Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXI, No. 10

SYNTHESIS OF 17α-[^{125}I]IODOETHYNYL-4,6-ANDROSTADIEN-17β-OL-3-ONE, AN ACTIVE-SITE-DIRECTED PHOTOAFFINITY RADIOLABEL FOR ANDROGEN-BINDING PROTEINS*

N. Scott Mason and Howard E. Smith**,
Department of Chemistry and
Center for Reproductive Biology Research,
Vanderbilt University,
Nashville, Tennessee 37235, U.S.A.,

Benjamin J. Danzo,
Departments of Obstetrics & Gynecology and Biochemistry and
Center for Reproductive Biology Research,
Vanderbilt University School of Medicine,
Nashville, Tennessee 37232, U.S.A.

and

Jeffrey A. Clanton,
Department of Radiology and Radiological Sciences,
Vanderbilt University School of Medicine,
Nashville,Tennessee 37232, U.S.A.

SUMMARY

17α-[^{125}I]Iodoethynyl-4,6-androstadien-17β-ol-3-one with a specific radioactivity of 24 Ci/mmol was prepared as an active-site-directed photoaffinity radiolabel for androgen-binding proteins. The iodinated steroid was formed by the silver nitrate-catalyzed reaction of *N*-[^{125}I]iodosuccinimide and 17α-ethynyl-4,6-androstadien-17β-ol-3-one in acetone. *N*-[^{125}I]Iodosuccinimide was prepared by reaction of silver succinimide with iodine-125 in dioxane, the latter being formed by oxidation of sodium iodide-125 with sodium nitrite-nitric acid in water-hexane.

Key Words: Androgen-Binding Proteins,17α-[^{125}I]Iodoethynyl-4,6-androstadien-17β-ol-3-one, Iodine-125, *N*-[^{125}I]Iodosuccinimide, Photoaffinity Radiolabeling

---

*Taken from the Ph.D. Dissertation of N.S.M., Vanderbilt University, May 1991.

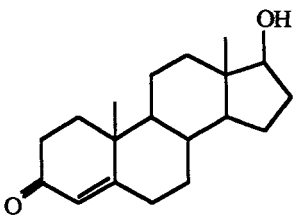
**To whom communications concerning this paper should be sent.

0362–4803/92/100729–10$10.00
© 1992 by John Wiley & Sons, Ltd.

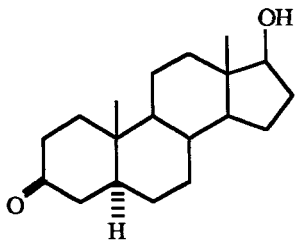
Received 17 January, 1992
Revised 22 June, 1992

INTRODUCTION

Androgen-binding protein (ABP) (1-4) and testosterone-binding globulin (TeBG) (5,6), the latter also referred to as sex hormone binding globulin (SHBG), are proteins present in several species, including man (7-10), and are responsible for the extracellular transport of the androgens, testosterone (**1**, 4-androsten-17β-ol-3-one) and 5α-dihydrotestosterone (**2**, 5α-androstan-17β-ol-

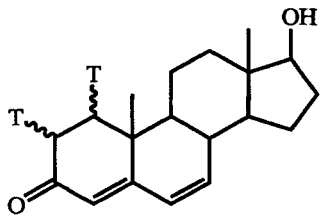


1

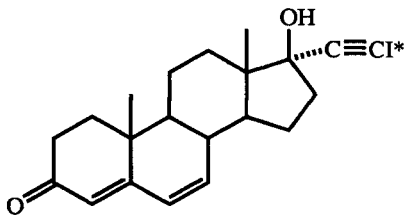


2

3-one, 5α-DHT). ABP is produced by the Sertoli cells of the testis (1,4) while TeBG is produced by the liver (11). Both proteins are considered to be involved in the regulation of male reproductive function, and we have previously reported the synthesis of [^{3}H]Δ^6-testosterone ([^{3}H]**3**,



[^{3}H]**3**



[^{125}I]**4**

[1ξ,2ξ-$^3H_2$]4,6-androstadien-17β-ol-3-one) as an active-site-directed photoaffinity radiolabel for study of the physical and biological properties of these androgen-binding proteins (12). Subsequently, we reported the use of [^{3}H]**3** in elucidating the physiochemical properties of ABP (13-15) and TeBG (16), and we have recently utilized [^{3}H]**3** as a probe for determining the amino acid sequence of the steroid binding domain of ABP (17).

Although testosterone (**1**) and 5α-DHT (**2**) are the endogenous ligands for androgen-binding proteins, these androgens can not be used as photoaffinity reagents for these proteins in cytosol. The latter has an intense absorption band centered at 270 nm with a long tail extending beyond 300

nm, thus, photoexcitation of 5α-dihydrotestosterone and testosterone, with their n $\rightarrow \pi^*$ carbonyl group transitions centered at 280 (ε_{max} 25) and 305 nm (ε_{max} 100), respectively, can not occur. In addition, a Pyrex filter with a cut-off at about 300 nm is necessary to protect the proteins from photodegradation during irradiation for photoaffinity labeling.

The extended conjugation of Δ^6-testosterone (3) which has a binding affinity for ABP approximately one-half that of testosterone (18), results in a carbonyl absorption band centered at 345 nm (ε_{max} 300). This absorption is sufficiently beyond the absorption band of cytosol and the cut-off of the Pyrex filter to permit photoexcitation of the unsaturated carbonyl group which results in covalent bond formation between [^{3}H]3 and ABP or TeBG (13,16). The covalently-bonded, radiolabeled steroid-protein complexes are then able to remain intact during electrophoresis under denaturing conditions and other manipulations.

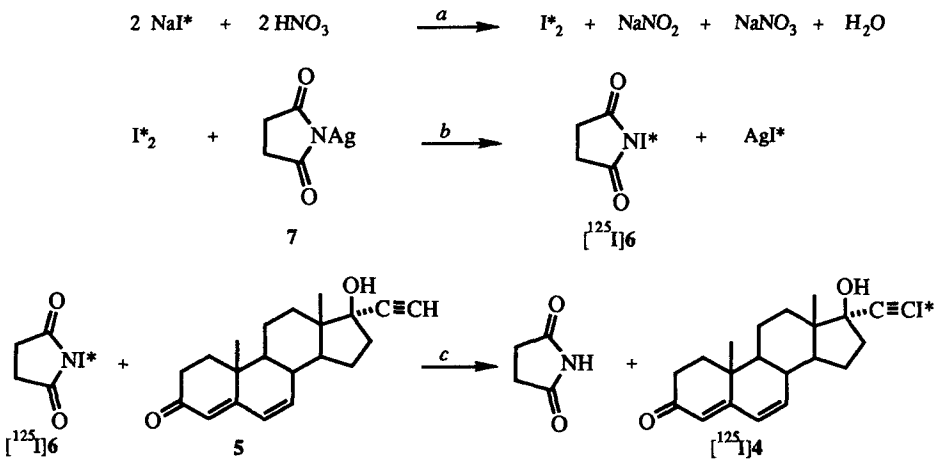
Although [^{3}H]Δ^6-testosterone-labeled androgen-binding proteins have had substantial use for the study of the physical properties and physiological role of androgen-binding proteins, an important extension of these studies would be the incorporation of iodine-125 into a Δ^6-testosterone or a Δ^6-testosterone analogue. Androgen-binding proteins covalently radiolabeled in this manner would have much greater specific activity than can be obtained with tritium-labeled compounds. Such a probe would be of great utility in studies geared to the examination of the *in vivo* tissue uptake of androgen-binding proteins and to the determination of the presence of tissue receptors.

An extensive study of the structural and configurational requirements for high binding affinity to androgen-binding proteins (19) revealed that substitution at the 17α position has little effect on the relative binding affinity of otherwise structurally and configurationally similar androgens. We have therefore prepared and studied the binding activity of a number of androgens, each with an extended unsaturated carbonyl group and with an iodine-containing 17α substituent (20), as potential active-site-directed photoaffinity labels for androgen-binding proteins. The work suggested the use of 17α-[^{125}I]iodoethynyl-4,6-androstadien-17β-ol-3-one ([^{125}I]**4**) as an iodine-125-labeled, active-site-directed photoaffinity ligand for ABP and other androgen-binding proteins. Compound **4** has a binding affinity for ABP about twice that of 5α-DHT (20), and [^{125}I]**4** can be prepared from its desiodo analogue, 17α-ethynyl-4,6-androstadien-17β-ol-3-one (**5**, Scheme I), in one synthetic step using *N*-[^{125}I]iodosuccinimide ([^{125}I]**6**) in the presence of a catalytic amount of silver nitrate (21). An outline of this synthesis is given in Scheme I. The photoaffinity labeling of ABP with [^{125}I]**4** will be reported elsewhere.

RESULTS AND DISCUSSION

As shown in Scheme I, treatment of 17α-ethynyl-4,6-androstadien-17β-ol-3-one (**5**) with *N*-iodosuccinimide (**6**) in acetone in the presence of a catalytic amount of silver nitrate gave 17α-iodoethynyl-4,6-androstadien-17β-ol-3-one (**4**). The same reaction using **5** and [^{125}I]**6** was used for the formation of [^{125}I]**4**.

Scheme I



a; Sodium nitrite-nitric acid in hexane-water. *b*; Dioxane as solvent. *c*; Silver nitrate in acetone.

The formation of *N*-[^{125}I]iodosuccinimide ([^{125}I]**6**) is also shown in Scheme I and began with commercially available, carrier free sodium iodide-125 (2200 Ci/mmol) which was reduced to a specific activity of 24 Ci/mmol or less by the addition of sodium iodide. Oxidation of sodium iodide-125 with sodium nitrite-nitric acid in water-hexane gave iodine-125 which was removed from the reaction as a violet solution in hexane. The radiochemical yield of the oxidation reaction and hexane removal process was usually greater than 80%. The hexane solution of iodine-125 was carefully dried and added to finely crushed silver succinimide (**7**). Since iodine does not react with silver succinimide in hexane, the hexane was replaced with dioxane. After reaction, the dioxane solution of [^{125}I]**6** was separated from the solid residue of silver iodide-125, and the dioxane solution of *N*-[^{125}I]iodosuccinimide ([^{125}I]**6**) was added to a conical reaction flask (Reacti-Vial, Pierce Chemical Co.) coated on the inside with a film of silver nitrate. The dioxane was removed by evaporation with a stream of nitrogen, and an acetone solution of 17α-ethynyl-4,6-androstadien-17b-ol-3-one (**5**) was added. The reaction was allowed to proceed for several hours, and [^{125}I]**4**

was purified by thin layer chromatography on silica gel (Figure 1) and removed from the silica gel by extraction with absolute ethanol. The radiochemical yield using 4.78 mCi of sodium iodide-125 (24 Ci/mmol) was 31%. Authentic **4** was added to an aliquot of the ethanol solution, and the mixture subjected again to TLC using hexane-ethyl acetate (1:1) as eluant (Figure 2). The location of the radioactive spot which accounted for 98% of the radioactivity on the plate coincided with that occupied by the authentic sample of **4** as visualized by ultraviolet light.

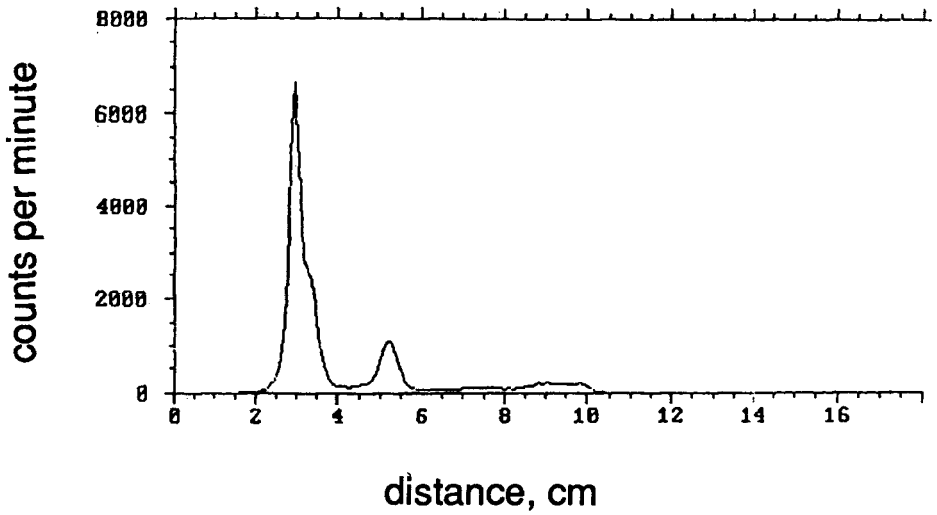


Figure 1. Thin layer chromatograph of the crude product from the silver-nitrate reaction of 17α-ethynyl-4,6-androstadien-17β-ol-3-one (**5**) and *N*-[^{125}I]iodosuccinimide ([^{125}I]**6**). The radiochromatogram was developed four times with hexane-ethyl acetate (7:3), and the peak centered at 5.19 cm (R_f = 0.29) represents 17α-[^{125}I]iodoethynyl-4,6-androstadien-17β-ol-3-one ([^{125}I]**4**.

In an experiment to demonstrate the chemical identity of *N*-[^{125}I]iodosuccinimide ([^{125}I]**6**) and 17α-[^{125}I]iodoethynyl-4,6-androstadien-17β-ol-3-one ([^{125}I]**4**), a dioxane solution of [^{125}I]**6** (20 μmol, 24 Ci/mmol) was used. The dioxane was evaporated, and a large excess of *N*-iodosuccinimide (**6**) (0.22 mmol) was added. Recrystallization of the mixture from dioxane-carbon tetrachloride gave pure [^{125}I]**6** with a specific activity of 3.4 mCi/mmol. This sample of [^{125}I]**6** was used to form 17α-[^{125}I]iodoethynyl-4,6-androstadien-17β-ol-3-one ([^{125}I]**4**), which, after recrystallization from acetone-hexane, was obtained in 35% yield with a specific activity of 3.4 mCi/mmol.

For a number of experiments with specific activity of sodium iodide-125 of 24 to 5.5 Ci/mmol, the radiochemical yield of 17α-[^{125}I]iodoethynyl-4,6-androstadien-17β-ol-3-one ([^{125}I]**4**)

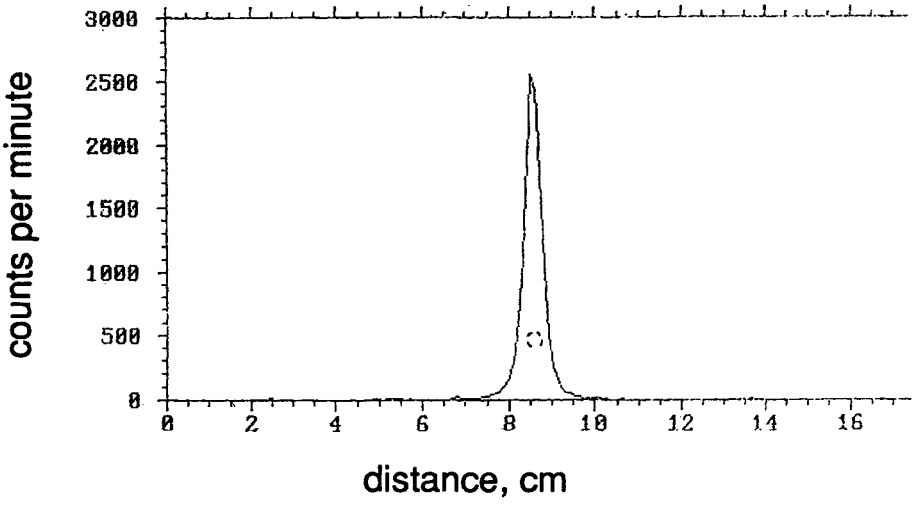


Figure 2. Thin layer chromatograph of purified 17α-[^{125}I]iodoethynyl-4,6-androstadien-17β-ol-3-one ([^{125}I]**4**). The radiochromatogram was developed one time using hexane-ethyl acetate (1:1). The broken circle represents an authentic sample of **4** which was codeveloped on the plate.

ranged from 5 to 31% with a radiochemical yield usually of 15%. For maximum radiochemical yield, it is very important that the solvents used in the reaction sequence in Scheme I be freshly purified as described below, no more than eight hours before use, and stored in dark bottles over 4-Å molecular sieves. It is also important that the silver succinimide used be free of gray or dark crystals. If not, it must be recrystallized before use.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are corrected. Optical rotatory powers were obtained with a Rudolph Autopol III automatic polarimeter and a 1-dm sample tube. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained in chloroform-*d* using a JEOL FX 90Q spectrometer operating at 90 MHz with tetramethylsilane as the internal standard.

For the radiosynthetic work described below, hexane and dioxane were freshly distilled from sodium and sodium benzophenone ketyl, respectively, under an atmosphere of nitrogen and stored over 4-Å molecular sieves. Acetone was also freshly distilled and stored over 4-Å molecular sieves. Thin layer chromatography for purifications and purity determinations was done with silica gel on glass plates (5 x 10 cm, silica gel 60-F254 250 μm thick, E. M. Science) using reagent grade

solvents as eluants. The radiochromatographs were analyzed with either a Nucleus, Inc. Quantum 8 multichannel pulse height analyzer or a Bioscan System 200 Imaging Scanner equipped with a Bioscan Autochanger 1000. All reactions and evaporations of solvent were done in conical Reacti-Vials (Pierce Chemical Co.) of an appropriate size.

17α-Iodoethynyl-4,6-androstadien-17β-ol-3-one (4) was prepared as reported elsewhere (20) by the silver-catalyzed reaction of 17α-ethynyl-4,6-androstadien-17β-ol-3-one (**5**) with *N*-iodosuccinimide (**6**) and had mp 184-186 °C, $[\alpha]^{25}_{D}$ -6° (*c* 0.500, $CHCl_3$), and a ^1H NMR spectrum identical with that reported (20).

17α-[^{125}I]Iodoethynyl-4,6-androstadien-17β-ol-3-one ([^{125}I]4), Carrier-added 91%. Sodium iodide-125 (4.78 mCi, 2200 Ci/mmol, 2.2 pmol, New England Nuclear, high concentration in 0.1 M NaOH), hexane (100 μL), aqueous sodium nitrate (100 μL, 0.20 M, 20 μmol), and nitric acid (100 μL, 1.2 M, 0.12 mmol) were added in sequence to aqueous sodium iodide (20 μL, 0.010 M, 0.20 μmol) in a 500-μL Reacti-Vial, and the layers were mixed. The violet hexane solution of iodine-125 was separated, and the aqueous mixture was extracted with hexane (2 x 100 μL). The combined hexane solutions (4.54 mCi, radiochemical yield 95%, specific activity 47 Ci/mmol, 0.097 μmol) were dried (Na_2SO_4) for ten min and was then added to a 500-μL Reacti-Vial containing dioxane (100 μL) and with finely crushed silver succinimide (**7**; 10.0 mg, 0.049 mmol) coating the inside of the flask. The film was prepared by introduction of an acetone solution of **7** into the flask and then evaporation of the acetone with a stream of nitrogen. To remove hexane, the reaction mixture was reduced in volume to approximately 100 μL by mild heating under a slow stream of nitrogen, keeping the refluxing solvent ring below the lip of the flask during the evaporation. Hexane was assumed to be sufficiently removed when the volume of solvent matched that of the initial volume of dioxane. The reaction flask was then sealed, and the reaction was allowed to proceed in the dark at room temperature for two hours with gentle agitation every 15 to 20 min. The dioxane solution of *N*-[^{125}I]iodosuccinimide ([^{125}I]**6**) was removed from the solid silver iodide-125 and excess silver succinimide and was transferred to a 500-μL Reacti-Vial with a film of silver nitrate (2.0 mg, 0.012 mmol) coating the inside of the flask. This film was also prepared by the introduction of an acetone solution of the silver nitrate into the flask, and then evaporation of the acetone with a stream of nitrogen. After removal of the dioxane solution of [^{125}I]**6**, the solid residue of silver iodide-125 and silver succinimide was washed with an additional portion of dioxane (100 μL), and the latter was also transferred to the flask containing the film of silver nitrate. The

dioxane was removed from the flask by evaporation using a stream of nitrogen, and an acetone solution of 17α-ethynyl-4,6-androstadien-17β-ol-3-one (**5**; 500 μL, 0.322 mM, 0.16 μmol) was added. The reaction mixture was concentrated to an approximate volume of 100 μL without heat under a stream of nitrogen. The mixture was allowed to stand at room temperature for four hours and then transferred to a thin layer chromatographic plate for purification. The TLC plate was developed (3 x) with hexane-ethyl acetate (7:3). The area on the plate corresponding to that occupied by an authentic sample of **4** was removed, and extraction of the silica gel with absolute ethanol (5 x 200 μL) gave a solution of [^{125}I]**4** (741 μCi, radiochemical yield 31%, specific radioactivity 24 Ci/mmol, 31 pmol). An aliquot of this solution (2 μL) was added to **4**, and the mixture subjected to TLC using hexane-ethyl acetate (1:1) as eluant. The location of the radioactive spot which accounted for 98% of the radioactivity on the plate coincided with that occupied by the authentic sample of **4** as visualized by ultraviolet light.

17α-([^{125}I]Iodoethynyl)-4,6-androstadien-17β-ol-3-one ([^{125}I]4), Carrier-added 6.5 x 10^5:1. *N*-[^{125}I]Iodosuccinimide ([^{125}I]**6**, 42.3 mg, 0.188 mmol, 0.640 mCi, specific radioactivity 3.40 mCi/mmol) and silver nitrate (2.0 mg, 12 μmol) were added to 17α-ethynyl-4,6-androstadien-17β-ol-3-one (**5**, 49.0 mg, 0.158 mmol) in acetone (2 mL) in a 3-mL Reacti-Vial, and the mixture was stirred for one hour. Thin layer chromatography [eluant: hexane-ethyl acetate (7:3); plate developed four times] of an aliquot (4 μL) showed no spot corresponding to that of **5** but a spot which comigrated with an authentic sample of **4**. The reaction mixture was transferred to a separatory funnel, aided by washing with ethyl acetate (3 x 1.5 mL). The combined organic solutions were washed with water (2 x 10 mL), and the aqueous washes were extracted with ethyl acetate (1 x 20 mL). The combined ethyl acetate solutions were dried (Na_2SO_4), and the solvent was evaporated without heating under a stream of nitrogen. Recrystallization of the residue from acetone-hexane gave [^{125}I]**4** (0.187 mCi, 24 mg, 29%, specific activity 3.40 mCi/mmol).

17α-Ethynyl-4,6-androstadien-17β-ol-3-one (5) was prepared as reported earlier (20) and had mp 260-262 °C, $[\alpha]^{24}_D$ -84° (*c* 1.00, $CHCl_3$) [lit. (22) 262-265 °C; $[\alpha]_D$ -85° ($CHCl_3$)], and a ^{1}H NMR spectrum identical with that reported earlier (20).

***N*-Iodosuccinimide (6)** was purchased from Aldrich Chemical Company, Inc. and was purified by dissolving it in a minimum volume of hot dioxane and then precipitation with carbon tetrachloride: mp 198-200 °C [lit. (23,24) mp 200-201 °C].

***N*-[^{125}I]Iodosuccinimide ([^{125}I]6), Carrier-added 6.5 x 10^5:1.** Sodium iodide-125 (4.85 mCi, 2200 Ci/mmol, 2.2 pmol, New England Nuclear, high concentration in 0.1 M NaOH) was placed in a 500-μL Reacti-Vial. Aqueous sodium iodide (20 μL, 0.010 M, 0.20 μmol) and hexane (50 μL) and then sequentially aqueous sodium nitrite (50 μL, 0.020 M, 1.0 μmol) and nitric acid (50 μL, 1.2 M, 60 μmol) were added, and the layers were mixed. The violet hexane layer was removed, and the aqueous layer was extracted with hexane (50 μL). The combined, hexane solutions of iodine-125 were dried (Na_2SO_4) for ten min and then assayed for radioactivity (3.82 mCi, radiochemical yield 79%, specific activity 49 Ci/mmol, 0.078 μmol). The hexane solution was added to dioxane (100 μL), and the hexane was removed by careful evaporation of the solution to 100 μL without heating under a stream of nitrogen. Finely crushed silver succinimide (**7**; 1.0 mg, 4.9 μmol) was added, and the reaction was allowed to proceed in the dark for two hours. The dioxane solution was removed from the solid, transferred to to a 5-mL Reacti-Vial, and evaporated to dryness at room temperature under a stream of nitrogen. *N*-Iodosuccinimide (**6**; 50 mg, 0.22 mmol) was added to the residue, and recrystallization to a specific radioactivity from dioxane-carbon tetrachloride (1:1) gave pure [^{125}I]**6** (0.640 mCi, radiochemical yield 26%, 42.3 mg, specific activity 3.40 mCi/mmol).

Silver succinimide (7) was formed as outlined earlier (23,24) by reaction of silver oxide with succinimide. It was recrystallized from water and dried at 105-110 °C (1 mmHg).

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (NIH) Training Grant HD-07043 in Reproductive Biology, Biochemistry, and Endocrinology to N.S.M. and by NIH grant HD-13813 to B.J.D. Assistance was provided by the Organic Chemistry Core Laboratory of the Center for Reproductive Biology Research. The Center is supported by the NIH grant HD-05797.

REFERENCES

1. Steinberger, A., Heindel, J.J., Lindsey, J.N., Elkington, J.S.H., Sanborn, B.M. and Steinberger, E. - Endocrine Res. Commun. *2* : 261 (1975)
2. Sanborn, B.M., Elkington, J.S.H., Tcholakian, R.K. and Steinberger, E. - Mol. Cell. Endocrinol. *3* : 129 (1975)
3. Tindall, D.J., Cunningham, G.R. and Means, A.R. - J. Biol. Chem. *253* : 166 (1978).

4. Schmidt, W.N., Taylor, Jr., C.A. and Danzo, B.J. - Endocrinology *108* : 786 (1981).
5. Corvol, P. and Bardin, C.W. - Biol. Reprod. *8* : 277 (1973)
6. Danzo, B.J. and Eller, B.C. - Mol. Cell. Endocrinol. *2* : 351 (1975)
7. Danzo, B.J., Orgebin-Crist, M.-C. and Toft, D.O. - Endocrinology *92* : 310 (1973)
8. Carreau, S., Drosdowsky, M.A. and Courot, M. - Intl. J. Androl. *2* : 49 (1979)
9. Danzo, B.J., Dunn, J.C. and Davis, J. - Mol. Cell. Endocrinol. *28* : 513 (1982)
10. Hsu, A.-F. and Troen, P. - J. Clin. Invest. *61* : 1611 (1978)
11. Mercier-Bodard, C., Radanyi, C., Roux, C., Groyer, M.T., Robel, P., Dadoune, J.P., Petra, P.H., Jolly, D.J. and Baulieu, E.E. - J. Steroid Biochem. *27* : 297 (1987)
12. Taylor, Jr., C.A., Danzo, B.J. and Smith, H.E. - J. Label. Compounds and Radiopharm. *17* : 627 (1980)
13. Taylor, Jr., C.A., Smith, H.E. and Danzo, B.J. - Proc. Natl. Acad. Sci. U.S.A. *77* : 234 (1980)
14. Taylor, Jr., C.A., Smith, H.E. and Danzo, B.J. - J. Biol. Chem. *255* : 7769 (1980)
15. Danzo, B.J., Taylor, Jr., C.A. and Eller, B.C. - Endocrinology *111* : 1270 (1982)
16. Danzo, B.J., Taylor, Jr., C.A. and Eller, B.C. - Endocrinology *111* : 1278 (1982)
17. Danzo, B.J., Parrott, J.A. and Skinner, M.K. - Endocrinology *129* : 690 (1991)
18. Danzo, B.J., Taylor, Jr., C.A. and Schmidt, W.N. - Endocrinology *107* : 1169 (1980)
19. Cunningham, G.R., Tindall, D.J., Lobl, T.J., Campbell, J.A. and Means, A.R. - Steroids *38* : 243 (1981)
20. Diaz Cruz, P.J., Mason, N.S., Danzo, B.J. and Smith, H.E. - Steroids in press
21. Hofmeister, H., Annen, K., Laurent, H. and Wiechert, R. - Angew. Chem. Int. Ed. Engl. *23* : 727 (1984).
22. Knox, L.H., Zderic, J.A., Ruelas, J.P., Djerassi, C. and Ringold, H.J. - J. Am. Chem. Soc. *82* : 1230 (1960)
23. Djerassi, C. and Lenk, C.T. - J. Am. Chem. Soc. *75* : 3493 (1953)
24. Benson, W.R., McBee, E.T. and Rand, L. - Org. Syn. *Coll Vol. 5* : 663 (1973)