SYNTHESIS OF F-18 LABELLED FLUORO-MELATONINS AND 5-HYDROXY-FLUORO-TRYPTOPHANS

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SUMMARY

Reaction of dilute [F-18]fluorine gas with either melatonin or 5-hydroxy-tryptophan in hydrogen fluoride at -70°C gives 6-fluoro-melatonin or 4- and 6-fluoro-5-hydroxytryptophan. They are of potential use in Positron Emission Tomography to image the binding sites for melatonin and to study the metabolism of serotonin.

Key words: Radiofluorination, F-18, Melatonin, Positron Emission Tomography

INTRODUCTION

Fluorinated 5-hydroxy-indoles have been prepared by the Abramovitch variation of the general Fischer synthesis for indoles (1). The synthesis is not suitable to prepare radiofluorinated indoles because it requires a lengthy sequence of reactions after the introduction of fluorine. Direct fluorination of 5-hydroxy-indoles would be preferred for work with F-18.

Recently, we have developed a simple technique for the direct radio-fluorination of activated aromatic compounds. For example, catechols dissolved in anhydrous hydrogen fluoride at low temperature react with dilute [F-18] fluorine gas to form [F-18]fluoro-catechols in good yields (2, 3). The fluorination reaction yields several isomers of the ring fluorinated catechol. This may be considered an advantage because after chromatographic separation, the isomers of novel fluorinated biomolecules, for example 2- and 6-fluoro-L-dopa, are available for biological investigations.

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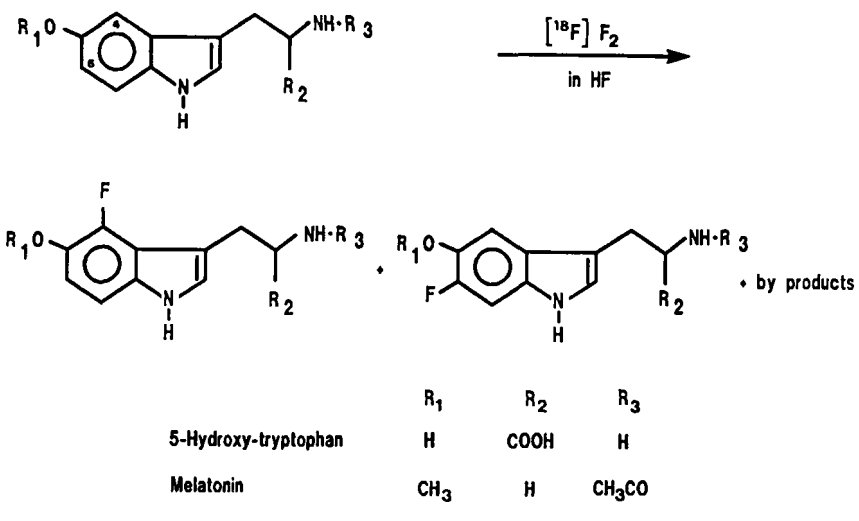
To explore further the scope of the direct radiofluorination we have applied it to 5-hydroxy-indoles. As examples we have deliberately chosen a fragile indole with three unprotected functional groups, 5-hydroxy-tryptophan, and the fairly stable indole, N-acetyl 5-methoxy-tryptamine, melatonin. Both are natural compounds and play an important role in the brain. 5-hydroxy-tryptophan is the metabolic precursor for the neurotransmitter serotonin. Melatonin is a neurohormone that is involved in the regulation of chronobiological rhythms, such as sleep and fertility (4).

The F-18 labelled fluoroanalogs of these key neurochemicals may be used in vivo for metabolic imaing with Positron Emission Tomography.

Scheme 1



	R_1	R_2	R_3
5-Hydroxy-tryptophan	H	COOH	H
Melatonin	CH_3	H	CH_3CO

MATERIALS

Melatonin and 5-hydroxy-tryptophan (5-HTP) were obtained from SIGMA. Anhydrous hydrogen fluoride (HF) was obtained from Matheson

and used without further purification. Fluorine-18 labelled fluorine gas, [F-18]F_2, was produced by the nuclear reaction $^{20}Ne(d,\alpha)\ ^{18}F$ when neon with 0.5% F_2 was irradiated with 15-MeV deuterons from the McMaster Tandem Van de Graaff Accelerator (5). The apparatus for handling HF and F_2 has been described earlier (2).

The ^{1}H- and ^{19}F-NMR spectra were recorded at 235 MHz and at field strength of 5.8719 tesla with a Bruker WM-250 spectrometer using a 5 mm probe at 12 ^{o}C. The melatonin samples were dissolved in DMSO and those of 5-HTP in DCl/D_2O to give a concentration of 0.1 molar. The external standards were TMS and $CFCl_3$ for ^{1}H- and ^{19}F-NMR, respectively.

The mass spectra were recorded using VG ZAB-E with fast atom bombardment ionization.

FLUORINATION PROCEDURE

Melatonin or 5-HTP (540 umol) was dissolved in HF (5 ml) and the solution was cooled to -70^{o}C. [F-18]F_2 (230 umol) in neon was passed through the solution at 80 ml/min. After the [^{18}F]F_2 had been passed through the substrate solution the ^{18}F contained in the reaction vessel was measured and taken as 100% for the calculation of the radiochemical yield. Then, the HF was evaporated.

When melatonin was the substrate, the residue was dissolved in 5 ml water-methanol (1:1) and evaporated to dryness. This procedure was repeated. Then the yellowish reaction mixture, dissolved in 3.0 ml water-ethanol (3:1); was separated by reversed-phase high pressure liquid chromatography (HPLC).

Table 1 summarizes the conditions. The eluate from the HPLC column was monitored continuously for UV-absorption at 280 um and for ^{18}F with a 120-ul-flow cell coupled to a NaI(Tl) scintillation detector.

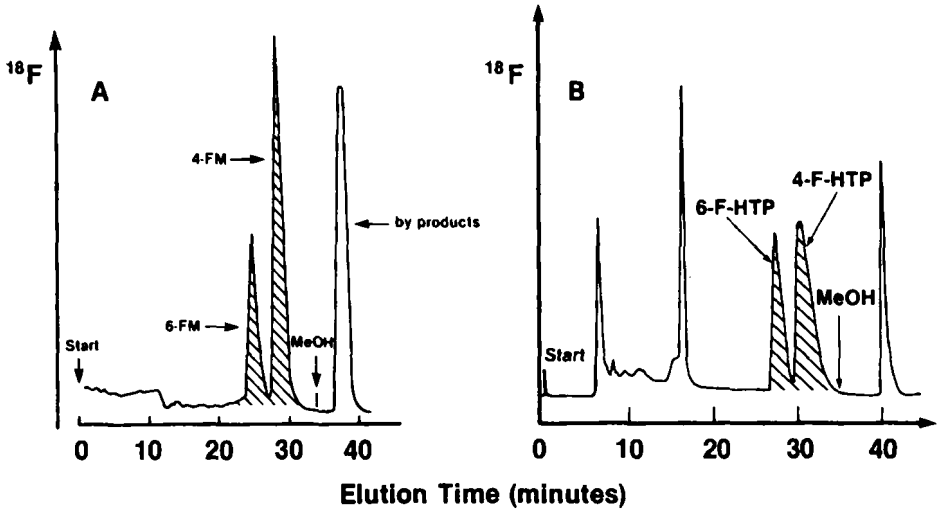
Table 1: HPLC - conditions and yield of products

Substrate	HPLC Column	HPLC Mobile Phase	Elution Times min	Products	Radiochem. Yield %
Melatonin	waters u Bonda-pak C-18 10 u 0.78x30 cm	water + 20% methanol at 3 ml/min	25	6-fluoro	8
			29	4-fluoro	19
5-HTP	Whatman Partisil ODS-2 10 u 0.9x50 cm	0.1% acetic acid + 3% methanol at 4 ml/min	27	6-fluoro	6.5
			31	4-fluoro	9

When 5-HTP was the substrate, the residue after the evaporation of HF was dissolved in 5 ml water and evaporated. This residue was re-dissolved in water and evaporated again. Finally, it was dissolved in 2 ml water and filtered through 0.4 u filter for HPLC separation.

A radiochromatogram of the HPLC separations on the analytical scale (0.05 ml aliquot of the reaction mixture) is shown in Fig. 1A and B. For preparative purposes the eluate was collected in fractions based upon the trace of the UV detector. The ^{18}F contained in the fractions was measured; the decay-corrected radiochemical yield for each product is given in Table 1. The synthesis with both substrates took 110 minutes. The specific activity was 235 mCi/mmol. The purity of each ^{18}F-indole preparation was analysed by analytical HPLC and found to be > 95%.

Figure 1: High pressure liquid chromatographic isolation of the fluoromelatonins (A) and fluoro-5-hydroxy-tryptophans (B)



4-FM = 4-fluoro-melatonin
6-FM = 6-fluoro-melatonin

4-F-HTP = 4-fluoro-5-OH-tryptophan
6-F-HTP = 6-Fluoro-5-OH-tryptophan

IDENTIFICATION OF THE PRODUCTS

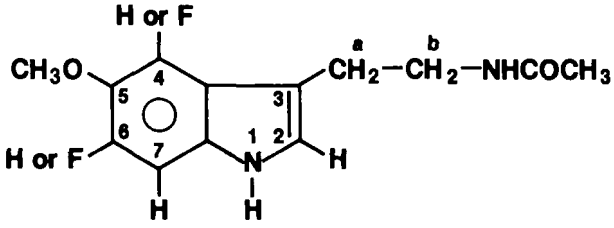
6-Fluoro-melatonin: The material of the 25-min peak (Fig. 1A) co-chromatographed with authentic 6-fluoromelatonin (1). Its ^{1}H- and ^{19}F-NMR spectra were identical to those of authentic 6-fluoromelatonin (Table 2).

4-Fluoro-melatonin: We have identified the material of the 29-min peak (Fig. 1A) as a fluoro-melatonin based on mass spectrometry. It showed a peak at *m/z* = 251.1 which corresponds to the protonated molecular ion (MH^+).

Comparison of the integrated H-NMR of melatonin with those of the 29-min material (Fig 1A) showed one less proton in the aromatic region of the latter material. Irradiation of the N-H proton in position 1 of the indole ring caused collapse of the doublet at 7.16 ppm of the proton in position 2. This indicated that fluorine did not enter in position 2. When the proton at 7.0 ppm (H-6 proton) was

irradiated the doublet at -147.8 ppm (J_{HF} = 7.8 Hz) in the ^{19}F-NMR spectrum collapsed to a singlet. ^{1}H-NMR of the 29-min material also showed an AB pattern for the aromatic protons (J=8.7 Hz) indicating that they are attached to adjacent carbons (C-6 and C-7). In 6-fluoromelatonin no coupling between H-4 and H-7 was observed. However, they were both coupled to fluorine at position 6 (Table 2). These observations confirm the presence of fluorine at carbon-4 in the material of the 29-minute peak.

Table 2: NMR-data of melatonin and fluoromelatonins

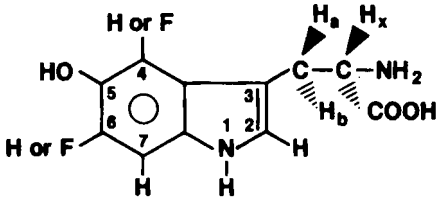


	CHEMICAL SHIFTS PPM							COUPLING CONSTANTS Hz			
	H_2	H_4	H_6	H_7	H_a	H_b	^{19}F	H_4-H_6	H_6-H_7	H_a-H_b	H-F
Melatonin	7.10	7.05	6.74	7.27	2.81	3.33	—	2.1	8.7	7.3	—
4-F-Melatonin	7.16	—	7.00	7.11	2.90	3.35	-147.8	—	8.7	7.3	7.8
6-F-Melatonin	7.10	7.21	—	7.21	2.80	3.32	-141.0	—	—	7.3	11.6 9.6

Solvent: DMSO (d_6)
Reference: DMSO (d_6) for ^{1}H
$CFCl_3$ for ^{19}F

4- and 6-Fluoro-5-HTP were identified on the basis of their ^{1}H- and ^{19}F-NMR data (Table 3).

Table 3: NMR-data of 5-hydroxy-tryptophan and its fluoro derivatives



	CHEMICAL SHIFTS PPM								COUPLING CONSTANTS Hz					
	H_2	H_4	H_6	H_7	H_a	H_b	H_x	^{19}F	H_4-H_6	H_6-H_7	H_a-H_b	H_a-H_x	H_b-H_x	H-F
5-HTP	—	6.90	6.50	7.04	3.01	3.08	4.02	—	—	8.34	15.16	6.16	5.78	—
4-Fluoro-5-HTP	6.58	—	6.27	6.51	2.99	2.96	3.74	-151.2	—	8.32	15.16	8.50	5.34	8.30
6-Fluoro-5-HTP	6.90	6.90	—	6.91	3.01	3.08	4.02	-147.6	—	—	15.16	6.20	5.80	8.50 11.80

Solvent: D_2O/DCl
Reference: D_2O for ^1H
$CFCl_3$ for ^{19}F

DISCUSSION

This is the first report of the direct fluorination of 5-hydroxy-indole derivatives. The reaction between fluorine and the 5-hydroxy-indoles in HF produced two structural isomers of each indole. 4- and 6-fluoro-melatonin and 4- and 6-fluoro-5-HTP (Scheme 1). Three of these compounds are novel fluoroanalogs; 6-fluoro-melatonin has been made before (1). It should be noted that in both cases the 4-fluoroisomer was the prominent product.

The formation of fluorocatechols during the reaction between F_2 and a catechol derivative in HF appears to be the result of the electrophilic action of fluorine. Nucleophilic sites, i.e. the preferred sites for electrophilic attack on the 5-hydroxy-indole, have been identified by kinetic measurements of the amount of hydrogen exchanged for deuterium in deuterated acid (6). The rate of this exchange is greater in position 4 than it is in position 6. It is therefore not surprising that electrophilic fluorine entered position 4 preferentially. The addition of BF_3 to the reaction mixture did not increase the yield of the fluoro-indoles. This is in

contrast to the fluorination of catechols (3).

The method can be used to produce either F-18 labelled fluoro-5-hydroxy-indole or fluoromelatonin in millicurie quantities. These quantities are required when the deposition of either of these compounds is to be studied in humans with Positron Emission Tomography.

Ultimately, the F-18 labelled fluoromelatonin is intended for melatonin receptor mapping in the human brain. This use as a tracer for natural melatonin requires, of course, that the tracer behaves biochemically like the tracee. Such biological equality needs to be established. One encouraging fact is known already; "the fluorine substitution for hydrogen in position 6 does not detectably interfere with activation of the pituitary melatonin receptor" (7, 8).

The specific activity of 235 mCi/mmol is not high enough to allow quantitation of the melatonin receptors. A material with > 100 Ci/mmol is usually required for receptor studies. Radiofluorinated melatonin with high specific activity could be produced by nucleophilic substitution of an appropriate leaving group by no-carrier added [^{18}F]fluoride. Work is in progress in our laboratory to synthesize material with high specific activity.

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