[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

THE SYNTHESIS OF 17α-ETHYNYLTESTOSTERONE-20,21-C^{14}

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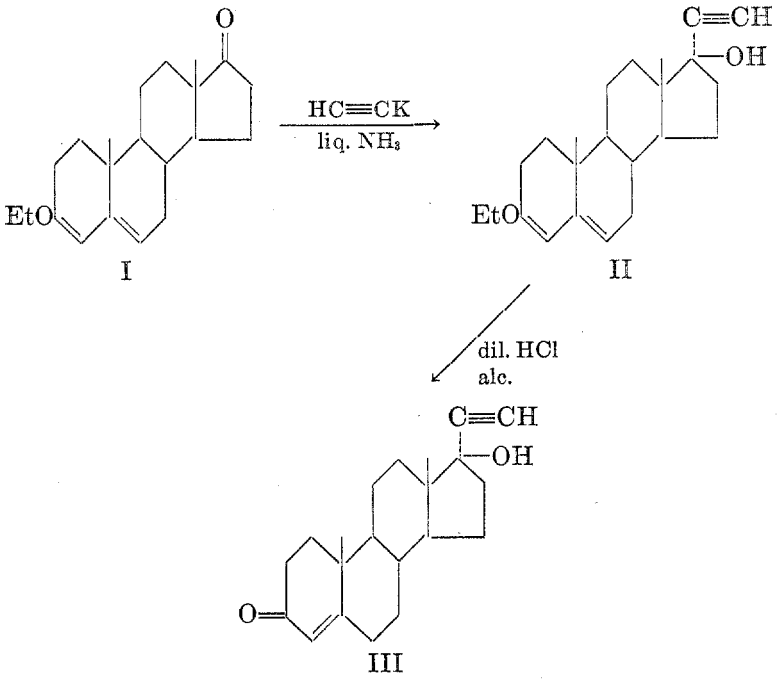
Among the steroid hormones structurally related to progesterone, one of the most interesting substances is 17α-ethynyltestosterone (III). Although this compound has not been isolated from animals, it possesses high progestational activity on oral administration. A study of the metabolism of 17α-ethynyltestosterone is of real importance, particularly in the field of endocrinology, because of its widespread clinical use. The introduction of radiocarbon in the ethynyl group of this synthetic hormone provides a labeled compound for biological investigations such as its possible conversion to progesterone. The synthesis (1) and a metabolic study (2) of radioprogesterone has been previously reported from this laboratory.



I II III

The classical method (3, 4) for the preparation of 17α-ethynyltestosterone involves the condensation of potassium acetylide with 3β-hydroxy-5-androsten-17-one (called dehydroandrosterone by Butenandt) to form 17α-ethynyl-5-androstene-3,17-diol and oxidation of this latter compound by Oppenauer's method. Because of the large excess of acetylene used and the unsatisfactory yield (60%) in the oxidation step, this method is unsuitable for labeling work. In fact, very few reactions among the steroids have been studied where the small

molecules have been conserved and the steroid intermediate used in excess. Many common reactions have to be completely reinvestigated before they are of value for labeling.

The method of Inhoffen (5) was, therefore, investigated as described in the patent literature. This method utilizes 3-ethoxy-3,5-androstadien-17-one (I) as the starting material which is condensed with potassium acetylide in liquid ammonia. Hydrolysis of the 3-enol ether (II) gives 17α-ethynyltestosterone (III). The original method employs a large excess (more than 16-fold) of potassium acetylide. After an extensive investigation of the reaction conditions, 17α-ethynyltestosterone-20,21-C^{14} was synthesized by using two millimoles of potassium radioacetylide in liquid ammonia solution with one-half of a millimole of 3-ethoxy-3,5-androstadien-17-one (I) under a nitrogen atmosphere at Dry Ice temperature and hydrolyzing the resulting enol ether with 0.1% hydrochloric acid in aqueous alcohol.

The isotopically labeled acetylene was generated from barium radiocarbide, measured and transferred by vacuum technique to a solution of potassium in liquid ammonia in order to produce the potassium acetylide. The method gave a 69% yield of 17α-ethynyltestosterone-20,21-C^{14} based on the steroid employed or about 13% based on the radioacetylene. The excess isotopic acetylene was recovered as silver acetylide. The radioethynyltestosterone had m.p. 263.5–265°; the specific rotation +22°, a maximum absorption at 240–242 mμ, and a specific activity of 3.8×10^5 counts/min./mg. or 0.6 microcurie/mg.

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EXPERIMENTAL

Apparatus. A small manifold was connected to a high-vacuum system, a manometer, two inlets for acetylene and nitrogen, and an outlet for attaching the reaction flask. All inlets and outlets were equipped with vacuum stopcocks and standard taper joints. The volume of the manifold was found to be 241 ml. The reaction flask, shown in Figure 1, was made from an 125-ml. Erlenmeyer flask equipped with a standard taper joint for attachment to the manifold, a magnetic stirrer, and an adaptor bearing a dropping-funnel and a stopper. The reaction flask was designed so that it would maintain a high vacuum, could be cooled with a liquid-air bath, and the contents vigorously stirred while evacuated. After this apparatus had been built and used, a somewhat similar design was published by Phibbs and Darwent (6) which permits overhead, variable, magnetic stirring under gas-tight conditions. The magnetic stirrer consisted of a small magnet enclosed in a glass tube sealed horizontally on top of the stirrer. The stirrer was supported by a glass to glass bearing and all was enclosed by a large stopper made from taper joints. The stirrer is operated by a variable speed motor attached to a horse-shoe magnet and mounted above the large stopper.

3-Ethoxy-3,5-androstadien-17-one (I). 4-Androstene-3,17-dione, prepared from 3-hydroxy-5-androsten-17-one by Oppenauer's method (7), was converted to the 3-enol ether with orthoformic ester and hydrochloric acid according to the procedure of Serini and Köster (8). 3-Ethoxy-3,5-androstadien-17-one crystallized from absolute alcohol, containing a small amount of pyridine, in the form of shiny plates, m.p. 149–151.6°.

17α-Ethynyltestosterone-20,21-C^{14} (III). The reaction flask, Figure 1, was connected to the manifold and the whole system was evacuated to 0.75 μ and shut off from the vacuum line. A tube containing 10 ml. of liquid ammonia, dried with potassium, was attached to an inlet and the ammonia was allowed to distill into the reaction flask by cooling it with Dry Ice. The whole system was then filled with dry nitrogen and 78 mg. (2 mmoles) of of potassium was added to the flask by removing the dropping-funnel and quickly replacing it. The ammonia was stirred for one minute to form the deep-blue potassium solution and then frozen with liquid air.

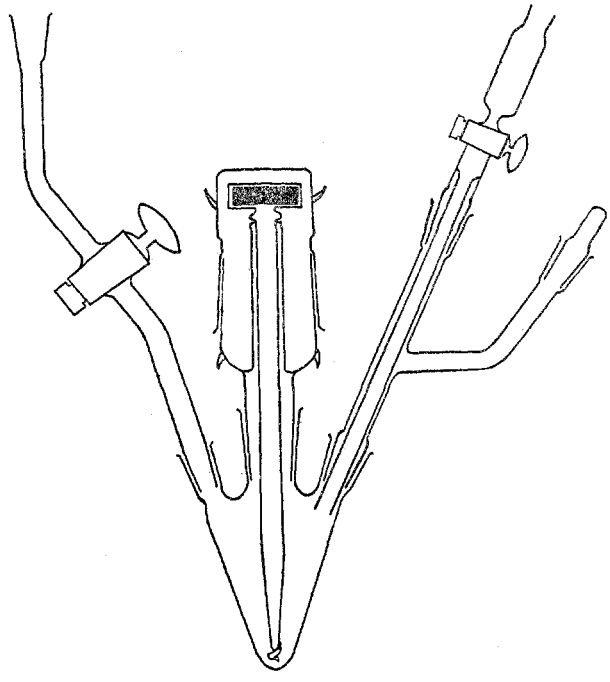

FIGURE 1. REACTION FLASK

The ampoule containing the barium radiocarbide[1] was opened and its contents were transferred to a 500-ml., wide-mouth Erlenmeyer flask which was tightly fitted with a rubber stopper carrying an inlet tube for nitrogen, a dropping-funnel, and a small condenser bearing the exit tube. Cold water (180 ml.) was cautiously added to cover the contents of the flask and the evolved gas was dried by passage through a trap cooled with Dry Ice and a U-tube filled with Dehydrite and then collected in a trap cooled with liquid air. After the evolution of acetylene and hydrogen had subsided, the water solution was heated to boiling for 15 minutes while a slow stream of nitrogen was passed through in order to sweep the acetylene into the collecting trap. The trap was removed from the train and attached to the manifold and the whole system was evacuated to 0.75 μ. The reaction flask was then shut off and the radioacetylene allowed to expand into the manifold

[1] Obtained from Tracerlab, 55 Oliver St., Boston 10, Mass. The sample contained about 1 millicurie of C^{14}; however, according to instructions received with the sample and previous correspondence, only about two thirds of the radiocarbon can be recovered as radioacetylene.

giving a pressure of 17 mm. *versus* 740 mm. barometric pressure at 27°. Since the combined volume of the manifold and the trap had been found to be 352 ml., the amount of radioacetylene was 0.33 mmoles. The stopcock leading to the reaction flask was then opened to let the acetylene condense in the flask and the mixture was allowed to warm up to room temperature while stirring. After the reaction had taken place, the mixture was again frozen with liquid air.

The radioactive acetylene was diluted with ordinary acetylene. Tank acetylene was passed through a train for the purification of acetylene as described by Conn, Kistiakowsky, and Smith (9). The purified acetylene was then passed through a large trap cooled with a Dry Ice-bath, two U-tubes containing anhydrous calcium chloride and Dehydrite respectively, and then collected in a trap cooled with liquid air. After a small amount of acetylene had condensed, the trap was attached to the manifold and the system evacuated to 1 μ. The acetylene was allowed to vaporize into the manifold until a pressure of 150 mm. was reached; the trap was then shut off, the reaction flask opened to the manifold and the mixture warmed up to room temperature and stirred. Since all of the potassium had not been consumed as indicated by the blue color, the reaction mixture was again frozen by the use of liquid air and an additional quantity of acetylene, which gave a pressure of 30 mm. in the manifold, was transferred. On warming up to room temperature and stirring, the solution was completely decolorized. The total amount of ordinary acetylene thus added was 2.38 mmoles.

At this point dry nitrogen was admitted to fill the system; the reaction flask was cooled in a Dry Ice-bath, and a solution of 158 mg. (0.5 mmole) of 3-ethoxy-3,5-androstadien-17-one in 6 ml. of benzene and 6 ml. of dry ether was added dropwise to the liquid ammonia solution of potassium acetylide. The mixture was stirred during the addition of the steroid but an incipient white precipitate formed. The mixture was stirred for an additional three hours. The flask was then transferred to a hood and lifted just above the cooling liquid to allow the ammonia to slowly evaporate. The exit gas was conducted through a mercury trap into a tube containing aqueous silver nitrate solution to recover the unreacted radioacetylene. After standing overnight, the reaction mixture was placed in an ice-bath and stirred for 30 minutes. To the reaction mixture was added 15 ml. of an ice cold, saturated ammonium chloride solution. The mixture was then stirred for 15 minutes while a stream of nitrogen was being passed through to carry the excess acetylene into the absorbing solution. The two layers were separated, the aqueous layer was extracted twice with ether, and the combined ethereal solution washed with water and a saturated sodium chloride solution and dried over sodium sulfate. A crystalline residue was obtained after removing the ether under reduced pressure. The crystalline material was the 3-ethoxy-17α-ethynyl-3,5-androstadien-17-ol (II) which was not characterized but carefully hydrolyzed to the final product.

To establish the best conditions for the hydrolysis of the enol ether, many model experiments were carried out. The tertiary carbinol at C-17 is rather sensitive to acid conditions which catalyze its rearrangement. The residue was dissolved in 6 ml. of absolute alcohol to which was added 2 ml. of 0.12 *N* hydrochloric acid and 1 ml. of water (final concentration of acid was 0.1%) and the resulting solution was allowed to stand overnight at room temperature. Crystals began to deposit after the first hour. The mixture was concentrated to about 5–6 ml. and cooled. The crystalline product was separated, washed with cold dilute alcohol, and dried. The yield was 107 mg. or 68.6% based on the steroid employed. The product melted at 263.5–265°, gave the specific rotation $[\alpha]_D^{25}$ +22.2° (5.3 mg. made up to 1 ml. with dioxane, α_D +0.118°; *l*, 1 dm.), $\lambda_{max.}$ at 240–242 mμ (absolute ethanol), and a specific activity of 3.8 x 10^5 counts/min./mg. or 0.6 microcurie/mg.

In one of the preliminary experiments, the product was crystallized from chloroform and absolute alcohol to give prisms melting at 264–266°, which were dried for 11 hours at 1 μ at 100°.

Anal. Calc'd for $C_{21}H_{28}O_2$: C, 80.77; H, 8.97.
Found: C, 78.63; H, 9.28.

The sample was then sublimed in a high vacuum (0.5 μ) at 190–200° which gave a white powder, m.p. 270–272°, $[\alpha]_D^{26}$ +22.7° (4.0 mg. made up to 1 ml. with dioxane, α_D +0.091°; l, 1 dm.).

Anal. Found: C, 80.44; H, 9.03.

The results indicate that the compound obtained from the hydrolysis of the condensation product was 17α-ethynyltestosterone hemihydrate (Calc'd for $C_{21}H_{28}O_2 \cdot 1/2H_2O$: C, 78.50; H, 9.03). Both melting points have been recorded in the literature (3, 4).

SUMMARY

A method has been developed for the preparation of 17α-ethynyltestosterone-20,21-C^{14} which involves the condensation of 3-ethoxy-3,5-androstadien-17-one with potassium radioacetylide followed by hydrolysis of the 3-enol ether. An easily constructed reaction flask was designed for the preparation.

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