TUMOR LOCALIZING AGENTS V.[1] RADIOIODINATED PREGNANES

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Previous studies with progesterone-4-^{14}C suggested that a radioiodinated analog might be useful for photoscanning the adrenal gland and associated tumors. 21-Iodoprogesterone was prepared by conversion of deoxycorticosterone to its mesylate and subsequent displacement with iodide. Depending on the conditions for mesylation, 21-chloroprogesterone could also be isolated. 21-Iodopregnenolone acetate was obtained in a similar manner from the 21-bromo analog which in turn was prepared in good yield from the enol acetate of pregnenolone acetate. The radioiodinated steroids were prepared by isotope exchange with sodium iodide-125 in refluxing acetone. Tissue distribution studies showed that the radioactivity concentrated mainly in the thyroid gland presumably as a result of rapid *in vivo* deiodination.

For the past several years an effort has been made in our laboratory to develop a radiopharmaceutical suitable for photoscanning the adrenal gland and associated tumors. As in the case of radioiodine and thyroid disease, such an agent would not only be of considerable diagnostic value but also have potential therapeutic applications.

A previous publication[2] in this series indicated that various radioiodinated analogs of 1-(*o*-chlorophenyl)-1-

(p-chlorophenyl)-2,2-dichloroethane (o,p'-DDD) behaved similar to the parent compound and concentrated in the adrenal cortex more than any other tissue shortly after administration. One of these agents is currently undergoing preclinical study for use as an adrenal photoscanning agent.

In an effort to find other agents that exhibit a predilection for the adrenal gland, our attention turned to studies dealing with radiolabeled steroids. At about this time, a publication by Bengtsson and coworkers[3] appeared dealing with the distribution of progesterone-4-^{14}C in male fetus. Whole body autoradiography revealed that the concentration of radioactivity in the adrenal cortex far exceeded the uptake by other fetal tissues. Similarly, Laumas and Farooq[4] studied tritiated progesterone in ovariectomized, estrogen-primed female rats and observed higher concentrations of radioactivity in the adrenals, brain, and pituitary than in uterus, vagina, blood or muscle.

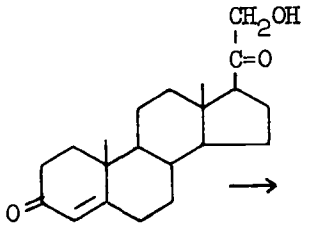
On the basis of this information, the synthesis and tissue distribution analysis of a progesterone analog labeled with a γ-emitting nuclide seemed warranted. Radioiodine-125 was selected as the γ-emitter for our initial studies because of its ready availability, low energy (35 kev photons), and suitable half-life (57 days). It was anticipated that this nuclide could be readily incorporated into the progesterone molecule by either direct displacement of other halides or by isotope exchange with the appropriate iodinated analog. Since α-haloketones are known to undergo these reactions readily, the synthesis of 21-halo-progesterones were selected for this study. Moreover, since

pregnenolone is the immediate precursor of progesterone and also of adrenal origin, the synthesis of 21-halogenated and radioiodinated pregnenolones was included for comparative purposes.

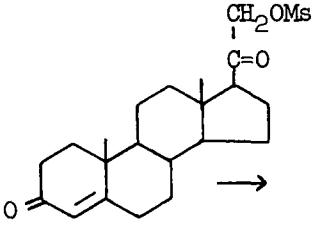
A search of the literature revealed that 21-iodoprogesterone (21-iodopregn-4-ene-3,20-dione, IIIb) had been previously prepared by Jensen[5] and by Takamura and coworkers[6]. Both investigators used the same pathway which involved conversion of deoxycorticosterone (I) to the mesylate (II) followed by displacement with iodide. The melting points reported by each group for these derivatives, however, were at considerable variance from one another. For example, Jensen's mesylate and 21-iodoprogesterone melted at 156-7° and 109-10°, respectively, whereas Takamura et al. reported melting points of 200-1° (dec.) and 125° (dec.) for the same compounds. The experimental in each case differed only in that mesylation in the former was conducted at 0° for 2 hours with the theoretical amount of methanesulfonyl chloride, whereas in the latter instance excess reagent was employed and the reaction was allowed to stand at 0° overnight.

We found that when the mesylation was carried out overnight (Method A) a high melting product (m.p. 201-3 dec.) was isolated but the elemental analysis and n.m.r. clearly established this product as 21-chloroprogesterone (21-chloropregn-4-ene-3,20-dione, (IIIa). Mesylation under Jensen's conditions (Method B), however, gave a product melting very close to that reported (164-165°) and displayed the characteristic protons for the CH_3SO_3 group in the

n.m.r. Displacement of this product with iodide afforded 21-iodoprogesterone (IIIb), melting exactly as reported by Jensen.



I



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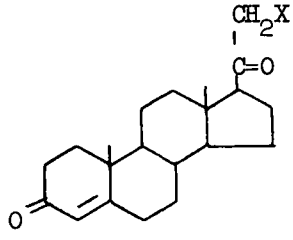
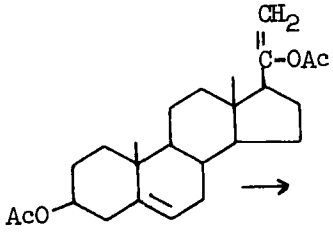
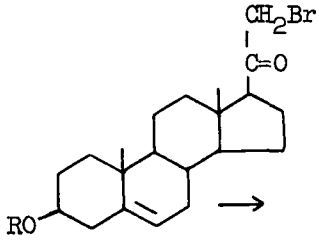


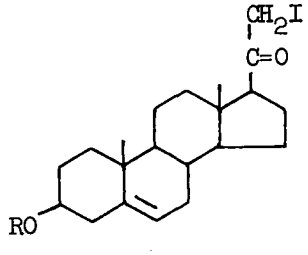
III a, X=Cl
b, X=I



IV



V a, R=Ac
b, R=H



VI a, R=Ac
b, R=H

For the synthesis of 21-iodopregnenolone (21-iodopregn-5-en-3β-ol-20-one, VIb), a method was required which took into account the instability of the iodoketones. It was reasoned, therefore, that the best approach to VIb would be by hydrolysis of the more stable 21-bromopregnenolone acetate (21-bromopregn-5-en-3β-ol-20-one acetate, Va) and subsequent displacement by iodide. A previous synthesis of Va involved bromination of the 20-ketal of pregnenolone acetate with N-bromosuccinimide and subsequent hydrolysis of the ketal[7]. We found, however, that superior yields of Va could be obtained directly from the enol acetate IV by treatment with hypobromous acid according to the method of Magerlein and coworkers[8]. Although the hydrolysis of Va to Vb

was accomplished in low yield with 20% HBr in dioxane, the sparing solubility of the halogenated pregnenolones in solvents suitable for injection prompted the use of the corresponding acetates for the initial tissue distribution studies. 21-Iodopregnenolone acetate (21-iodopregn-5-en-3β-ol-20-one acetate, VIa) was readily prepared from Va by treatment with sodium iodide in acetone. This product agreed with that previously reported by Djerassi et al.[9] which was prepared by reaction of IV with N-iodosuccinimide.

In contrast with the chloro (IIIa) and bromo (Va) derivatives, the n.m.r. spectra for the iodinated steroids IIIb and VIa exhibited a four line AB pattern for the C-21 methylene protons. The increased size of the iodine atom apparently was sufficient to restrict rotation about the 20-21 bond thus rendering the protons nonequivalent[10]. The geminal coupling constant was -10 cps.

The incorporation of radioiodine was readily accomplished by isotope exchange with iodide-125 in acetone. In the case of 21-iodoprogesterone, 92% exchange was effected by heating at the reflux temperature for 4 hours. Chemical and radiochemical purity of the final products were established by melting point and thin layer chromatography (t.l.c.). Contamination of the products with inorganic iodide-125 was readily observed by radioscanning the t.l.c. plates since inorganic iodide fails to move in the solvent systems employed and the radioactivity appears at the origin. All samples were recrystallized until free of contamination with radioiodide.

Preliminary tissue distribution studies with the radioiodinated steroids were carried out by investigators in the Nuclear Medicine

Unit of the University of Michigan[11]. 21-Iodoprogesterone-^{125}I (200 μc) in ethanol was administered I.V. to a 10.4 Kg. dog and the animal sacrificed after 6 hours. Analysis of 18 tissues showed the concentration of radioactivity in c.p.m./mg. to be in the order: thyroid>>stomach>>spleen>intestine>lung>breast>other tissues. The 21-iodopregnenolone acetate-^{125}I in dimethyl sulfoxide precipitated in the blood immediately after I.V. administration to rats. I.M. administration to rats in the same solvent, however, showed no significant tissue localization except in thyroid. The high levels of radioactivity found in the thyroid was interpreted as indicative of considerable in vivo deiodination of these compounds. Studies are now in progress to find positions on the steroid nucleus which are less prone to deiodination.

EXPERIMENTAL[12]

21-Hydroxypregn-4-ene-3,20-dione methanesulfonate (II) Method A.--A mixture of deoxycorticosterone (I, 2.0 g), methanesulfonyl chloride (0.7 ml) and pyridine (9 ml) was kept at 0-5° overnight. The dark reaction mixture was poured into ice water and the precipitate collected by filtration. Decolorization of an ether solution with Norit and removal of the solvent afforded a crude product (650 mg), mp 165-175° (dec). Several recrystallizations from methanol-acetone gave a pure product identified as 21-chloropregn-4-ene-3,20-dione (IIIa), mp 201-3° (dec) and nmr peaks at 0.72 (C-18 methyl), 1.20 (C-19 methyl), 4.1 (C-21 protons), and 5.74 ppm (C-4 proton).

Anal. Calcd for $C_{21}H_{29}ClO_2$: C, 72.30; H, 8.38. Found: C, 72.29; H, 8.26.

Method B.--The same as above except that reaction mixture was poured into ice-water after 2 hours. Recrystallization of the crude product afforded II (1.6 g), mp 160-163°. Recrystallization from acetone-cyclohexane furnished an analytical

sample, mp 164-165° (reported[5] 156-157°) and nmr peaks at 0.73 (C-18 methyl), 1.21 (C-19 methyl), 3.23 (CH_3-SO_3), 4.80 (C-21 protons) and 5.72 ppm (C-4 proton).

Anal. Calcd for $C_{22}H_{32}O_5S$: C, 64.69; H, 7.90. Found: C, 64.56; H, 7.82.

21-Iodopregn-4-ene-3,20-dione (IIIb).--A solution of II (525 mg) and sodium iodide (612 mg) in acetone (20 ml) was refluxed for 30 minutes on the steam bath. A few drops of 10% sodium thiosulfate solution was added and the reaction mixture poured slowly into ice water. A quantitative yield of IIIb, mp 107.5-110° (dec) was isolated. Recrystallization from methylene dichloride-hexane gave an analytical sample, mp 109-110° (dec) (reported[5] 109-110°) and nmr peaks at 0.71 (C-18 methyl), 1.20 (C-19 methyl), 3.77 and 3.90 (C-21 protons, Jgem = 11 cps), and 5.77 ppm (C-4 proton).

Anal. Calcd for $C_{21}H_{29}IO_2$: C, 57.28; H, 6.63. Found: C, 57.24; H, 6.50.

21-Bromopregn-5-en-3β-ol-20-one acetate (Va).--To a solution of 3β,20-diacetoxypregna-5,20-diene (IV) (1 g, 2.5 mM) in t-butylalcohol (40 ml) was added a solution of N-bromosuccinimide (0.45 g, 2.5 mM) in t-butylalcohol (40 ml). To this solution was added dropwise with stirring 1.083 N-sulfuric acid (1.2 ml). After 1 hour, a few drops of 10% sodium bisulfite solution was added and the stirring continued for an additional hour. The solution was concentrated by distillation under reduced pressure and the residue crystallized from methanol and water. The crystals were collected by filtration and air-dried to give a quantitative yield of crude product, mp 106-112.5°. Several recrystallizations from methanol afforded 0.45 g (yield, 50%); mp 145-147° (reported[7] 143-4.5°) and nmr peaks at 66 (C-18 methyl), 1.02 (C-19 methyl), 2.01 (CH_3CO_2-), 3.91 (C-21 protons), 4.33-4.92 (C-3 proton, multiplet), and 5.37 ppm (C-5 proton).

Anal. Calcd for $C_{23}H_{33}BrO_3$: C, 63.14; H, 7.60. Found: C, 63.14; H, 7.64.

Hydrolysis of Va was effected by warming 1.6 g in dioxane (27 ml) and 20% HBr (3 ml) at 50° for 8 hours and allowing to stand overnight at room temperature. The solution was poured into ice water and the mixture extracted with ether. The ether solution was warmed with water, dried, and the solvent removed. Recrystallization of the crude product from acetone-heptane furnished 21-bromopregn-5-en-3β-ol-20-one (Vb, 0.4 g), mp 148-149.5°. Chromatography of the residue using silica gel and elution with benzene containing increasing concentrations of ethyl acetate gave starting material (270 mg) and an equal quantity of hydrolyzed product. Recrystallization of the latter

from acetone-heptane and then from aqueous methanol gave an analytical sample, mp 147-151.5° and nmr peaks at 0.67 (C-18 methyl), 1.02 (C-19 methyl), 3.90 (C-21 protons), and 5.34 ppm (C-5 proton).

Anal. Calcd for $C_{21}H_{31}BrO_2$: C, 63.76; H, 7.90. Found: C, 63.74; H, 7.95.

21-Iodopregn-5-en-3β-ol-20-one acetate (VIa).--A solution of Va (0.5 g) in acetone (13 ml) was refluxed with sodium iodide (0.6 g) for 5 hours. A slight iodine color was noted, which was discharged completely by the addition of 10% sodium thiosulfate solution. Water was added and the precipitate collected by filtration and air-dried to give crude VIa (0.41 g, 74.5%), mp 129-132° (dec). Recrystallization from methanol gave 0.25 g, mp 132-134° (dec) (reported[9] mp 140-141°) and nmr peaks at 0.62 (C-18 methyl), 1.02 (C-19 methyl), 1.95 (CH_3CO_2), 2.93 (C-17 proton, triplet, J = 9 cps), 3.66 and 3.77 (C-21 protons, Jgem = 10 cps), 4.27-4.72 (C-3 proton, multiplet), and 5.37 ppm (C-5 proton).

Anal. Calcd for $C_{23}H_{33}O_3I$: C, 57.03; H, 6.87. Found: C, 56.92; H, 6.75.

Radioiodinated Steroids - Isotope Exchange:

A. A solution of IIIb (100 mg) and $Na^{125}I$ (2 mc) in acetone (4 ml) was stirred and heated at gentle reflux for 4 hours under an atmosphere of nitrogen. The condensor was removed and the solution concentrated to 1/4 its original volume. The solution was poured into cold water (25 ml) and the precipitate collected by filtration. The product was washed well with water and recrystallized from aqueous acetone. Two more recrystallizations afforded 20 mg, mp 110-112° (dec), specific activity 18.4 μc/mg (92% exchange). T.l.c., using ethyl acetate:benzene (9:1), showed a single spot coincident with the radioactive area on scanning (R_f 0.58).

B. A solution of VIa (100 mg) in acetone (3 ml) was added to the residue obtained by evaporating a solution containing 117.5 μc of $Na^{125}I$ under a stream of nitrogen. The mixture was stirred at 50° for 1 hour and the solvent evaporated under nitrogen. The residue was vigorously stirred with water (4 ml) and the precipitate collected by filtration. This washing was repeated twice and the product recrystallized from methanol. This gave the radioiodinated product as crystals (87.4 mg), mp 129-130° with a specific activity of 0.89 μc/mg (76% exchange).

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11. The authors are grateful to Drs. W. H. Beierwaltes, V. Varma and H. Cohn at the University of Michigan Nuclear Medicine Unit for providing us with this information.

12. Melting points were taken on a Fisher-Johns melting point apparatus and are corrected. Elemental analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich. The nmr spectra were obtained with a Varian A-60 spectrometer in $CDCl_3$ at a concentration of 10%, with tetramethylsilane as internal reference. Thin layer chromatograms (t.l.c.) were run with 1-in. wide Eastman Chromagrams, Type K301R, with fluorescence indicator, developed with benzene and spots detected with uv light and iodine vapor. Chromagrams of radioiodinated compounds were scanned with an Atomic Associates RCS-363 radiochromatogram scanner. The specific activities were determined with an Atomic Associates well scintillation counter Model 810c, scintillation spectrometer Model 530 and Beckman liquid scintillation system Teletype Model 33.