[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

# THE SYNTHESIS OF PROGESTERONE-21-$C^{14}$

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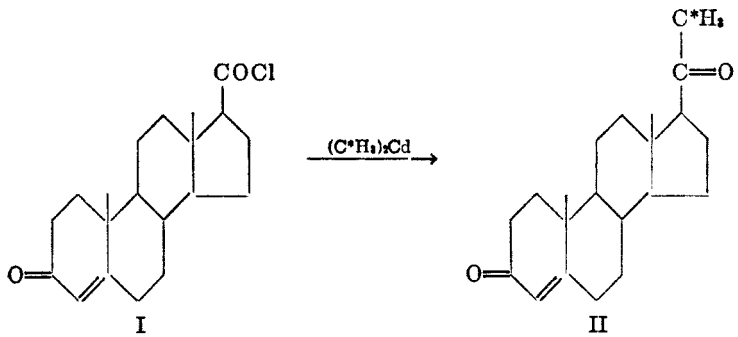
As a part of a program to study the intermediary metabolism of steroids, radioprogesterone (II) has been prepared. This labeled hormone was made by the alkylation of 3-keto-4-etiocholenoyl chloride with radioactive dimethylcadmium.

Several procedures for the labeling of progesterone in the side chain were examined critically. The method of Butenandt and Schmidt-Thomé (1) beginning with the cyanhydrin reaction on β-dehydroandrosterone acetate was investigated extensively. However, we failed to achieve consistently the high yields obtained by these authors. Efforts to prepare 17-chloro(or bromo)-3β-acetoxy-5-androstene by chlorination of 3β-acetoxy-5-androsten-17-ol (2) or degradation of silver 3β-acetoxy-5-etiocholenate with bromine (3) gave rearranged products. Carbonation (4) of these halides would give 3β-acetoxy-5-etiocholenic acid-20-$C^{14}$ which could then be alkylated with dimethylcadmium (5).




I II

Our synthesis utilizes 3-keto-4-etiocholenoyl chloride (I), whose preparation has recently been described by Wilds and Shunk (6). While the alkylation of this 3-keto acid chloride with dimethylcadmium gives somewhat smaller yield than the alkylation of 3β-acetoxy-5-etiocholenoyl chloride (5), avoidance of two steps after introduction of the radiocarbon justifies the one-step procedure.

Isotopic methanol was converted to methyl bromide by an adaptation of Tolbert's procedure (7) for the preparation of methyl iodide. Using a simplified vacuum line, the methanol was quantitatively converted to methyl bromide when allowed to stand with phosphorus tribromide for four hours. The methyl bromide was purified by distilling through a column packed with both acid and alkali absorbents. The first part of the packing was porcelain chips soaked with concentrated sulfuric acid, and the second part was pellets of sodium hydroxide.

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Portions of the methyl bromide were transferred using vacuum technique to a flask, containing the ether and magnesium, cooled with liquid air. After each addition, the flask was shut off from the system and the reaction was allowed to take place at room temperature. The Grignard reagent was prepared in about an 80% yield. The methylmagnesium bromide was converted to dimethylcadmium by the usual procedure of adding anhydrous cadmium chloride. The acid chloride in dry benzene was then added to the ether solution.

The reaction mixture was worked up in the usual way and the product was chromatographed on activated alumina, molecularly distilled, and finally crystallized from acetone-hexane. This gave a 29% yield of progesterone-21-$C^{14}$ based on the radiomethanol employed. The labeled progesterone melted at 122.1–125.6°, gave a specific rotation of +191° and a specific activity of $2.5 \times 10^4$ counts/sec./mg.

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*Note added in proof:* Dr. T. F. Gallagher has informed us that Dr. H. B. MacPhillamy of the Ciba Pharmaceutical Products, Inc. has carried out essentially the same synthesis of radioprogesterone at the Sloan-Kettering Institute for Cancer Research.

## EXPERIMENTAL

*Radiomethyl bromide.* The conversion of methanol to methylmagnesium bromide was accomplished in a small manifold equipped with three outlet tubes fitted with appropriate standard taper joints, a storage bulb, a closed-end manometer of about 100 mm. length and a connection to a high vacuum line. All outlets were equipped with stopcocks. The volumes of the manifold (288 cc.) and storage bulb (217 cc.) were known and utilized to determine the quantities of gas manometrically.

Two portions of methanol, 0.06 cc. of ordinary dry methanol and 0.59 mmol. of radioactive methanol,[2] were attached to the manifold and frozen in liquid air and the system was evacuated to 0.5 $\mu$. The isotopic methanol was transferred at low pressure to the storage bulb and then allowed to evolve into the manifold-storage bulb system to register 18 mm. pressure, equivalent to 0.59 mmol.[3] Similarly, the ordinary methanol was transferred, and when it was allowed to expand into the manifold-storage bulb system, the pressure recorded was 65 mm. or equivalent to 2.1 mmol. of methanol.

After the methanol was returned to the storage bulb, a 45-cc. reaction bulb fitted with a stopcock and joint and charged with 0.23 cc. of phosphorus tribromide was attached to the

[2] The radioactive methanol containing 1 mc. in 0.59 mmol. was obtained from the United States Atomic Energy Commission, Oak Ridge, Tennessee.

[3] In the 505-cc. manifold-storage bulb system at *ca.* 30° the millimoles of gas were calculated as follows: $\frac{303}{273} \times \frac{500}{22.4} \times \frac{18}{760} = 0.59$ mmol.

manifold, cooled in liquid air and evacuated to 3 $\mu$. The methanol was distilled into the reaction bulb and the stopcock was shut. The bulb was removed from the line and was allowed to stand at room temperature (*ca.* 30°) for four hours. This bulb was then attached to the manifold with a drying tube, packed so that the evolving methyl bromide passed in order through porcelain chips saturated with concentrated sulfuric acid and sodium hydroxide pellets. After evacuation of the system to 3 $\mu$, the methyl bromide was allowed to evolve slowly, eventually heating the bromide reaction mixture up to 0° and condensing the methyl bromide in the storage bulb with liquid air. On allowing the methyl bromide to expand into the manifold-storage bulb system, the pressure was 66 mm., indicating a quanititative yield of bromide.

*Progesterone-21-C*$^{14}$ (*II*). A two-neck, 50-cc. flask containing 60 mg. (2.5 mmol.) of magnesium and a small magnetic stirrer were attached to one of the outlets. Eleven cubic centimeters of dry ether was added and a drop of 0.1 *M* methylmagnesium bromide solution to assure dryness. The ether solution was frozen in liquid air and the system was evacuated to 0.5 $\mu$. Twenty-nine per cent of the methyl bromide was frozen in this flask, the flask shut off from the manifold and the reaction mixture was warmed to room temperature. The reaction was stirred for thirty minutes. The remainder of the bromide was added in three additional portions (41%, 20%, and 9%) in the same manner. In one run the Grignard reagent was filtered and shown by titration (8) to have been formed in an 80% yield.

At this point nitrogen was admitted into the system and the reaction was continued at atmospheric pressure in a nitrogen atmosphere. Freshly dried cadmium chloride (0.26 g., 1.4 mmol.) was added and the mixture was stirred for two hours at room temperature. The acid chloride (I) was prepared from 595 mg. of sodium 3-keto-4-etiocholenate by the method of Wilds and Shunk (6) employing oxalyl chloride. The 3-keto-4-etiocholenoyl chloride (I) dissolved in 10 cc. of benzene was added to the dimethylcadmium solution. The resulting gummy mixture was stirred for ten hours at room temperature and heated under reflux for three hours more. Then 5 cc. of 1:24 sulfuric acid was added and boiling was continued for an hour to effect decomposition of the complex and to rearrange any isoprogesterone which might have been formed (9). The aqueous phase was separated and extracted with two portions of benzene. After washing successively with water, 5% potassium carbonate solution, water, and saturated sodium chloride solution, the extracts were dried with sodium sulfate; and the solvent was removed to give 375 mg. of oil. The carbonate washes were acidified to give 212 mg. of acid; m.p. 175–195°.

The crude oil was chromatographed on 10 g. of acid-washed alumina (80–200 mesh, activated at 250°). After developing with 50 cc. of benzene the progesterone was eluted with 50 cc. of 1:4 ethyl acetate-benzene to furnish 290 mg. of partly purified progesterone. Molecular distillation of this product at 0.5 $\mu$. pressure gave three fractions: A, 214 mg. at 120–155°; B, 31 mg. at 155–190° and C, 20 mg. at 190–200°. Recrystallization of A from 3 cc. hexane-0.5 cc. acetone furnished 147 mg. of progesterone as heavy prisms, probably in the $\beta$-form: m.p. 122.1–125.6°; $[\alpha]_D^{29}$ +191° (6.7 mg. made up to 2 cc. with 95% ethanol, $\alpha_D^{29}$ 1.28°, l, 2 dm.). The literature values are: m.p. 121° ($\beta$-form); 128.5° ($\alpha$-form); $[\alpha]_D^{20}$ +191.5° (absolute ethanol) (10). Further work-up on fractions B and C and the mother liquors gave an additional 46 mg. (total yield, 193 mg., 29.2%, based on methanol used).

In preliminary runs the yield of progesterone (based on methanol) was found to be 25% with a 1:1 ratio of methanol to acid chloride and 7.6% with a ratio 5:1. In this last case the yield was 43%, based on the acid chloride. In comparable runs on the alkylation 3 $\beta$-acetoxy-5-etiocholenoyl chloride (5), we obtained 31% yield of pregnenolone acetate using a 1:1 ratio and a 17% yield using a 4:1 ratio of methanol to acid chloride.

## SUMMARY

**The preparation of isotopic methyl bromide on a millimole scale has been described. This radioactive methyl bromide has been converted to dimethyl-**

cadmium and used to alkylate 3-keto-4-etiocholenoyl chloride to furnish progesterone-21-$C^{14}$.

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