately 70-90 ml/min for 30 minutes. Hydrogen fluoride was then removed by vacuum distillation. A brown residue was obtained. This was dissolved in 1 M HCl and transferred into a round bottomed flask. The solvent was evaporated on a rotary evaporator and the residue was washed with water and evaporated again. The final residue was dissolved in 2 ml of water and filtered through a 0.4 μ filter for HPLC separation.

2-Fluorotyrosine: Reaction Scheme II

Compound (1) (150 mg) was dissolved in 6-8 mL of trifluoroacetic acid. The solution was kept at 0-4^{0}C and 0.5% fluorine in neon was bubbled through at 70-90 ml/min for 30 min. The reaction mixture was transferred into a round bottomed flask and the solvent was evaporated under vacuum. The yellowish oily residue that was obtained was dissolved in 48% HBr and refluxed for 25 minutes at 145^{0}C. The HBr was evaporated, and the residue was washed with water (2 x 5 ml portions) and evaporated to remove any traces of acid. The final residue was dissolved in 2 ml of water and filtered through 0.4 μ filter for HPLC analysis.

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