acid was added, and shaking was continued for 3 more hr. The insoluble barium salts were filtered off and washed with water and acetone, and the combined filtrate and washings were concentrated *in vacuo* to 5 ml.

Barium formate (200 mg.) was obtained from the concentrate by the addition of 50 ml. of acetone. It was purified by three crystallizations from water-acetone, then steam distillation from acid solution, and reconversion to the barium salt. The pure salt was plated on copper planchets from water solution.

The aqueous acetone filtrate from the original precipitation of the barium formate was concentrated to dryness. 1,3-Dithiolane 1,1,3,3-tetroxide was extracted from the residue with boiling acetone and crystallized from isopropyl alcohol. After three recrystallizations, it was plated from acetone solution with collodion as a binder. The following activities were found (corrected for selfabsorption):

	c.p.m. per mmole diluted inositol
All carbons (counted as disulfone)	38,300
Carbon 2 (dithiolane tetroxide)	37,500
Carbons 1,3,4,5,6 (barium formate)	500

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Convenient New Synthesis of Pregnenolone-4-C^{14}

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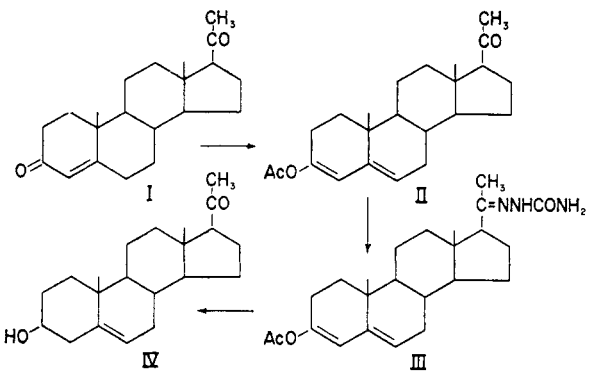
A convenient new synthesis of pregnenolone-4-C^{14} from progesterone-4-C^{14} is described.

Although progesterone-4-C^{14} has been widely used as a substrate for the study of steroid biosynthesis, the Δ^4-3-keto moiety in ring A of progesterone (I), in fact, already represents an advanced step in the biosynthetic pathway from pregnenolone (IV) which contains a Δ^5-3β-ol system. The various metabolic pathways concerned with the Δ^5-double bond and the 3β-hydroxyl group could not be elucidated with progesterone-4-C^{14}, and for a better understanding of these metabolic processes, pregnenolone-4-C^{14} would be the logical substrate.

The previously published method for the synthesis of pregnenolone-4-C^{14} by Milan Uskokovic, *et al.*[1] was rather involved and the over-all yield was very low. Since progesterone-4-C^{14} is readily available, it was decided to transform it to pregnenolone-4-C^{14} by a suitable synthesis which forms the subject of the present paper. The various stages involved in the synthesis were first carried out with nonradioactive material, and when optimum conditions were established the synthesis was repeated with the radioactive steroid.

Progesterone (I) was converted to its 3-enol acetate (II) in 67% yield by treating with acetic anhydride and acetyl chloride as described by Westphal.[2] It has been shown previously by Wendler, *et al.*[3] that, where the reduction of a particular ketonic group was not desirable it could be effectively protected as its semicarbazone derivative, and that the subsequent reduction of the semicarbazone derivative with lithium borohydride



does not reduce the semicarbazone linkage whereas it reduces other carbonyl functions. Consequently, the protected ketonic function could readily be regenerated by removing the semicarbazone group with pyruvic acid. Accordingly, the 20-keto group in II was then protected as the 20-semicarbazone by treating II in pyridine solution with semicarbazide hydrochloride dissolved in aqueous methanol to give 3-acetoxy-$\Delta^{3,5}$-pregnadiene-20-one-20-semicarbazone (III) in 85% yield. The enol acetate function in III was then reduced with lithium borohydride[4] in tetrahydrofuran-dimethylformamide solution to give a mixture of 3α- and 3β-hydroxy-Δ^5-pregnen-20-ones as their semicarbazones. Without further purification the semicarbazone group was removed with pyruvic acid to give a mixture of 3α- and 3β-hydroxy-Δ^5-pregnene-20-ones. The 3β-hydroxy-Δ^5-pregnene-20-one (IV) was selectively isolated in 55% yield through a digitonide formation and subsequent split of the digitonide with pyridine. The over-all

(1) M. Uskokovic, R. I. Dorfman, and M. Gut, *J. Org. Chem.*, **23**, 1947 (1958).

(2) U. Westphal, *Ber.*, **70B**, 2128 (1937); *Chem. Abstr.*, **32**, 2141[3] (1938).

(3) N. L. Wendler, Huang-Minlon, and M. Tishler, *J. Am. Chem. Soc.*, **73**, 3818 (1957).

(4) See L. F. Fieser and M. Fieser, *Steroids*, Reinhold, New York, 1959, p. 439.

yield of pregnenolone from progesterone was 30–32%. Starting with progesterone-4-C^{14} pregnenolone-4-C^{14} was prepared following the above sequence of reactions and all the radioactive intermediates and final product were found to be identical (melting points and infrared spectra) with the nonradioactive authentic samples.

EXPERIMENTAL[5]

Melting points. All melting points were determined on samples dried under high vacuum at 60° for 24 hr. and were uncorrected.

Absorption spectra. The ultraviolet absorption spectra were determined in methanol with a Cary Recording Spectrophotometer (Model 11 MS). The infrared absorption spectra were determined in potassium bromide disk on a Perkin-Elmer (Model 21) Infrared Spectrometer.

3-Acetoxy-$\Delta^{3,5}$-Pregnadiene-20-one (II). This compound was prepared in 67% yield as described by Westphal.[2] The crude product was crystallized once from methanol containing a trace of pyridine to give 2-acetoxy-$\Delta^{3,5}$-pregnadiene-20-one (II), m.p. 132–133°, $(\alpha)_D^{26}$ − 24° ($CHCl_3$), $\lambda_{max}^{CH_3OH}$ 235 mμ, (ϵ = 19,535), γ_{max}^{KBr} 1757, 1705, and 1220 cm.$^{-1}$ (Lit.[2] m.p. 138°, $(\alpha)_D^{20}$ − 41.9°).

3-Acetoxy-$\Delta^{3,5}$-pregnadiene-20-one-20-semicarbazone (III). To a solution of 1.3 g. of enol acetate II in 5 ml. of pyridine, a solution of 3.5 g. of semicarbazide hydrochloride in 15 ml. of methanol and 5 ml. of water was added and the mixture was warmed at 70–75° for 15 min. The precipitated semicarbazone 1.3 g. (85% yield) was filtered and was crystallized from dimethylformamide to give analytically pure 3-acetoxy$\Delta^{3,5}$-pregnadiene-20-one-20-semicarbazone (III), m.p. 255–257° (dec.), $(\alpha)_D^{22}$ − 47.7° (dioxane), $\lambda_{max}^{CH_3OH}$ 232 mμ, (ϵ = 29,650), γ_{max}^{KBr} 3565, 3347, 3280, 3220, 1760, 1695, 1670, 1430, and 1220 cm.$^{-1}$

Anal. Calcd for $C_{24}H_{35}O_3N_3$: N, 10.1. Found: N, 9.98.

3β-Hydroxy-Δ^5-pregnene-20-one (pregnenolone) (IV). *Lithium borohydride reductions.* A solution of 1 g. of semicarbazone III in 80 ml. of tetrahydrofuran and 10 ml. of dimethylformamide was added dropwise at room temperature to a stirred solution of 940 mg. of lithium borohydride in 60 ml. of tetrahydrofuran. The stirring was continued at 25° for 2 hr. and then an additional quantity of 470 mg. of lithium borohydride was added and stirring continued for two more hours. At the end of this period the excess lithium borohydride was decomposed with dilute acetic acid and the solution was concentrated under vacuum at 40° almost to dryness. The residue was then triturated with water and the precipitated reduction product was filtered, washed with water and then dried in a vacuum desiccator overnight.

(5) Analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

Removal of the semicarbazone group. The above dried reduction product was dissolved in 15 ml. of glacial acetic acid and 4 ml. of water. To this solution 2.6 g. of anhydrous sodium acetate and 2.4 ml. of 90% pyruvic acid were added and the mixture was heated under nitrogen atmosphere at 80° for 4 hr. At the end of this period the mixture was concentrated under vacuum to a small volume and then diluted with water and extracted with ether. The ether extract was washed with saturated sodium bicarbonate solution, and with water until neutral and then dried over anhydrous sodium sulphate. Evaporation of the dried ether solution gave 0.641 g. of a mixture of 3α- and 3β-hydroxy-Δ^5-pregnene-20-ones.

Separation of 3β-hydroxy-Δ^5-pregnene-20-one through digitonin precipitation. To a solution of the aforementioned mixture of pregnene compounds in 30 ml. of 80% ethanol, a solution of 2.8 g. of digitonin in 160 ml. of 80% ethanol was added and set aside at room temperature for 20 hr. The resulting digitonide was centrifuged, washed with 80% ethanol and with ether to remove free steroid. The digitonide was dissolved in 50 ml. of pyridine and allowed to stand for 15 hr. Most of the pyridine was then removed under vacuum at 40° and the residue was diluted with 200 ml. of ether. The precipitated digitonin was centrifuged and washed twice with ether. The combined ether washings and the first supernatant were washed with 5% hydrochloric acid, water, saturated sodium bicarbonate solution, and finally with water until neutral. The ether solution was dried over anhydrous sodium sulfate and evaporated to dryness to give 0.355 g. (55%) of 3β-hydroxy-Δ^5-pregnen-20-one (IV) (pregnenolone). The analytical sample melted at 190–191°, $(\alpha)_D^{29}$ + 25.7° ($CHCl_3$), γ_{max}^{KBr} 3500, and 1695 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.69; H, 10.19. Found: C, 79.63; H, 10.21. Further identity was established through a mixture melting point determination and the comparison of the infrared spectrum with that of an authentic sample.

Radio-pure pregnenolone-4-C^{14} (m.p. 189–190°), was synthesized in 32.5% over-all yield, essentially by the same procedure worked out above by starting with 108.7 mg. of progesterone-4-C^{14} with a specific activity of 4.51 microcuries per milligram. The specific activity of the final product was 4.4 microcuries per milligram as determined on a constant recording Packard Tri-Carb Scintillation Counter. All the intermediates and the final product in the radiosynthesis were identical with those of the nonradioactive intermediates and final product described above as shown by mixed melting point determinations and infrared spectra comparisons.

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