SYNTHESIS OF 21-DIAZOPROGESTERONE-6,7-$^{3}H_2$

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SUMMARY

The preparation of 21-diazoprogesterone-6,7-$^{3}H_2$ is described. Progesterone was dehydrogenated with chloranil to give Δ^{6}-dehydroprogesterone. Degradation of the pregnane side-chain with sodium hypobromite gave the corresponding carboxylic acid. Catalytic reduction with carrier-free tritium followed by treatment with oxalyl chloride and then diazomethane afforded 21-diazoprogesterone-6,7-$^{3}H_2$ with a specific activity of 42 Ci/mmol.

Key Words: Catalytic Tritiation, 21-Diazoprogesterone.

INTRODUCTION

In a previous study (1), 21-diazo derivatives of corticosteroids exhibited reasonable affinities for intracellular receptors and functional activity in a toad bladder assay. In addition, covalent photoaffinity labeling of plasma proteins was obtained with 9α-bromo-21-diazo-21-deoxycorticosterone-1,2-$^{3}H_2$ (25 mCi/mmol) (1) and 21-diazo-21-deoxycorticosterone-6,7-$^{3}H_2$ (7 Ci/mmol) (2). The utility of the 9α-bromo analog was limited, however, by its low specific activity. This report describes the procedure used to synthesize high-specific-activity 21-diazoprogesterone-6,7-$^{3}H_2$.

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RESULTS AND DISCUSSION

The reaction sequence used to introduce tritium into 21-diazoprogesterone is shown in Figure 1. Progesterone (1) was treated with chloranil to give Δ^6-dehydroprogesterone (2). Degradation of the pregnane side-chain with sodium hypobromite in t-butyl alcohol gave 3-oxoandrosta-4,6-diene-17β-carboxylic acid (3). Tritium was introduced by catalytic tritiation in benzene-tetrahydrofuran solvent in the presence of 5% Pd-$BaSO_4$ using carrier-free tritium gas and vacuum line techniques in a microhydrogenation apparatus. Slight over-reduction of the starting material (3) is desirable since it is easier to separate the Δ^4-3-ketone from the saturated 3-ketone than from the $\Delta^{4,6}$-3-keto starting material. Analysis of the crude preparation by UV spectroscopy indicated the absence of the starting material (3) and about 40% yield of product 4. Without purification, tritium-labeled 4 was treated with oxalyl chloride in anhydrous benzene and lyophilized on a vacuum line. The residue (acid chlorides) was taken up in anhydrous benzene and treated with

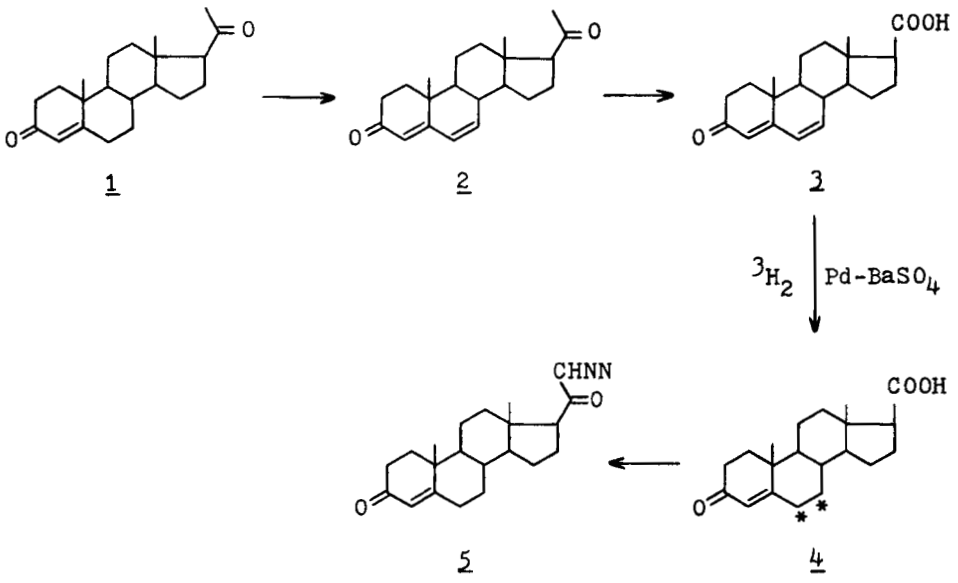

Fig. 1. Pathway in the synthesis of 21-diazoprogesterone-6,7-$^3H_2$

ethereal diazomethane. After purification by thin layer chromatography (TLC), pure 21-diazoprogesterone-6,7-$^3H_2$ (5) was obtained with a specific activity of 42 Ci/mmol. A radio-purity of greater than 96% was indicated by TLC in several solvent systems. Identity was confirmed in several ways. Photolysis at 253.7 nm and 300 nm gave the same results with tritium-labeled 5 as with authentic 5 (Figure 2). Note the rapid (<1 min) change in absorbance with the diazo compound only. Second, the methyl ester 6 (Figure 3) formed on photolysis of the labeled material 5 in methyl alcohol was identical to the compound prepared from authentic 5. Third, when treated with HCl, labeled 5 gave 21-chloroprogesterone (7), with R_f value on TLC identical to that of authentic compound 7 (3). Finally under the same experimental procedure using hydrogen instead of tritium, non-radioactive 21-diazoprogesterone (5) was obtained from compound 3. It was identical to authentic compound 5 prepared by the method previously described (1).

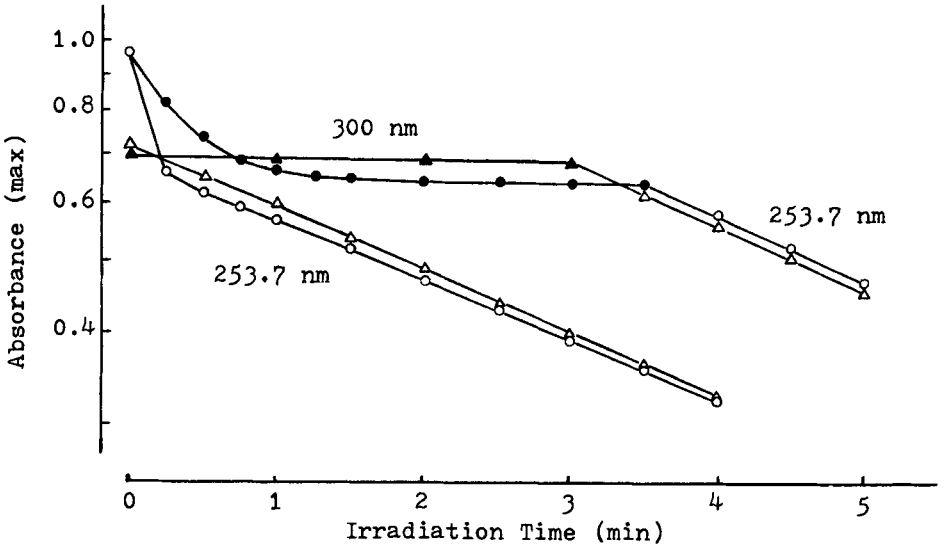

Fig. 2. Effect of UV irradiation on absorbance of progesterone (1) and 21-diazoprogesterone (5). Absorbance (max) of 1 after irradiation at 253.7 nm (Δ) and at 300.0 nm (▲), and of 5 at 253.7 nm (o) and at 300.0 nm (●).

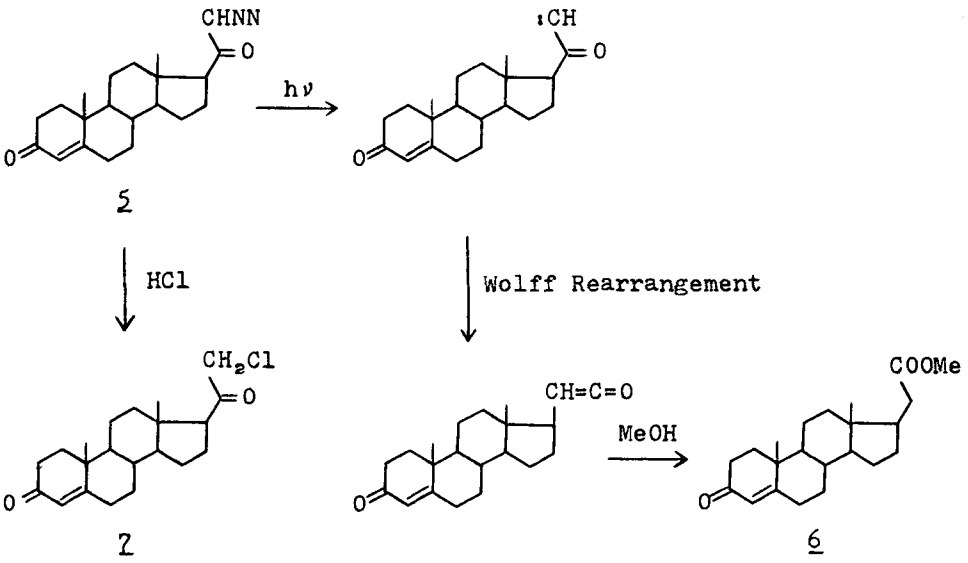

Fig. 3. Photo-chemical reaction of 21-diazoprogesterone.

Pure 21-diazoprogesterone-6,7-$^{3}H_2$ appeared to be quite stable when kept in pure ethyl alcohol at -15° C and protected from light.

Covalent labeling of progesterone-binding globulin with tritium-labeled 21-diazoprogesterone has been achieved and the results will be published shortly. 21-Diazoprogesterone was also examined for binding to the progesterone receptor components of chick oviduct (4). By Scatchard analysis the K_d was found to be $1.384X10^{-8}M$ and therefore the possibility of labeling the progesterone receptor of chick oviduct is available.

EXPERIMENTAL

Precursor steroids were purchased from Searle. The conventional reagents and solvents were of the highest purity available. When necessary, the solvents used in preparations of tritium-labeled steroids were redistilled or purified (5). Tritium was freshly released from uranium tritiide by heating and contained no decay product.

Melting points were determined with a Thomas-Hoover apparatus equipped with a corrected thermometer. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, California. UV spectra were obtained on a Beckman DB-G Grating Spectrophotometer.

Analytical thin layer chromatography (TLC) was carried out with 0.25-mm layers of precoated silica gel on 5X20-cm or 5X10-cm glass plates with or without F-254 (E. Merck). Plates were prewashed by ascending chromatography in methyl alcohol and reactivated just before use by heating in an oven at 105° C for 15 min (5). Plates were developed in the following solvent systems: (A) benzene-ethyl acetate (3:2, v:v); (B) benzene-ethyl alcohol (9:1, v:v); (C) chloroform-ethyl acetate (5:1, v:v); (D) chloroform-ethyl alcohol (25:1, v:v); (E) ether-dichloromethane (5:1, v:v); (F) cyclohexane-ethyl acetate (1:1, v:v). Spots or bands on TLC plates were visualized under 254 nm UV illumination by fluorescence quenching or located by scanning on Varian Radio Scanner LB 2722.

Radioactivity measurements were performed with a Searle Mark III Liquid Scintillation System. Appropriate aliquots of samples were dissolved in 12 ml of scintillation solvent composed of 4 g of PPO and 50 mg of POPOP per 1 l of toluene (New England Nuclear). The counting efficiency (57-60%) for each sample was determined by internal standardization using toluene-^3H (New England Nuclear) as an absolute standard.

Δ^6-Dehydroprogesterone (Pregna-4,6-diene-3,20-dione) (2) -- A mixture of 8 g (25 mmoles) of progesterone (1) and 10 g (40 mmoles) of chloranil in 300 ml of t-butyl alcohol was heated with stirring at reflux temperature for 3 hr. The excess chloranil was filtered off and the filtrate was evaporated under reduced pressure. The oily residue was taken up in 300 ml of ether which was washed three times with 1% sodium hydroxide solution, and several times with water. The solvent was evaporated and the crude residue was

crystallized from acetone-hexane to yield the dienone 2, 3.3 g (42%); mp 137-141° C; λ_{max} 285 nm (lit. mp 139-141° C (6)).

3-Oxoandrosta-4,6-diene-17β-carboxylic acid (3) -- A cold NaOBr solution (from 1.2 g of NaOH in 10 ml of H_2O treated at 0° C with 0.5 ml of Br_2) was added dropwise (35 min) to a stirred solution of 1.0 g (3.2 mmoles) of 2 in 60 ml of t-butyl alcohol and 15 ml of H_2O (cooled in ice-water bath). The reaction mixture was stirred at 3-12° C for 1.5 hr, at 30° C for 2 hr, and at room temperature overnight. After being treated with solid sodium bisulfite (0.1 g), t-butyl alcohol was removed by evaporation under reduced pressure. The aqueous solution was diluted with 100 ml of H_2O, extracted once with 50 ml of ether, and cooled by addition of ice chips. After acidification with HCl, the white precipitate was collected by filtration, washed with H_2O and air dried, to give the carboxylic acid 3, 0.77 g (77%); mp 223° C (dec). An analytical sample was obtained after two crystallizations from acetone, mp 267-270° C (dec). Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34. Found: C, 76.62; H, 8.31.

Photolysis of 21-diazoprogesterone (5) and progesterone (1) -- Compound 5 was prepared by the method previously described (1), mp 175-178° C, λ_{max} 247 nm (ϵ $2.3X10^4$) with a shoulder at 280 nm. Photolysis was performed as follows: A solution of an appropriate amount of steroid in methyl alcohol ($4X10^{-5}$ M) was irradiated in a quartz cell (1 cm light path) on a rotating stage of a Rayonet Photochemical Reactor at room temperature, at 253.7 nm and 300 nm. Absorbance (max) was determined at 242-247 nm with UV spectrophotometer. The results are shown in Figure 2.

21-Diazoprogesterone-6,7-$^3H_2$ (21-Diazopregn-4-ene-3,20-dione-6,7-$^3H_2$) (5) -- A microhydrogenation apparatus (from Lawrence Radiation Laboratory, capacity about 13 ml) equipped with a magnetic stirring bar in a pear shaped flask, a stopcock with extended arm and a device for addition of the catalyst in the

closed apparatus by turning the stopcock 180 degrees during the reaction, was connected to a vacuum line and a tritium generator. Forty milligrams of 5% Pd-$BaSO_4$ was placed on the addition device and a solution of 8.3 mg (0.026 mmole) of 3 in 1.0 ml of anhydrous benzene and 0.4 ml of anhydrous tetrahydrofuran was introduced into the pear shaped flask and frozen in liquid nitrogen. The apparatus was evacuated under high vacuum and carrier-free tritium gas was introduced into the system to give 750 mm Hg pressure. After the system was brought back to room temperature, the catalyst was added to the solution and the reaction mixture was stirred at room temperature for 45 min. The reaction mixture was frozen in liquid nitrogen and the excess tritium was pumped off. The catalyst was removed by centrifugation and the clear solution mixed with ethyl alcohol (0.5 ml) was lyophilized on a vacuum line. UV measurement indicated the absence of the starting material 3 (λ_{max} 285 nm). The yield of the product 4 (λ_{max} 242 nm) was 3.2 mg (38%). Without purification, a portion of tritium-labeled products containing about 1.6 mg ($5X10^{-3}$ mmole) of 4 was suspended in 1 ml of anhydrous benzene and treated with 0.050 ml of freshly distilled oxalyl chloride at 5° C. The reaction mixture was stirred at 19° C for 30 min and lyophilized on a vacuum line. The resulting acid chlorides taken up in 3 ml of anhydrous benzene were added through a filter to ethereal diazomethane (cooled in Dry Ice-acetone) prepared from 235 mg (1.8 mmoles) of N-methyl-N'-nitro-N-nitrosoguanidine in an Aldrich MNNG-Diazomethane Kit (7). The reaction mixture was stirred at -15° C for 30 min, at 20° C for 30 min, and evaporated under reduced pressure. The product 5 was purified on TLC plates in solvent system A. The recovery of pure 5 was 5.52×10^{-4} mmole (11%) and the total radioactivity was 23.9 mCi. The specific activity of 5 was found to be 43 Ci/mmol. At least 99% radiopurity was indicated by radio scanner on TLC plates in solvent systems B, C, D, E, and F. A portion of the purified 5 was

purified again on TLC plates in solvent system B and the specific activity was found to be 42 Ci/mmol. A radio-purity of greater than 96% was obtained by counting 0.25-cm sections of Eastman Chromagram Sheet, 13179 silica gel without fluorescent indicator (No. 6061) which was developed with benzene-ethyl acetate (2:1, v:v). Identity of the purified 5 was confirmed by (i) photolysis at 253.7 nm and 300 nm, which gave the same results with tritium-labeled 5 as with authentic 5 (Figure 2); (ii) the product (methyl ester 6) formed by photochemical Wolff Rearrangement (1) of labeled and unlabeled 5 in methyl alcohol gave identical R_f values on TLC; (iii) when treated with HCl, both yielded 21-chloroprogesterone (7), with R_f values on TLC identical to that of authentic 7 (3); (iv) when hydrogen was used instead of tritium under the same experimental procedure, non-radioactive 5 was obtained from 3. This compound was identical to the authentic 5 prepared by the method previously described (1).

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