chromium trioxide–sulfuric acid reagent.[14] The mixture was kept at 0° for 7 minutes, then diluted with 30 ml. of salt water. Chloroform extraction followed by washing until neutral, drying and evaporation provided 0.65 g. of gummy crystals which after recrystallization from ethyl acetate gave 0.36 g., m.p. 240–245°. Several further recrystallizations from ether–acetone led to the pure sample, m.p. 265–267°, $[\alpha]_D$ −21°. Mixture melting points and infrared comparison established identity of this compound with a specimen prepared according to (a).

5α,6α-Oxido-17α-ethynyl-19-norandrostane-3β,17β-diol (XIX).—To one liter of chloroform containing 13.8 g. of 17α-ethynyl-Δ⁵-19-norandrostene-3β,17β-diol (XVIII)[11] was added one liter of a 0.5 *N* ether monoperphthalic acid solution. After being kept in a refrigerator for 20 hours the mixture was washed with cold aqueous sodium bicarbonate followed by water. After drying and evaporation there remained 13 g. of a white froth which was crystallized from ether–acetone to provide 8.70 g., m.p. 198–200°. By chromatography of the mother liquors on 100 g. of neutral alumina an additional 0.85 g. was obtained. Recrystallization from ether–acetone yielded the analytical sample, m.p. 202–205°, $[\alpha]_D$ −63°.

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92; O, 15.17. Found: C, 75.95; H, 8.93; O, 15.45.

6β-Fluoro-17α-ethynyl-19-norandrostane-3β,5α,17β-triol (XXa).—By the method previously described 1.3 g. of the 5α,6α-epoxy-3β-ol (XIX) was treated with boron trifluoride to yield 0.76 g., m.p. 225–230°. Recrystallization from ether–acetone provided the pure sample m.p. 244–246°, $[\alpha]_D$ −54°.

Anal. Calcd. for $C_{20}H_{29}O_3F$: C, 71.39; H, 8.69; F, 5.64. Found: C, 70.89; H, 8.62; F, 5.49.

6β-Fluoro-17α-ethynyl-19-norandrostane-3β,5α,17β-triol 3-acetate (XXb) was prepared from XXa under the usual acetylating conditions of pyridine and acetic anhydride at room temperature for 15 hours. The resulting product obtained in 95% yield was recrystallized several times from acetone, m.p. 250–252°, $[\alpha]_D$ −52°.

Anal. Calcd. for $C_{22}H_{31}O_4F$: C, 69.81; H, 8.25; O-acetyl, 11.38. Found: C, 69.27; H, 7.99; O-acetyl, 11.57.

6β-Fluoro-17α-ethynyl-19-nortestosterone (XXI).—To 25 ml. of acetic acid containing 1.0 g. of the ketofluorohydrin XII was added 2.0 ml. of concentrated hydrochloric acid. After 65 min. at room temperature, the mixture was diluted with 60 ml. of ice-water and the resulting crystals were collected and washed almost to neutrality with water. Drying in air then provided 0.65 g. of tan crystals which were adsorbed on 20 g. of neutral alumina. By benzene elution there was obtained 0.24 g. of crystals which were repeatedly recrystallized from methanol to provide the analytical sample, m.p. 184–187°; λ_{max}^{EtOH} 234 mμ, log ε 4.10; R.D., *c* 0.059 (dioxane): $[\alpha]_{700}$ −54.7°, $[\alpha]_{589}$ −113°, $[\alpha]_{365}$ −1285°, $[\alpha]_{310}$ +556°, $[\alpha]_{305}$ +25.7°.

Anal. Calcd. for $C_{20}H_{25}O_2F$: C, 75.92; H, 7.96. Found: C, 75.58; H, 8.00.

APARTADO POSTAL 2679, MEXICO, D. F.

(14) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Stereospecific Syntheses of 11-Deuterated Steroids

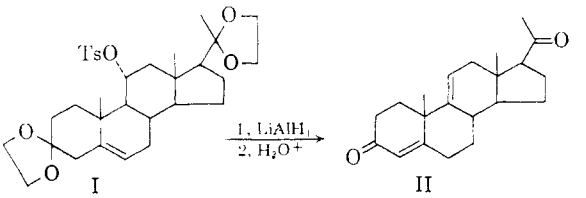
BY E. J. COREY AND G. A. GREGORIOU[1]

RECEIVED NOVEMBER 22, 1958

The synthesis of pregnane-3,20-dione-11β-*d* has been accomplished by the sequence: pregnane-3,11,20-trione → pregnane-3,11,20-trione-3,20-bis-ethylene ketal → pregnane-3,20-dione-11β-ol-11α-*d*-3,20-bis-ethylene ketal ($LiAlD_4$) → $\Delta^{9:11}$-pregnene-3,20-dione-11-*d* ($POCl_3$–C_5H_5N, followed by HOAc) → pregnane-3,20-diol-11β-*d* (Pt, H_2, HOAc followed by deacetylation with $LiAlH_4$) → pregnane-3,20-dione-11β-*d* (CrO_3–HOAc). Pregnane-3,20-dione-9α,11α,12α-$d_{2.8}$ has been prepared by the route: $\Delta^{9:11}$-pregnene-3,20-diol ($LiAlH_4$) → pregnane-3,20-diol-9α,11α,12α-$d_{2.80}$ (D_2, DOAc, Pt) → pregnane-3,20-dione-9α,11α,12α-$d_{2.80}$ (CrO_3).

Recently we have determined the stereochemical course of the enzyme-catalyzed hydroxylation of steroids at C_7 or C_{11} through the use of compounds labeled stereospecifically with deuterium or tritium at those positions,[2,3] and in the preceding paper the methods employed for the synthesis of 7-labeled steroids have been described. This article is concerned with another part of this work, the synthesis of epimeric 11-deuterated pregnane-3,20-diones.

At the outset two methods seemed worthy of consideration for the stereospecific introduction of hydrogen isotope at C_{11}—nucleophilic displacement by hydride and addition of hydrogen to a $\Delta^{9:11}$- or $\Delta^{11:12}$-olefin. It was anticipated that application of the former approach to 11β-substituted steroids would result in the occurrence of elimination, either during attempts to prepare reactive derivatives for displacement, or during the reaction with nucleophile. Consequently, displacement was first studied with a reactive 11α-substituted compound, progesterone bisethylene ketal 11α-toluenesulfonate (I). Attempts to replace toluenesulfonate in I by hydrogen using lithium aluminum hydride in ether were unsuccessful, however, due to rapid elimination, and $\Delta^{9:11}$-dehydroprogesterone (II) was isolated in 60% yield after acid hydrolysis. Because elimination proceeded so readily and because it was clear that displacement of an 11α-toluenesulfonate from the backside would be retarded enormously by the angular methyl groups (C_{18} and C_{19}), no further studies were made of the displacement approach.



I II

The strong shielding of the β-side of C_{11} by the angular methyl groups is a highly desirable effect in the introduction of hydrogen isotope at C_{11} by an addition process and this was exploited in the successful syntheses of 11α- and 11β-labeled preg-

(1) Alfred P. Sloan Foundation Fellow 1956–1958.

(2) S. Bergstrom, S. Lindstedt, B. Samuelsson, E. J. Corey and G. Gregoriou, THIS JOURNAL, **80**, 2337 (1958).

(3) E. J. Corey, G. A. Gregoriou and D. H. Peterson, *ibid.*, **80**, 2338 (1958).

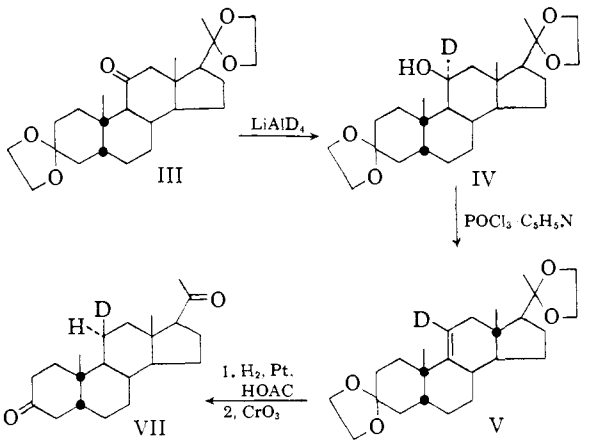


Fig. 1.

nane-3,20-diones which are outlined in Figs. 1 and 2. The first step in the synthesis of pregnane-3,20-dione-11β-*d* (VII) was conversion of pregnane-3,11,20-trione to the 3,20-bisketal III. The exposed 11-ketone was then reduced with lithium aluminum deuteride to the 11β-hydroxy derivative IV (infrared max. 2118 cm.$^{-1}$) which was dehydrated by phosphorus oxychloride–pyridine to $\Delta^{9:11}$-pregnene-3,20-dione-11-*d* 3,20-bisethylene ketal (V) (infrared max. 2255 cm.$^{-1}$).

Difficulties were encountered in the catalytic reduction of the $\Delta^{9:11}$-olefin V. Lengthy treatment (several days) of an acetic acid solution of V in the presence of active Adams catalyst was required before the reduction approached completion. During this treatment hydrolysis of the bisethylene ketal groups and reduction to the 3,20-diol took place along with some acetylation of this alcohol. However, the crude product VI was deacetylated with lithium aluminum hydride, and was oxidized back to the dione by means of chromic oxide in acetic acid. Chromatographic purification of the crude oixdation product gave pregnane-3,20-dione-11β-*d* (VII) having 1.07 atoms deuterium/molecule.

The synthesis of the 11α-*d* epimer was based on a similar series of reactions. Unlabeled $\Delta^{9:11}$-pregnene-3,20-dione 3,20-bisethylene ketal was prepared from pregnane-3,11-20-trione by the method used for the preparation of the C_{11}-labeled compound V. The two ketal groups were removed by heating in acetic acid–water and the crude dione VIII was reduced with lithium aluminum hydride to the diol IX prior to catalytic reduction with deuterium in order to avoid incorporation of deuterium alpha to the ketone groups through enolization. The alcohol IX was reduced catalytically with deuterium in acetic acid-*d* and in the presence of Adams catalyst. The reduction was very slow and had not proceeded to completion when it was discontinued after 6 days. The crude product which had been partly acetylated was deacetylated with lithium aluminum hydride and was oxidized with sodium dichromate and chromic oxide in a two-phase system composed of benzene and water containing some acetic acid. The crude product gave, after chromatography, pregnane-3,20-dione-9α,11α,12α-$d_{2.83}$ (X). The incorporation of more than two atoms of deuterium by the catalytic reduction of some steroidal olefins including the $\Delta^{9:11}$-double bond has been observed before and has been discussed.[4] The distribution of deuterium in the dione X is expected to be: 9α:d_1, 11α:d_1 and 12α:$d_{0.83}$. The compound exhibited broad absorption in the C–D stretching region of the infrared due to the overlap of several peaks. The assignment of orientation to the deuterium or deuterium atoms in pregnane-3,20-dione-11β-*d* (VII) and pregnane-3,20-dione-9α,11α,-12α-$d_{2.83}$ (X) is based on the reasonable assumption that reduction of the $\Delta^{9:11}$-olefin proceeds by addition of hydrogen or deuterium from the less hindered α-side of the steroid.

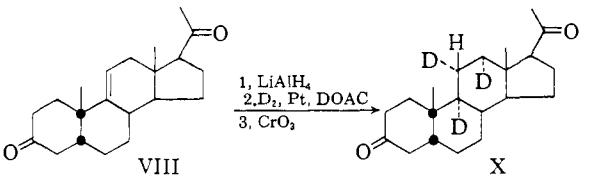


Fig. 2.

The two epimeric 11-deuterated pregnane-3,20-diones VII and X were used in the study of 11α-hydroxylation by *Rhizopus nigricans* and as previously described[3] no deuterium loss could be detected in the formation of 11α-hydroxypregnane 3,20-dione from the β-deuterated VII, whereas one atom of deuterium was lost in the 11α-hydroxylation of X. The accompanying paper[5] presents our present views on the nature of the enzyme-catalyzed hydroxylation reaction.

Experimental[6]

11α-Hydroxyprogesterone Bis-(ethylene Ketal).—A mixture of 11α-hydroxyprogesterone (2.0 g.), ethylene glycol (4 ml.), *p*-toluenesulfonic acid monohydrate (0.1 g.) and 60 ml. of benzene was heated at reflux under vigorous magnetic stirring for 16 hours. The water formed during the reaction was removed continually by means of a Soxhlet extractor containing calcium carbide. The reaction mixture was then cooled and poured into an ether–aqueous sodium bicarbonate mixture. The ether layer was washed with 5% aqueous sodium bicarbonate and then with water, was dried over sodium sulfate, was evaporated to dryness *in vacuo*, and the crude product was crystallized from ethanol–hexane to give 1.51 g. (60%) of 11α-hydroxyprogesterone bis-(ethylene ketal), m.p. 213–216°.

11α-Hydroxyprogesterone Bis-(ethylene Ketal) *p*-Toluenesulfonate.—A solution of 1.15 g. of the 11α-hydroxy bisketal in 10 ml. of dry pyridine was treated with 1.1 g. of *p*-toluenesulfonyl chloride and the mixture was allowed to stand at room temperature for 3 days. At the end of this period precipitation of the crystalline *p*-toluenesulfonate was effected by the addition of more water and the product was collected by filtration, dissolved in ether–chloroform and washed with 5% aqueous sodium hydroxide followed by water. Evaporation and recrystallization from acetone–hexane gave 1.04 g. (65%) of the *p*-toluenesulfonate, m.p. 136–138° dec.

Lithium Aluminum Hydride Reduction of 11α-Hydroxyprogesterone Bis-(ethylene Ketal) *p*-Toluenesulfonate.—A solution of 0.95 g. of the tosylate I in 14 ml. of 1:1 ether–benzene was added dropwise over a period of 7 minutes to a solution (at reflux) of 1.0 g. of lithium aluminum hydride in 70 ml. of 1:1 ether–benzene. The reaction mixture was

(4) D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **77**, 139 (1955).

(5) E. J. Corey and G. A. Gregoriou, *ibid.*, **81**, 3127 (1959).

(6) We are indebted to Mr. J. Nemeth for the deuterium analyses (see ref. 5) and to Dr. R. H. Levin of the Upjohn Co. for gifts of steroids.

maintained at reflux for an additional 8 minutes and then the excess of the hydride was decomposed with ethyl acetate followed by water. Filtration through Celite, washing with 5% aqueous sodium hydroxide and with water, followed by drying over sodium sulfate and evaporation to dryness *in vacuo*, gave 0.62 g. of crude product (theoretical 0.66 g.). The infrared spectrum of this material indicated the absence of *p*-toluenesulfonate ester and of alcohol. The crude product was chromatographed through 50 g. of Merck alumina. Careful elution with cyclohexane–benzene mixtures failed to give any sharp separation. The purest fractions melted at 168–174°. Most of the product (440 mg.) obtained from the chromatographic fractions was mixed with 12 ml. of acetone and 3 ml. of a 20% solution of sulfuric acid in water. The reaction mixture was stored at room temperature for 11 hours and was then worked up by dilution with water, extraction with methylene chloride, washing with aqueous sodium carbonate followed by water, drying over magnesium sulfate and evaporation to dryness to give crude product, m.p. 116–120°, wt. 0.33 g. (65%). One crystallization from cyclohexane gave material melting at 120–122°. This product, however, was not progesterone (lit.[7] m.p. 121°) but $\Delta^{9,11}$-dehydroprogesterone (II) (lit.[8] m.p. 127–128°, lit.[9] m.p. 120–122° when prepared by acid-catalyzed dehydration of 11β-hydroxyprogesterone). The identity was established by mixed melting point determinations with authentic samples of progesterone and $\Delta^{9,11}$-dehydroprogesterone and by comparison of their infrared absorption spectra.

An authentic sample of $\Delta^{9,11}$-dehydroprogesterone was prepared from 11-ketoprogesterone by conversion to the 3,20-bisethylene ketal followed by lithium aluminum hydride reduction to 11β-hydroxyprogesterone bisethylene ketal. Treatment of this product with hydrochloric acid in acetic acid (hot) resulted in hydrolysis of the two ethylene ketal groups and in elimination of the alcohol to give the $\Delta^{9,11}$-olefin.[8,9] Chromatographic purification gave $\Delta^{9,11}$-dehydroprogesterone, m.p. 120–122°.

Lithium Aluminum Deuteride Reduction of 11α-Hydroxyprogesterone Bis-(ethylene Ketal) *p*-Toluenesulfonate.—A solution of 0.15 g. of the tosylate I in 5 ml. of anhydrous tetrahydrofuran was added to a solution of 0.13 g. of lithium aluminum deuteride in 11 ml. of the same solvent at reflux. After 4 minutes at reflux the reaction mixture was cooled and was worked up by evaporation, addition of ether, washing with 4% aqueous hydrochloric acid, 5% aqueous sodium hydroxide, and water, drying over magnesium sulfate, and evaporation to dryness *in vacuo*.

The infrared spectrum of this crude product in the C–D stretching region indicated that if any C–D bond had formed it was present in the product to an extent of less (and probably much less) than 0.1 atom deuterium/molecule.

Pregnane-3,11,20-trione 3,20-Bis-(ethylene Ketal) (III).—A mixture of 2.2 g. of pregnane-3,11,20-trione (m.p. 157–159°), ethylene glycol (4 ml.), *p*-toluenesulfonic acid monohydrate (0.09 g.) and 60 ml. of benzene was heated at reflux for 21 hours under vigorous magnetic stirring. The water formed during reaction was removed continually by means of a Soxhlet extractor containing calcium carbide. The reaction mixture was worked up as described for ketal I and the crude product was crystallized from hexane to give 1.93 g. (70%) of pregnane-3,11,20-trione 3,20-bis-(ethylene ketal), m.p. 144–145.5°. Further purification of part of the product gave pure diketal, m.p. 146.3–146.9° (lit.[10] m.p. 125.4–127°).

Pregnane-11β-ol-11α-*d*-3,20-dione 3,20-Bis-(ethylene Ketal).—A solution of 3.35 g. of pregnane-3,11,20-trione 3,20-bis-(ethylene ketal) in 80 ml. of ether was added over a period of 10 minutes to a solution of 0.615 g. of lithium aluminum deuteride in 100 ml. of ether at reflux. The reaction mixture was maintained at reflux for 40 minutes before the excess of the deuteride was decomposed with ethanol and water. The organic layer was cooled with ice and was washed successively with 4% aqueous hydrochloric acid, 5% aqueous sodium hydroxide and with water. Drying over magnesium sulfate and evaporation to dryness gave 3.3 g. of a solid residue which exhibited hydroxyl absorption in the infrared but no carbonyl absorption. The infrared spectrum of this product in the C–D stretching region showed a band at 2118 cm.$^{-1}$. The C–D band was a very broad one, which seems to be rather general (our experience) for steroid α-deuterio alcohols. The orientation of the C_{11}-hydroxyl group is probably beta as is general for products of hydride reduction of 11-ketosteroids. The crude product was used without purification in the next step.

$\Delta^{9,11}$-Pregnene-3,20-dione-11-*d* 3,20-Bis-(ethylene Ketal) (V).—A solution of 2.65 g. of the crude alcohol, prepared above, in 29 ml. of dry pyridine was treated with 5.2 ml. of phosphorus oxychloride (reagent grade) and was allowed to stand at room temperature for 34 hours. The reaction mixture was then poured into a mixture of ice, ether and an excess of 7% aqueous sodium hydroxide. The organic layer was washed with more sodium hydroxide to ensure the removal of phosphoric acid, then with 5% aqueous sulfuric acid (ice-cold), followed by sodium hydroxide, and with water. It was dried over magnesium sulfate and evaporated to dryness *in vacuo*. The crude product (2.35 g., 93%) had no hydroxyl absorption and no carbonyl absorption in the infrared. The latter appears if in the work-up the reaction mixture is not made basic immediately, which results in the hydrolysis of one of the ketal groups which is very labile. The crude $\Delta^{9,11}$-olefin V was used without purification in the next step.

Pregnane-3,20-diol-11β-*d* (VI).—The catalytic reduction of the $\Delta^{9,11}$-double bond of the olefin V proved to be difficult but was forced almost to completion (*ca.* 93%) by exposure for 3 days using 2.1 g. of the olefin V in 125 ml. of acetic acid in the presence of a total of 1.2 g. of platinum oxide (Baker, lot 8369-2) used in two portions. The solution was then concentrated *in vacuo* to small volume, taken up in ether–methylene chloride, washed with aqueous sodium carbonate and water, dried over magnesium sulfate and evaporated to dryness *in vacuo*. Partial acetylation of the alcohol groups had also taken place during reduction as suggested by the infrared absorption spectrum of the product. Deacetylation was accomplished by reaction of the product with 0.8 g. of lithium aluminum hydride in 350 ml. of ether for 2 hours. The reaction mixture was worked up as usual to give crude pregnane-3,20-diol-11β-*d*, probably as a mixture of epimeric diols.

Pregnane-3,20-dione-11β-*d* (VII).—The diol obtained above was dissolved in 90 ml. of acetic acid and was treated and stirred with a solution of 1.45 g. of chromic oxide in 70 ml. of 90% acetic acid at 18° for 30 minutes and at 27° for 60 minutes. Ethanol was added to destroy the excess of the oxidizing agent and after 30 minutes the mixture was concentrated to small volume *in vacuo*, taken up in ether–methylene chloride and was washed successively with 4% hydrochloric acid, 5% aqueous sodium hydroxide and water. The crude product (m.p. 90–105°) was chromatographed through a 2.5 cm. (diameter) column, packed with 150 g. of Merck acid-washed alumina (lot no. 51186) suspended in cyclohexane. Benzene–cyclohexane (2:1) was first used for elution, followed by benzene and benzene–ether, giving three distinct fractions. The infrared absorption spectra of these fractions, in the order in which they were obtained, had carbonyl absorption intensities corresponding to a monoketone, diketone and triketone. The first and the third fraction were not investigated any further. The fractions from the chromatography containing the diketone were combined and crystallized from cyclohexane to give 0.39 g. of pure pregnane-3,20-dione-11β-*d* (m.p. 120–122°) which after sublimation melted at 119–120.5° (lit.[11] m.p. 123°). Mixed melting point with authentic pregnane-3,20-dione gave no depression.

Pregnane-11β-ol-3,20-dione 3,20-Bis-(ethylene Ketal).—Pregnane-3,11,20-trione 3,20-bis-(ethylene ketal) (4.68 g., m.p. 145.2–146.3°) was reduced by reaction with lithium aluminum hydride (0.85 g.) in 250 ml. of ether at reflux for 40 minutes. The product, pregnane-11β-ol-3,20-dione 3,20-bis-(ethylene ketal), was isolated in quantitative yield.[12]

(7) A. Butenandt and J. Schmidt, *Ber.*, **67**, 1901 (1934).

(8) G. Rosenkranz, O. Mancera and F. Sondheimer, THIS JOURNAL, **76**, 2227 (1954).

(9) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **24**, 351 (1941); P. Hegner and T. Reichstein, *ibid.*, **26**, 715 (1943).

(10) E. P. Oliveto, T. Clayton and E. B. Hershberg, THIS JOURNAL, **75**, 186 (1953).

(11) A. Butenandt, *Ber.*, **63**, 659 (1930).

(12) B. J. Magerlein and R. H. Levin, THIS JOURNAL, **75**, 3654 (1953).

$\Delta^{9(11)}$-Pregnene-3,20-dione 3,20-Bis-(ethylene Ketal) (VII).—The alcohol prepared above was treated with 10 ml. of phosphorus oxychloride in 60 ml. of pyridine at room temperature for 60 hours.

It was worked up by the method described for the preparation of the 11-deuterated isomer V. The crude $\Delta^{9(11)}$-pregnene-3,20-dione 3,20-bis-(ethylene ketal) weighed 4.28 g.

$\Delta^{9(11)}$-Pregnene-3,20-dione.—Most of the crude olefin VII prepared above (4.1 g.) was mixed with 100 ml. of acetic acid and 8 ml. of water and heated on a steam-bath for 45 minutes. Most of the acid was then distilled off *in vacuo* and the residue was taken up in ether–methylene chloride, washed with 5% aqueous sulfuric acid (to ensure removal of even traces of pyridine if any were still present), 5% aqueous sodium hydroxide and water, dried over magnesium sulfate and the solution was evaporated to dryness to give crude $\Delta^{9(11)}$-pregnene-3,20-dione (3.22 g.).

$\Delta^{9(11)}$-Pregnene-3,20-diol (IX).—The dione prepared above was reduced with 0.87 g. of lithium aluminum hydride in 400 ml. of ether at reflux for 2 hours. The reaction mixture was worked up as usual, to give a quantitative yield of crude $\Delta^{9(11)}$-pregnene-3,20-diol (actually a mixture of epimeric alcohols).

Catalytic Reduction of $\Delta^{9(11)}$-Pregnene-3,20-diol with Deuterium.—The diol IX was dissolved in 85 ml. of acetic acid-*d* and was hydrogenated with deuterium gas in the presence of 1.2 g. of platinum oxide (Baker, lot no. 8369-2) with shaking for 20 hours. The solution was transferred to another flask and fresh catalyst (1.0 g.) was added. The hydrogenation was continued for 72 hours and again fresh catalyst (0.51 g.) was added and shaking was continued for 60 more hours. The uptake of deuterium had slowed down very much at the end of this period. Most of the acetic acid-*d* was then distilled off *in vacuo* and the residue was taken up in methylene chloride–ether. It was washed with aqueous sodium hydroxide and water, dried over magnesium sulfate and evaporated to dryness. A positive tetranitromethane test and spectroscopic data indicated that the reduction had not proceeded to completion (*ca.* 80–85% completed).

The partly acetylated product obtained was deacetylated (all but 0.65 g.) with 0.4 g. of lithium aluminum hydride in 1.8 liters of ether. It was worked up as usual to give 2.31 g. of the deuterated pregnane-diol.

Pregnane-3,20-dione-9α,11α,12α-d_3.—Part of the deuterated pregnane-diol prepared above (1.7 g.) was mixed with 280 ml. of benzene cooled to 7°. A solution of 4.0 g. of chromic oxide and 4.0 g. of sodium dichromate dihydrate in 48 ml. of water and 10 ml. of acetic acid was cooled to 7° and was added to the steroid. The two-phase system was stirred at that temperature for 2 hours and then at 14° for an additional 3 hours. The organic layer was then separated, was washed successively with water, aqueous sodium hydroxide, water, and was evaporated to dryness to give crude product (1.61 g.). This product was chromatographed through 190 g. of acid-washed alumina (Merck) (column diameter, 2.5 cm.) suspended in cyclohexane. Benzene–ether (29:1) was the eluent used. Three main products were obtained. The best fractions of the first product melted at 149–152° and showed no C–D absorption. This product was unreduced $\Delta^{9(11)}$-pregnene-3,20-dione [lit.[3] m.p. 148–150°, m.p. 153–155° (Kofler)] and weighed *ca.* 0.19 g. The second product was deuterated pregnane-3,20-dione. About 0.4 g. of essentially pure dione was obtained (m.p. 117–120°) in addition to several intermediate fractions. The over-all yield from pregnane-3,11,20-trione was 16%. The dione was crystallized from cyclohexane and sublimed to give 0.24 g. of pure compound, m.p. 119–120.5°.

The deuterium content of the deuterated pregnane-dione XV obtained was 2.83 atoms deuterium/molecule. The theoretical maximum deuterium content should be 2 atoms per molecule. The excess (0.83 atom/molecule) is due to incorporation of deuterium at the 12-position in accord with previous observations,[4] and the product is formulated as pregnane-3,20-dione-9α,11α-d_2-12α-$d_{0.83}$ (X).

URBANA, ILL.

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

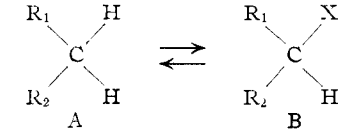
Stereospecific Syntheses of the 7-Deuterio- and 7-Tritiocholesterols. The Mechanism of Enzyme-catalyzed Hydroxylation at a Saturated Carbon Atom

BY E. J. COREY AND GEORGE A. GREGORIOU[1]

RECEIVED NOVEMBER 22, 1958

Cholesterol-7-α-*t* and cholesterol-7-α-*d* have been synthesized stereospecifically by the sequence: 7α-bromo-6-ketocholestanyl acetate → 7α-^{n}H-6-ketocholestanyl acetate (Zn–^{n}HOAc) → 7α-^{n}H-6β-hydroxycholestanyl acetate ($NaBH_4$) → 7α-^{n}H-cholesterol ($POCl_3$–C_5H_5N, followed by $LiAlH_4$). Cholesterol-7β-*t* and cholesterol-7β-*d* have been prepared by a similar process starting with 5α,7β-$^{n}H_2$-7α-bromo-6-ketocholestanyl acetate using unlabeled acetic acid in the debromination step. The facts presently available regarding the mechanism of enzyme-catalyzed hydroxylation at a saturated carbon atom are summarized and a substitution mechanism consistent with these is proposed.

The mechanism of enzyme-catalyzed hydroxylation at a saturated carbon atom[2] has been of particular interest because of the utility of this process for the controlled oxygenation of steroids and because of the fact that many oxygenated natural products are produced in this way. Since the stereochemistry of this reaction is an intrinsic feature of mechanism which can be determined by use of hydrogen isotope, as discussed previously for the general transformation A ⇄ B,[3] such studies have been carried out in these laboratories with a number of steroids. For the specific case of the 7α-hydroxylation reaction which occurs during the biosynthesis of cholic acid from cholesterol it was necessary to obtain cholesterol stereospecifically labeled in the 7α- and 7β-positions by hydrogen isotope and as a result two different syntheses were devised. One of these has already



been outlined in brief in a previous report on the bioöxidation studies using the 7-labeled cholesterols.[4] The first part of this paper deals with the details of this synthetic work and the latter part is concerned with the mechanistic implications of the results of the enzymatic studies.

The more successful and practical synthesis of the two 7-deuterio- and 7-tritio-cholesterols followed

(1) Alfred P. Sloan Foundation Fellow 1956–1958.

(2) For a recent review see P. Talalay, *Physiol. Rev.*, **37**, 362 (1957).

(3) E. J. Corey, M. G. Howell, A. Boston, R. L. Young and R. A. Sneen, THIS JOURNAL, **78**, 5036 (1956).

(4) S. Bergstrom, S. Lindstedt, B. Samuelsson, E. J. Corey and G. Gregoriou, *ibid.*, **80**, 2337 (1958).