Synthesis of Ring-Fluorinated Serotonins and Melatonins

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The Abramovitch adaptation of the Fischer indole synthesis was used to prepare 6-fluoro- and 4,6-difluoroserotonin and the corresponding melatonins. Appropriate fluorinated anisidines led, after diazotization, coupling with 2-oxopiperidine-3-carboxylic acid, and cyclization of the products, to the corresponding β-carbolines. Cyclization of the unsymmetrical mono-fluorophenylhydrazone produced only one of two possible isomers. Ring opening of the β-carbolines and decarboxylation gave 6-fluoro- and 4,6-difluoro-5-methoxytryptamines, demethylation of which with boron tribromide produced 6-fluoro- and 4,6-difluoroserotonin. Acetylation of the methoxy tryptamines gave the corresponding fluorinated melatonins. As a result of the electronic effects of fluorine substitution, the phenol acidities of the serotonin analogs are considerably enhanced.

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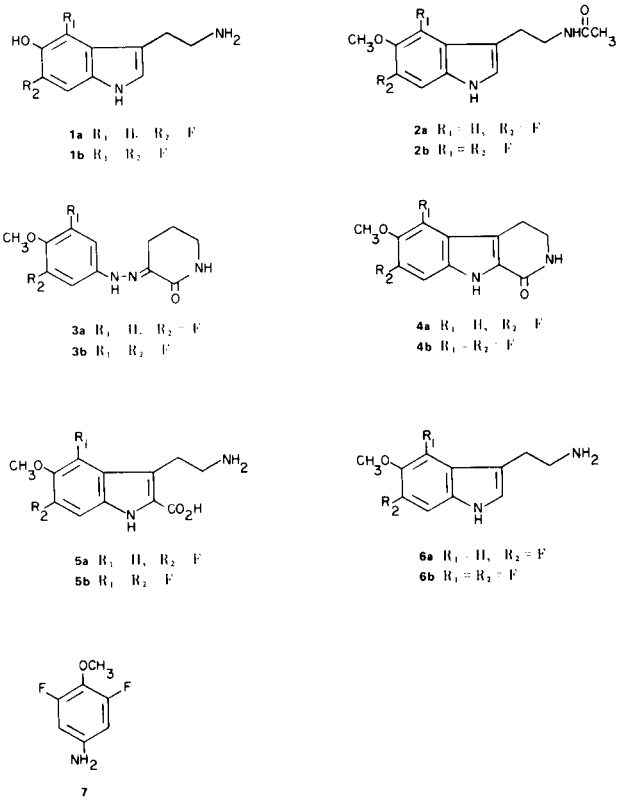
As the biochemical and pharmacological studies of 5-hydroxytryptamine (serotonin) increase in scope, the importance of this amine in a host of physiological functions becomes more and more evident (1). In experiments designed to identify mechanisms and sites of action of serotonin, structurally related tryptamines have been investigated (2). Replacement of the hydroxyl group with other functions results in analogs which usually possess decreased activity, suggesting the importance of this part of the molecule in interaction with receptor sites (2). That the phenolic group is indeed involved in amine/receptor interactions is suggested further by the presence of this group in a wide range of biogenic amines. While many 5-substituted tryptamines have been examined as analogs of serotonin, few compounds have been studied which contain both a 5-hydroxyl group and an additional substituent at C-4, 6, or 7 (3). This report describes the synthesis of 6-fluoro (**1a**) and 4,6-difluoroserotonin (**1b**), analogs designed to probe the effect of variations in the physico-chemical properties of the 5-hydroxyl groups, *eg.* enhanced phenol acidity and intra-molecular hydrogen bonding, the alterations in biochemical behavior already observed with ring-fluorinated tyramines and dopamines (4) making this approach seem worthy of pursuit. The preparation of the analogous melatonins, **2a** and **2b**, also is described.

The Abramovitch adaption (5) of the Fischer indole synthesis was envisioned as a convenient route to indoles having the desired functionality (Scheme). Since this

Scheme



route was used by Abramovitch for the preparation of 6-methoxytryptamine, no difficulties were foreseen in the

key cyclization step. Thus, formic acid-catalyzed cyclization of the phenylhydrazone (**3a**) prepared by coupling diazotized 3-fluoro-4-methoxyaniline (6) and 2-oxopiperidine-3-carboxylic acid produced a single ketocarboline (**4a**). ^{1}H-nmr analysis of this product showed *ortho* and *para* proton-fluorine spin interactions, without discernible proton-proton coupling, and thus established the structure as **4a**. The formation of only one of the two possible isomeric ketocarbolines apparently results from the directive influence of the fluorine substituent, its high electronegativity being more effective at an *ortho vs para* carbon atom (7). This result is not surprising in light of the electrophilic character of the aromatic substitution step of the cyclization (8). Base-catalyzed ring opening of **4a**, and acid-catalyzed decarboxylation of the resulting amino acid **5a** produced 5-methoxy-6-fluorotryptamine (**6a**). In order to effect the hydrolysis of **4a** (as well as of **4b**), more forcing conditions were required than described by Abramovitch for the preparation of 5-methoxytryptamine (5). The increased stability of **4** to base may be due to the effect of fluorine in increasing the acidity of the indole NH, the resulting negative charge protecting the carbonyl group from attack by hydroxide ion. Similarly, the amino acids **5a** and **5b** were more resistant to decarboxylation, possibly because the fluorine renders the same nitrogen atom more difficult to protonate, with protonation a presumed requirement for decarboxylation. Demethylation of **6a** with boron tribromide (9) proceeded cleanly to give 6-fluoroserotonin (**1a**), isolated as the creatinine sulfate salt. To our knowledge, this is the first use of boron tribromide for demethylation of methoxy indoles, a method which appears to be superior to reported procedures (10).

Coupling of diazotized 3,4-difluoro-4-methoxyaniline (11) with 2-oxopiperidine-3-carboxylic acid produced phenylhydrazone **3b**; cyclization of **3b** with formic acid gave a good yield of the difluoroketocarboline **4b**. The ^{1}H nmr spectrum of **4b** displayed one aromatic proton, split into a symmetrical quartet by *ortho* and *para* fluorine coupling. Base-catalyzed ring opening of **4b** and decarboxylation of the product in acid afforded 4,6-difluoro-5-methoxytryptamine (**6b**). This compound was demethylated with boron tribromide to give 4,6-difluoroserotonin (**1b**), isolated as its creatinine sulfate salt.

The electronic effects of the fluorine substituents are reflected in the ionization constants (phenol acidity) of **1a** and **1b**, as determined spectrophotometrically. Thus, **1a** and **1b** give p*K* values of 9.07 and 7.97, respectively, which may be compared with 10.73 (12) for serotonin itself. Fluorine substitution should also influence the hydrogen bonding properties of the phenols as well as their oxidation potentials. The biological consequences of such variations are difficult to predict: a variety of studies are in progress and results will be reported separately.

Melatonin, a metabolite of serotonin, is produced *in vivo* by *N*-acetylation and *O*-methylation. One route for the metabolism of melatonin, a compound which itself has a range of biological activities (13), is initiated by hydroxylation at the 6-position (14). The fluorinated analogs of melatonin, **2a** and **2b**, were prepared by acetyla-

Table I

Physical and Analytical Data

Compound	M.p. °C	Formula	Analyses, % Calcd. C	H	N	Found C	H	N
1a·Creatinine Sulfate Monohydrate	205-208	$C_{14}H_{22}FN_5O_7S$	39.71	5.24	16.54	39.99	5.24	16.84
1b·Creatinine Sulfate Monohydrate	216-221	$C_{14}H_{21}F_2N_5O_7S$	38.09	4.80	15.87	38.08	4.87	15.61
2a	152-154	$C_{13}H_{15}FN_2O_2$	62.39	6.04	11.19	62.49	6.04	10.95
2b	107-108	$C_{13}H_{14}F_2N_2O_3$	58.20	5.26	10.44	58.48	5.28	10.32
3a	167-175 dec.	$C_{12}H_{13}FN_3O_2$	57.59	5.24	16.79	57.58	5.53	17.00
3b	216-217	$C_{12}H_{12}F_2N_3O_2$	53.73	4.51	15.69	53.53	4.89	16.11
4a	>300	$C_{12}H_{11}FN_2O_2$	61.53	4.73	11.96	61.37	4.82	11.82
4b	212-214	$C_{12}H_{10}F_2N_2O_2$	57.14	4.00	11.11	57.23	3.85	11.08
5a(a)	263-266 dec.	$C_{12}H_{13}FN_2O_3$						
5b	252-257 dec.	$C_{12}H_{12}F_2N_2O_3$	53.33	4.48	10.37	53.05	4.60	10.30
6a·Picrate	195-210	$C_{17}H_{16}FN_5O_8$	46.68	3.69	16.02	46.90	3.84	15.65
6b·Picrate	208-222	$C_{17}H_{15}F_2N_5O_8$	44.84	3.32	15.38	44.94	3.29	15.17
7·Hydrochloride	225-228	$C_7H_8ClF_2NO$	42.98	4.12	7.16	42.79	4.28	7.01

(a) Could not be recrystallized. See Text.

Table II
Ultraviolet Spectral Data (a)

Compound (Solvent) (b)	λ max (nm)	ε
1a(A)	292	5760
1a(B)	315	5040
1b(A)	265 (shoulder)	4620
1b(B)	305	2690

(a) Spectra were recorded on a Cary Model 15 recording spectrophotometer. (b) Solvents: A, 0.05 *M* phosphate buffer, *p*H 6; B, 0.05 *N* sodium hydroxide.

tion of **6a** and **6b**. These analogs are being studied to determine the effect of ring fluorination on the biological activity and stability of melatonin.

EXPERIMENTAL

Microanalyses and mass spectra were provided by the Microanalytical Services and Instrumentation Section of this Laboratory, under the direction of Dr. David F. Johnson. Homogeneities of all compounds were checked by mass spectrometry and tlc. Physical and analytical data are given in Table I. Nmr spectra were recorded on a Varian A60 spectrometer with trifluoroacetic acid as solvent and tetramethylsilane as an internal reference.

2,3-Dioxopiperidine-3-(3-fluoro-4-methoxy)phenylhydrazone (**3a**).

The procedure is patterned after that described by Abramovitch, *et al.*, for the synthesis of 6-methoxytryptamine (5). A solution of 3.21 g. (0.017 mole) of 3-fluoro-4-methoxyaniline hydrochloride (6) in 26 ml. of water and 5.3 ml. of concentrated hydrochloric acid was cooled to 0° and diazotized with 1.75 g. of sodium nitrite in 5 ml. of water. Excess nitrite was decomposed with urea, the solution was neutralized at 0° with 10% aqueous sodium carbonate, and was filtered into a cold solution of 2-oxopiperidine-3-carboxylic acid; the latter acid was prepared from 3.3 g. (0.019 mole) of the ethyl ester by overnight saponification at room temperature with 40 ml. of 0.75 *N* sodium hydroxide. The reaction mixture was brought to *p*H 3.5 with acetic acid and was stirred at 4° for 15 hours. Filtration gave 4.15 g. (97%) of **3a**. This was recrystallized from 3% methanol/ethyl acetate.

7-Fluoro-6-methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline (**4a**).

Phenylhydrazone **3a**, 3.83 g. (0.015 mole), was cyclized by heating its solution in formic acid (20 ml.) on a steam bath for 30 minutes. Dilution with water, filtration, and recrystallization of the solid from acetic acid afforded 1.32 g. of fluorocarboline **4a** (38%). The nmr spectrum showed two aromatic protons at 7.25 ppm (d), $J(meta)_{HF}$ = 8 Hz, and at 7.19 ppm (d), $J(ortho)_{HF}$ = 11 Hz.

6-Fluoro-5-methoxytryptamine-2-carboxylic Acid (**5a**).

A solution of 1.32 g. of **4a** (5.6 mmoles) in 12 ml. of 4.2 *N* potassium hydroxide and 18 ml. of ethanol was heated at reflux for 15 hours. The ethanol was removed by evaporation, water was added, and the solution was acidified with acetic acid. The solution was chilled in ice and the precipitated amino acid (**5a**) was collected by filtration to give 1.28 g. (91%). Attempts to recrystallize this material were thwarted by its tendency to recyclize when its solutions were heated.

6-Fluoro-5-methoxytryptamine (**6a**).

A suspension of 1.50 g. (6 mmoles) of **5a** in 25 ml. of 3 *N* hydrochloric acid was heated at reflux overnight. The cooled solution was made basic with 30% sodium hydroxide and was extracted with ethyl acetate until the aqueous phase was free of product, as determined by tlc. The ethyl acetate extract was dried (sodium sulfate) and solvent was evaporated to give 850 mg. (69%) of **6a**. The picrate was recrystallized from ethanol.

6-Fluoroserotonin (**1a**).

A solution of 318 mg. (1.52 mmoles) of **6a** in 5 ml. of methylene chloride was cooled to dry ice temperature, and 0.2 ml. of boron tribromide was added with rapid stirring and protection from moisture. A brown solid quickly separated; the dry ice bath was removed and the reaction flask was allowed to warm to room temperature. Water (20 ml.) was added and stirring was continued for 5 minutes. The methylene chloride layer was removed and the aqueous solution was adjusted to *p*H 6 with 5% sodium hydroxide. The solution was lyophilized and the residue was triturated with hot isopropanol and filtered. The insoluble inorganic salts were washed twice with hot isopropanol, and the combined filtrate and washings were evaporated. The residue was dissolved in 2 ml. of 1/1 acetone-water and this solution added to a hot solution of 194 mg. of creatinine sulfate in 2 ml. of 1/1 acetone-water. An additional 2 ml. of acetone was added and, upon cooling, needles separated. Filtration gave 317 mg. (50%) of **1a**•creatinine sulfate monohydrate, recrystallized from acetone/water.

3,5-Difluoro-4-methoxyaniline (**7**) (11).

To a solution of 19.3 g. (0.15 mole) of 2,6-difluorophenol (15) in 50 ml. of cold acetic acid was added 7.9 ml. of fuming nitric acid. After being stirred for 1 hour, the reaction mixture was diluted with ice water to give 17.5 g. (67%) of 2,6-difluoro-4-nitrophenol, m.p. 100-101.5°, lit. m.p. 105-105.5° (16), purified by sublimation. This product was dissolved in 300 ml. of benzene and a solution of 21.5 g. (0.145 mole) of 3-methyl-1-*p*-tolyltriazene was added, in portions, over 0.5 hour. The reaction mixture was washed with 3 x 50 ml. of 1 *N* hydrochloric acid, 3 x 50 ml. of 10% sodium carbonate, once with 50 ml. of water, and the benzene layer was dried over sodium sulfate. Removal of solvent and sublimation gave 17 g. (90%) of 2,6-difluoro-4-nitroanisole, m.p. 33-35.5°, lit. m.p. 37-38° (11). The product was dissolved in 200 ml. of ethanol and was hydrogenated over platinum. After removal of the catalyst by filtration, addition of 20 ml. of 6 *N* hydrochloric acid effected the precipitation of 14 g. of **7**•hydrochloride. Concentration of the filtrate produced more material and the overall yield was essentially quantitative.

2,3-Dioxopiperidine-3-(3,5-difluoro-4-methoxy)phenylhydrazone (**3b**).

The procedure described for the preparation of **2a** was followed. 3,5-Difluoro-4-methoxyaniline hydrochloride (14 g., 0.07 mole) provided, after recrystallization from methanol/ethyl acetate, 9 g. (47%) of **3b**.

5,7-Difluoro-6-methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline (**4b**).

Cyclization of 9 g. (0.033 mole) of **3b** in 100 ml. of formic acid (as described for **3a**) afforded, after recrystallization from aqueous acetic acid, 6.1 g. (73%) of **4b**. The nmr spectrum displayed one aromatic proton at 7.01 ppm (q), $J(ortho)_{HF}$ = 10 Hz, $J(para)_{HF}$ = 1.8 Hz.

4,6-Difluoro-5-methoxytryptamine-2-carboxylic Acid (**5b**).

A solution of 2.5 g. (0.01 mole) of **4b** in 20 ml. of 4.2 *N* potassium hydroxide and 30 ml. of water was heated at reflux for 20 hours. Isolation of the product (as for **5a**) gave 2.3 g. (80%) of **5b**, recrystallized from ethanol.

4,6-Difluoro-5-methoxytryptamine (**6b**).

Decarboxylation of 1 g. (3.7 mmoles) of **5b** in 20 ml. of refluxing 3 *N* hydrochloric acid for 20 hours gave 800 mg. (96%) of **6b**. The picrate was recrystallized from ethanol.

4,6-Difluoroserotonin (**1b**).

Demethylation of 226 mg. of **6b** (1 mmole), as described for the preparation of **1a**, gave 130 mg. (29%) of **1b**·creatinine sulfate monohydrate, recrystallized twice from water.

6-Fluoromelatonin (**7a**).

To a solution of 700 mg. (3.38 mmoles) of **6a** in 10 ml. of ethyl acetate was added 0.35 ml. of acetic anhydride. The mixture was stirred at room temperature for 1 hour, 25 ml. of ethyl acetate were added, the solution was washed 3 times with 10% sodium bicarbonate, once with water, and was dried over sodium sulfate. Removal of solvent and recrystallization from aqueous ethanol gave 604 mg. (72%) of **7a**.

4,6-Difluoromelatonin (**7b**).

By the same procedure, 300 mg. (1.32 mmoles) of **6b** was acetylated to give **7b** in 49% yield, recrystallized from benzene.

p*K* Determinations.

Ionization of the phenolic groups of **1a** or **1b** results in a decrease in intensity and a bathochromic shift in the ultraviolet spectrum (Table II). Aliquots (300 μl) of a 1.4×10^{-3} *M* solution of **1a** or **1b** were diluted to 3.00 ml. with 0.05 *M* phosphate buffer and the degree of ionization determined by measuring the absorption at that wavelength corresponding to the largest difference in optical density between phenol and phenolate anion (315 nm for **1a** and 305 nm for **1b**). Determinations were made for at least 3 *p*H values with agreement to at least $\pm$ 0.05 *p*H units.

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