PREPARATION OF RADIOLABELED PREGNENOLONE ANALOGS. 21-FLUORO-PREGNENOLONE-21-^{18}F, 21-FLUOROPREGNENOLONE-3-ACETATE-21-^{18}F, 21-FLUOROPREGNENOLONE-7-^{3}H, AND 21-FLUOROPREGNENOLONE-3-ACETATE-7-^{3}H.

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SUMMARY

21-Fluoropregnenolone-3-acetate-21-^{18}F (4b) and 21-fluoro-pregnenolone-21-^{18}F (8) were synthesized by nucleophilic substitution of the iodo group of the corresponding 21-iodo analogs by F-fluoride in an acetonitrile solution of 18-Crown-6. 21-Fluoropregnenolone-3-acetate-7-^{3}H (10b) and 21-fluoropregnenolone-7-^{3}H (9b) were synthesized by reacting 21-ethoxalyl pregnenolone-7-^{3}H (6b) with perchloryl fluoride.

Key Words: 21-Fluoropregnenolone-21-^{18}F, 21-Fluoropregnenolone-3--acetate-21-^{18}F, 21-Fluoropregnenolone-7-^{3}H, 21-Fluoro-pregnenolone-3-acetate-7-^{3}H, 18-Crown-6, and Fluorine-18

INTRODUCTION

The iodinated cholesterol derivative, 6β-methyl-^{131}I-iodo-19--norcholesterol, is currently the agent of choice for detection of various adrenal dysfunctions.[1-3] However, the disadvantages associated with this radiopharmaceutical are the long post-injection latent periods necessary before imaging (~3 days) resulting in high doses of radiation to the adrenals (24.5 rads/mCi), liver (2.4 rads/mCi), ovaries (8.0 rads/mCi), testes (2.3 rads/mCi), and total body (1.2 rads/mCi).[4]

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It is known that pregnenolone is a precursor in the biosynthesis of cortisol and corticosterone as well as androgens in the adrenal glands, and its metabolism involves the 17β-acetyl substituent[5,6]. Therefore, selective accumulation of pregnenolone in the adrenals might be anticipated. In addition it has been demonstrated that maximum uptake of ^{14}C-labeled pregnenolone occurs five minutes post-injection, and activity decreases rapidly thereafter.[7] To the best of our knowledge fluorinated steroids have not previously been studied as adrenal imaging agents. The radiolabeled fluorinated analogs 4b and 8 of pregnenolone might reasonably be expected to display similar uptake and distribution properties. Moreover the incorporation of ^{18}F could lead to significant advantages. For example, its short half-life should lead to minimal radiation exposures to patients; and being a positron emitter it should be amenable to positron emission tomography for detection. Thus the synthesis of the ^{18}F-labeled steroids 4b and 8 was undertaken. To determine the magnitude of metabolism of the ^{18}F-labeled steroids 4b and 8, the tritiated fluorosteroids 9b and 10b were synthesized and used in dual isotope animal distribution studies. With tritium labeled at carbon-7 and fluorine-18 labeled at carbon-21, the ratios of the % dose/g values of $^{18}F/^{3}H$ can be used to monitor adrenal metabolism as well as other organ and tissue metabolism.

Although many fluorinated steroids have been reported in the literature, the synthesis of fluorine-18 labeled steroids is complicated by the lack of suitable fluorinating agents incorporating ^{18}F. We chose to approach the problem by using the readily available form of fluorine-18, aqueous fluoride ion for the nucleophilic substitution of a suitable leaving group. Thus ^{18}F-labeled steroids 4b and 8 were synthesized from the

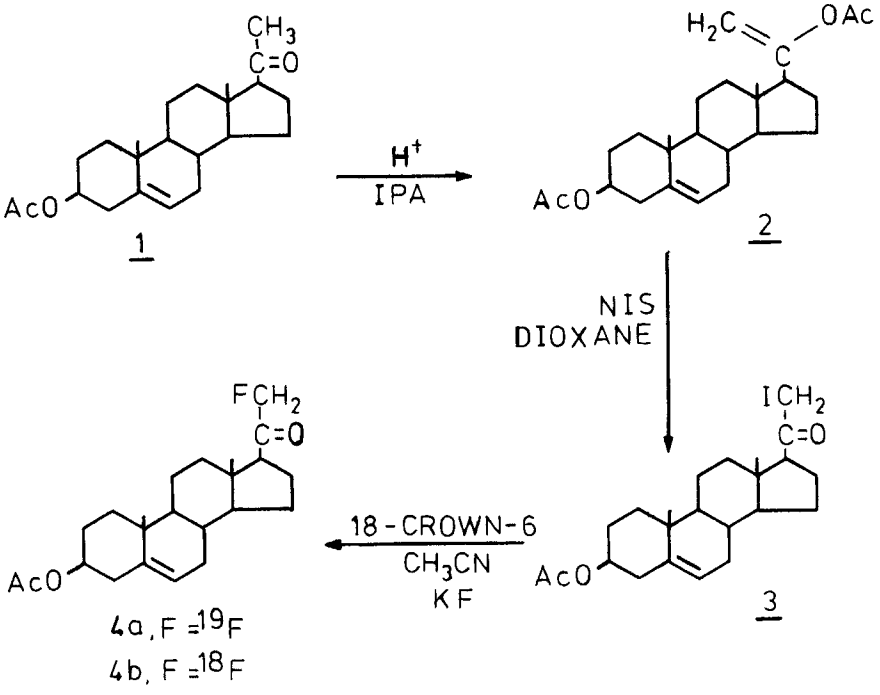
corresponding steroid in which iodine had been incorporated as a leaving group.

RESULTS AND DISCUSSION

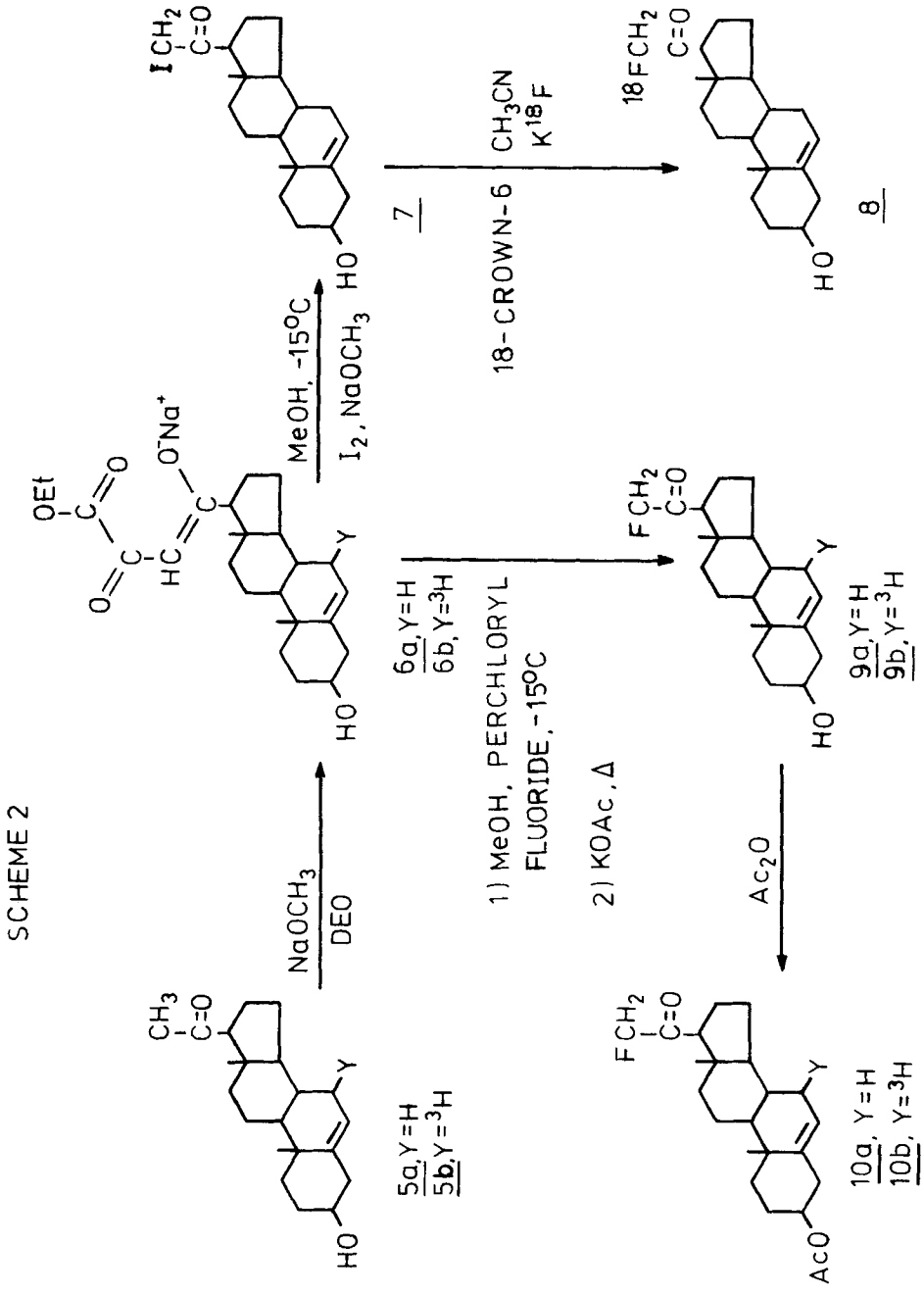
The iodinated steroid 21-iodopregnenolone-3-acetate (3) was synthesized from 5,20(21)-pregnadiene-3β,20-diol acetate (2) in a dioxane solution of N-iodosuccinimide[8]; and 2 from pregnenolone-3--acetate (1) in isopropenyl acetate with a catalytic amount of sulfuric acid[9] (Scheme 1). While 3 served as a precursor for the preparation of 4a and 4b, it proved impossible to use it as a precursor for the preparation of 8 since all attempts to remove the 3-acetate group also removed the 21-iodo group. Thus 21-iodo-pregnenolone (7) was synthesized by an alternate procedure from 21-ethoxalyl-pregnenolone (6a) using a methanolic solution of iodine and sodium methoxide, and 6a was synthesized from pregneno-lone (5a) using ethyl oxalate and sodium methoxide in benzene[10] (Scheme 2).

The nucleophilic substitution of a fluorine for an iodine made use of an acetonitrile solution of 18-Crown-6 (1.4.7.10.13,16-hexaoxacyclooctadecane), 21-iodopregnenolone-3--acetate (3), and excess potassium fluoride which was refluxed to give a 50% yield of 4a. This method was adapted for the synthesis of the ^{18}F-labeled steroids 4b and 8, but using molar amounts of potassium fluoride twice that of precursor steroid. Thus 21-fluoro-5-pregnen3β-ol-acetate-21-^{18}F (4b) was prepared with a radiochemical yield of 33.7% and a radiochemical purity of 95%, and 21-fluoro-5-pregnen-3β-ol-21-^{18}F (8) was prepared with a radiochemical yield of 20% and a radiochemical purity of 97%.

SCHEME 1



The tritium labeled fluoropregnenolones 9b and 10b were synthesized by fluorinating 21-ethoxalylpregnenolone-7-^{3}H (6b) with perchloryl fluoride in methanol and treating with potassium acetate.[11] The crude 21-fluoropregnenolone-7-^{3}H was acetylated with acetic anhydride to produce 21-fluoropregnenolone-3-acetate-7-^{3}H (10b) which was purified by thin layer chromatography (Scheme 2). Purified 21-fluoropregnenolone-7-^{3}H (9b) was obtained by alkaline hydrolysis of 10b followed by preparative TLC. The radiochemical yield of 9b was 0.44 mCi (82%, 199 mCi/mmol.).


SCHEME 2
NaOCH3
DEO
5a, Y=H
5b, Y=3H
MeOH, -15°C
I2, NaOCH3
7
18-CROWN-6
CH3CN
K18F
8
1) MeOH, PERCHLORYL FLUORIDE, -15°C
2) KOAc, Δ
6a, Y=H
6b, Y=3H
9a, Y=H
9b, Y=3H
Ac2O
10a, Y=H
10b, Y=3H

EXPERIMENTAL

Solvents and various reagents were commercially available from common sources and were used without further purification. The fluorine-18 was produced in a water target by $^{16}O(^{3}He,p)^{18}F$ reaction at Sloan Kettering Memorial Cancer Institute, New York City. The fluorine-18 activity was measured with a Capintec CRC-2N dose calibrator.

21-Fluoro-5-pregnen-3β-ol-20-one acetate (4a). A solution of 18-Crown-6 (0.30 g, 1.14 mmol) and potassium fluoride (0.15 g, 1.56 mmol) in 60 ml acetonitrile was refluxed for 30 min. Steroid 3 (0.50 g, 1.04 mmol) was then added with 20 ml acetonitrile. The resulting solution was refluxed for 1.5 h and allowed to cool. To the reaction solution was added 150 ml ethyl ether, and the resulting solution was washed twice with 75 ml portions of water. The ether layer was dried over magnesium sulfate and evaporated to dryness. The product was crystallized from isopropanol-hexane (1:4 v/v) yielding 0.2 g (51.5%) of 4a as a white solid: mp 157-157.5°C (Lit.[12] mp 155-156°C); ir (KBr) 1726 cm^{-1} (3 O-C=O and 20 C-O); pmr ($CDCl_3$), δ, 0.67 (s, 3, 18-CH_3), 1.01 (s, 3, 19-CH_3), 2.01 (s, 3, 3 O-C=O), 2.31 (bd, 2, J=7.6 Hz, 4-CH_2-), 2.73 (dt, 1, J=2.9, 9.0 Hz, 17-CH-), 4.70 (q, 1, J=16.1, 48 Hz, 21-CH-), 4.76 (q, 1, J=16.1, 48 Hz, 21-CH-), 4.53 (bsg, 1, 3-CH-), 5.34 (bd, 1, -CH=).

21-Fluoro-5-pregnen-3β-ol Acetate-21-^{18}F (4b). Aqueous ^{18}F (22.7 mCi/8 ml, 1.05 x 10^{-11} g/mCi) was added to potassium fluoride (6 mg, 0.10 mmol) and evaporated to dryness. Acetonitrile (4ml) and 18-Crown-6 (0.027 g, 0.10 mmol) were added, and the

resulting mixture was refluxed for 15 min. The 21-iodopregnenolone-3-acetate (3) (0.025 g, 0.05 mmol) and one ml acetonitrile was added, and the resulting solution was refluxed for 70 min., and then cooled. Ether (40 ml) was added, and the resulting solution was washed twice with 20 ml portions of water. The ether layer was evaporated under reduced pressure. The resulting 4b was dissolved in 20 ml ethanol, the radiochemical yield after correcting for 3 hours of radioactive decay was 2.4 mCi (33.7%, 47.7 mCi/mmol calculated from the amount of fluoride ion which reacted). A thin layer chromatogram of the ethanolic solution of 4b was developed in dichloroethane-ethyl acetate (19:1 v/v) as the solvent system. The activity of 4b at R_f 0.40 indicated a radiochemical purity of 95%.

21-Fluoro-5-pregnen-3β-ol-20-one (9a). Steroid 6a (2.96 g, 6.3 mmol) was added to 76 ml absolute methanol, and the reaction mixture was cooled to -20°C in a DRY ICE-methanol bath. Without further addition of DRY ICE to the methanol bath, perchloryl fluoride was bubbled slowly into the reaction suspension for 15 min. The reaction solution was allowed to stir for an additional 20 min. The reaction solution was evaporated to 50 ml by heating. Potassium acetate (3 g, 20.6 mmol) was added to the reaction solution, and the mixture was refluxed for 1 hr. Ether (150 ml) was added to the reaction mixture which was then washed three times with 150 ml portions of water. The ether phase was dried over sodium sulfate and evaporated to dryness. The product was crystallized from acetone, yielding 1.05 g (50%) of 9a as a white solid: mp 180-181°C (Lit.[11,12] mp 184-185°C and 178.5-179.5°C, respectively); pmr ($CDCl_3$), δ, 0.67 (s, 3, 18-CH_3), 1.02 (s, 3, 19-CH_3), 2.25 (bd, 2, J=7.5 Hz, 4-CH_2-), 2.70 (dt, 1, J=2.9, 9.0

Hz, 17-CH-), 3.5 (bsg, 1, 3-CH-), 4.70 (q, 1, J=16.1, 48 Hz, 21-CH-), 4.76 (q, 1, J=16.1, 48 Hz, 21-CH-), 5.32 (bd, 1, 6-CH=).

21-Fluoro-5-pregnen-3β-ol-21-^{18}F (8). Steroid 8 was synthesized using the same procedure used for 4b. Potassium fluoride (4.8 mg, 0.083 mmol), 18-Crown-6 (21.8 mg, 0.083 mmol), 21-iodopregnenolone (7) (18.3 mg, 0.041 mmol), 4 ml acetonitrile, and 15.6 mCi aqueous ^{18}F were reacted to give 8 with a radiochemical yield of 1.03 mCi (20%, 24.9 mCi/mmol calculated from the amount of fluoride ion which reacted). Thin layer chromatography using a solvent system of chloroform-methanol 37:4 v/v) indicated a 97% radiochemical purity of 8, R_f 0.58.

Sodium 21-ethaoxalyl-5-pregnen-3β-ol-7-^{3}H (6b). A solution of pregnenolone-7-^{3}H (5b) (5.66 mCi, 17.2 Ci/mmol) was evaporated to dryness with a stream of nitrogen, carrier pregnenolone (5a) (9 mg, 0.028 mmol), fresh sodium methoxide (5mg, 0.09 mmol), benzene (0.1 ml) dried over sodium wire, and diethyl oxalate (0.04 ml, 0.29 mmol) were added rapidly in that order. The reaction mixture was heated for 20 sec at 70°C and then stirred with a magnetic stirrer for 15 min. The reaction mixture containing 6b was washed with three 0.5 ml portions of benzene, and the product was collected by suction filtration, yielding 21.3 mg of crude 6b as a yellowish solid.

21-Fluoro-5-pregnen-3β-ol-20-one-7-^{3}H (9b,). To the crude 6b (21.3 mg) was added 0.4 ml absolute methanol. The reaction suspension was cooled in a DRY ICE-methanol bath to -15°C. Without further addition of DRY ICE to the methanol bath,

perchloryl fluoride was bubbled slowly into the suspension for 13 min; and the reaction solution was allowed to stir for an additional 20 min. Potassium acetate (10 mg, 0.10 mmol) was added, and the reaction mixture was refluxed for 1 h during which time two 0.3 ml portions of methanol were added to prevent dryness. After 1 h the reaction mixture was allowed to evaporate to dryness leaving a yellow paste. The yellow paste was extracted with three 0.5 ml portions of chloroform. The chloroform solution was evaporated to dryness, and quantitation revealed 4.4 mCi (78%) of product. Steroid 9b was purified by acetylation (See below) followed by deacetylation. To 10b (0.540 mCi) was added one ml of methanol and sodium methoxide (5 mg, 0.13 mmol). This solution was stirred for 20 h at room temperature. The reaction solution and unlabeled 21-fluoropregnenolone (9a) were spotted on a thin layer chromatogram and developed in dichloroethane-ethyl acetate (19:1 v/v). The R_f value for 9b was 0.07-0.12. The silica gel was extracted with methanol three times to give a radiochemical yield of 0.444 mCi (82.2%, 199 mCi/mmol calculated from the specific activity of 5b and the amount of carrier added). The radiochemical purity was greater than 98%.

21-Fluoro-5-pregnen-3ß-ol-20-one acetate-7-^{3}H (10b). Steroid 9b (4.4 mCi) was added to 0.1 ml carbon tetrachloride and 0.5 ml acetic anhydride. After 1 h of heating at 105°C, the reaction solution was evaporated to dryness with a stream of dry nitrogen. The product was purified by thin layer chromatography using dichloroethane-ethyl acetate (19:1 v/v) as the solvent system. The R_f range of 10b was 0.33-0.41 as determined from a separate spotting of 4a. Extraction of 10b from the silica gel with methanol three times gave a radiochemical yield of 1.06 mCi (24%,

199 mCi/mmol calculated from the specific activity of 5b and the amount of carrier added) with a radiochemical purity of greater than 98%.

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