10.02. Oxidation of this compound (150 mg) in acetone solution (10 ml) with Jones reagent[12] (4 drops) and work-up *via* ethyl acetate gave the ketolactone **6** (110 mg), obtained from aqueous ethanol as needles: mp 232.5–233°; $[\alpha]^{23}D$ −79° (*c* 1.0, $CHCl_3$); ir (KBr) 1750 (γ-lactone C=O), 1718 cm^{-1} (6-ring ketone C=O); nmr τ 3.78, 4.26 (2 H, AB q, J = 4 Hz, C-6 and C-7 H), 4.89 (2 H, m, C-22, -23 H), 5.93 (1 H, t) and 6.35 (1 H, m, C-3′ H's), 7.11 (1 H, d, J = 5, C-1′ H), 7.25 (1 H, m, C-2′ H), 8.91 (3 H, s, C-19 CH_3), 9.24 (3 H, s, C-18 CH_3). *Anal.* Calcd for $C_{32}H_{46}O_3$: C, 80.29; H, 9.69. Found: C, 80.42; H, 9.67.

Registry No.—**4**, 22965-85-1; **5**, 22965-86-2; **6**, 22965-87-3; **7**, 3930-58-3; **11**, 22965-89-5; **12**, 22965-90-8; **13**, 22965-91-9; **14**, 22950-89-6.

Acknowledgments.—We thank Dr. J. A. Moore for assistance with ORD measurements. This work was supported in part by Institutional Research Grant No. IN 40-H to The University of Michigan from the American Cancer Society, to whom we express our thanks.

Studies on the Heterolytic Fragmentation of Pregnane-16,20-diol Derivatives to Androst-16-enes[1]

MICHIO MATSUI[2] AND DAVID K. FUKUSHIMA

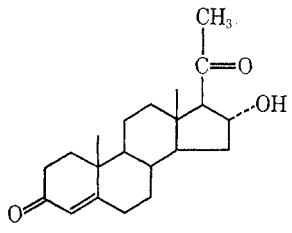
Institute for Steroid Research, Montefiore Hospital and Medical Center, New York, New York 10467

Received July 11, 1969

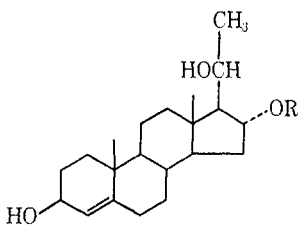
Heterolytic fragmentation of pregn-4-ene-3β,16α,20β-triol 16-tosylate and 16-mesylate with potassium *t*-butoxide afforded androst-4,16-dien-3β-ol as the principal product. In addition, 16β,20β-epoxypregn-4-en-3β-ol, 3β-hydroxypregn-4-en-20-one, and pregna-4,16-diene-3β,20β-diol were obtained. Sodium 3β,20β-dihydroxypregn-4-en-16α-yl sulfate was recovered unchanged under the same condition. Only poor yields of the fragmentation product, 5α-androst-16-en-3β-ol, were obtained from the C-20 epimers of 5α-pregnane-3β,16β,20-triol 3-acetate 16-mesylate. In addition, 3β-hydroxy-5α-pregnan-20-one and the corresponding 5α-pregn-16-ene-3β,20-diol were obtained; there was no evidence for 16,20-oxetane formation from the 16β-mesylates. The stereochemistry of the fragmentation reaction is discussed. The possibility of 16α-hydroxyprogesterone as an intermediate in the biochemical transformation of progesterone to Δ^{16}-C_{19} steroids by boar testis homogenate was examined.

The stereospecific heterolytic fragmentation[3] of the C-20 epimers of 20-chloro-16β-hydroxypregnanes to 16,17-secopregn-17(20)-en-16-als has been reported by Adam and Schreiber.[4] In the present investigation the stereochemical requirements of the fragmentation of 16,20-dihydroxypregnane derivatives have been studied using the 16-mesylate and sulfate derivatives.

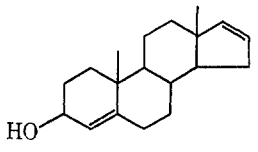
The compounds for fragmentation studies were prepared in essentially similar manner. Pregn-4-ene-3β,16α,20β-triol 16-tosylate (**2a**) was synthesized by tosylation of 16α-hydroxyprogesterone (**1**), followed by reduction with sodium borohydride. The 16-mesylate **2b** and the sodium sulfonoxy derivative **2c** were prepared from **1** with methanesulfonyl chloride and trimethylamine sulfur trioxide, respectively. In all instances sodium borohydride reduction led to the predominant formation of the 3β,20β isomers; only trace amount of the 20α epimer appeared to be present. However, with the 20-keto-16β-mesylate the reduction was not stereoselective. Thus reduction of 3β,16β-dihydroxy-5α-pregnan-20-one 3-acetate 16-mesylate afforded both 5α-pregnane-3β,16β,20β-triol 3-acetate 16-mesylate (**7a**) and its 20α epimer **7b** in a 3:2 ratio. The orientation of the C-20 hydroxyl group was assigned from their nmr spectra. The epimer in which the C-18 methyl proton signals appeared at δ 0.97 was assigned the 20β-hydroxy structure **7a** and that which had δ 0.83, the 20α structure **7b** by comparison with the nmr data obtained from the C-20 epimers of 20-hydroxypregn-4-en-3-one and 5α-pregnane-3β,20-diol 3-monoacetate. Confirmation was subsequently



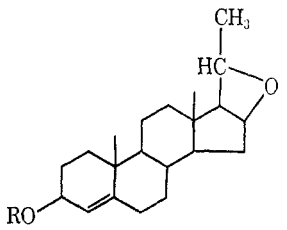
1



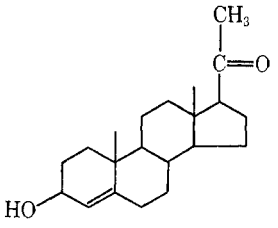
2a, R = $CH_3C_6H_4SO_2$
b, R = CH_3SO_2
c, R = SO_3Na



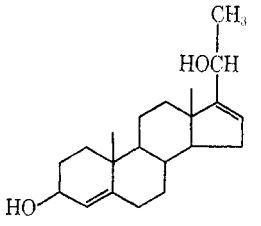
3



4a, R = H
b, R = CH_3CO



5



6

achieved upon isolation of the known 5α-pregn-16-ene-3β,20α-diol (**10b**) from the fragmentation reaction of **7b**.

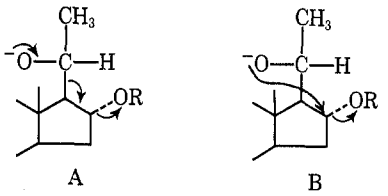
The fragmentation reaction was carried out with potassium *t*-butoxide in *t*-butyl alcohol under reflux for 1 hr. Tosylate **2a** gave an array of products from which four compounds were characterized. The fragmentation product androst-4,16-dien-3β-ol (**3**) was the princi-

(1) This investigation was supported by a grant from the American Cancer Society and Grant CA-07304 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) Visiting Scientist 1967–1969, on leave from Tokyo Biochemical Research Institute, Japan.

(3) C. A. Grob and P. W. Schiess, *Angew. Chem. Intern. Ed. Engl.*, **6**, 1 (1967).

(4) G. Adam and K. Schreiber, *Ann.* **709**, 191 (1967).



A B

Figure 1.

pal component, isolated in 29% yield. It was identified by the multiplet centered at δ 5.77 due to the vinyl protons on C-16 and C-17 and the absence of the C-21 methyl proton signals in the nmr spectrum. It was further characterized by its synthesis from androst-4,16-dien-3-one.

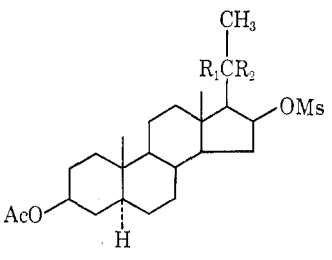
A compound, **4a**, which had a slightly slower mobility on tlc than the fragmentation product **3** was a syrup and therefore characterized as its acetate **4b**. Based on the elemental analysis of **4b** and nmr spectra of **4a** and **4b**, the compound and its derivative were assigned the structures of 16β,20β-epoxypregn-4-en-3β-ol (**4a**) and its 3-acetate **4b**. Evidence for the oxetane ring was derived from the doublet at δ 1.27 (J = 6 cps) for the C-21 methyl protons and the quartet at δ 2.90 for the C-16 methine proton.

The third product was shown to have a ketone by its absorption band at 1695 cm^{-1} and singlets at δ 0.63 and 2.10 for the methyl protons of C-18 and C-21, respectively. The melting point and optical rotation were in good agreement with those reported for 3β-hydroxypregn-4-en-20-one (**5**). Additional proof of structure was obtained by oxidation to progesterone with dichlorodicyanobenzoquinone, DDQ.

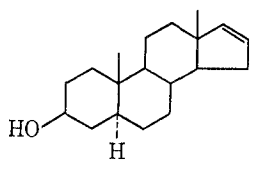
The most polar product isolated was the elimination product, pregna-4,16-diene-3β,20β-diol (**6**), which exhibited a narrow multiplet at δ 5.60 for the vinyl proton at C-16 as well as a broad singlet at δ 5.30 for the C-4 vinyl proton. Confirmation of the structure was achieved by oxidation of **6** to pregna-4,16-diene-3,20-dione with DDQ or with chromium trioxide.

Fragmentation reaction of the 16α-mesylate **2b** gave the same four produts as that from tosylate **2a** but with slightly different quantitative results. The 16-sulfate ester **2c** did not undergo fragmentation and was recovered unchanged. The C-20 hydroxy epimers of the 16β-mesylate **7a** and **7b** yielded many products but only three compounds could be isolated in sufficient amounts for characterization. These products were similar to those obtained from the 16α derivatives. The fragmentation product 5α-androst-16-en-3β-ol (**8**) was obtained in poor yields from **7a** and **7b**. A ketonic compound, singlets at δ 0.60 and 2.08 due to C-18 and C-21 methyl protons, respectively, and absorption at 1705 cm^{-1}, was demonstrated to be 3β-hydroxy-5α-pregnan-20-one (**9**). The elimination product from **7a** was 5α-pregn-16-ene-3β,20β-diol (**10a**), whereas from **7b** it was the 3β,20α isomer **10b**. Both of these compounds were transformed to 5α-pregn-16-ene-3,20-dione for structure verification. No oxetane derivative analogous to **4** found in the reaction with the 16α derivatives was isolated from either of the 16β-mesylates.

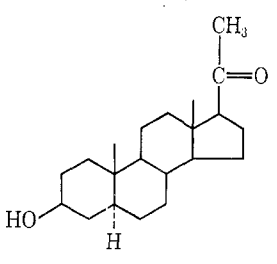
Wharton and Hiegel[5] have demonstrated the importance of geometry in the fragmentation reaction. In the studies with 1,10-decalindiol monotosylates the compounds with *anti*-periplanar bonds[6] yielded over 90% fragmentation product, whereas the isomer with *syn*-clinal bonds gave less than 6% under forcing conditions. The 16α derivatives **2** have *anti*-clinal bonds and the concerted fragmentation reaction, Figure 1A, proceeds relatively well but not so well as expected from compounds with *anti*-periplanar bonds. However with the meslyate in the β orientation at C-16 as in **7**, the bonds involved are *syn* periplanar and poor yields of the fragmentation product, as predicted, were obtained. The stereochemistry at C-20 played no role in the geometry necessary for fragmentation for both **7a** and **7b** gave essentially similar results.



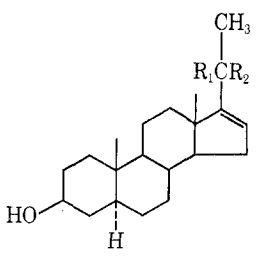
7a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH



8



9



10a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH

Oxetane ring formation during similar fragmentation reactions have been reported by Clayton, Henbest, and Smith[7] with cholestane-3,5-diol 3-monotosylates and more recently by Zurflüh and coworkers[8] in their studies on the synthesis of juvenile hormones. Heckendorn and Tamm[9] proposed that oxetane ring formation in fragmentation of A-nor steroids proceeded by the intermediate formation of the anion of the hydroxyl group which attacked the back side of the carbon carrying the tosylate (or mesylate) group with the displacement of this group. Based on this mechanism, Figure 1B, the stereochemical assignment of the 16β,20β-oxetane ring in **4** has been made. With the 16β-mesylates **7a** and **7b**, the ethyl side chain is also β oriented and the intermediate anion is unable to attack the α face of C-16. Hence no oxetane derivative was observed in the potassium *t*-butoxide reaction of these compounds.

It was demonstrated that the 20-keto derivatives **5** and **9** did not arise from the Δ^{16}-20-hydroxyl products by double bond migration from C-16 to C-17(20) and subsequent ketonization. Under the conditions of the fragmentation reaction, pregna-4,16-diene-3β,20β-diol (**6**) was recovered unchanged. It is suggested that the

(5) P. S. Wharton and G. A. Hiegel, *J. Org. Chem.*, **30**, 3254 (1965).

(6) W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960).

(7) R. B. Clayton, H. B. Henbest, and M. Smith, *J. Chem. Soc.*, 1982 (1957).

(8) R. Zurflüh, E. N. Wall, J. B. Siddall, and J. A. Edwards, *J. Amer. Chem. Soc.*, **90**, 6224 (1968).

(9) R. Heckendorn and Ch. Tamm, *Helv. Chim. Acta*, **51**, 1068 (1968).

20-ketone arose from a 1,3-hydride shift or a double 1,2-hydride shift as depicted in Figure 2.

5α-Androst-16-en-3α-ol has been isolated from boar testes[10] and from human urine.[11] The C_{21} precursors of Δ^{16}-C_{19} steroids have been shown to be progesterone and 3β-hydroxypregn-5-en-20-one,[12,13] but the biochemical pathway involving the removal of the two-carbon side chain and introduction of the unsaturation has not been elucidated. In the present paper the chemical formation of Δ^{16}-C_{19} steroids from 16,20-oxygenated pregnane derivatives has been demonstrated. The possibility of the enzymatic formation of Δ^{16}-C_{19} steroids *via* a 16-oxygenated progesterone intermediate was therefore examined. Incubations of 4-^{14}C-labeled 16α-hydroxyprogesterone **1**, its mesylate, and its sodium sulfate ester with boar testis homogenate following the procedure of Gower and Ahmad[12] afforded no trace of Δ^{16}-C_{19} steroids, whereas in the control incubations with progesterone-4-^{14}C the total yield of various Δ^{16}-C_{19} steroids was at least 10%.

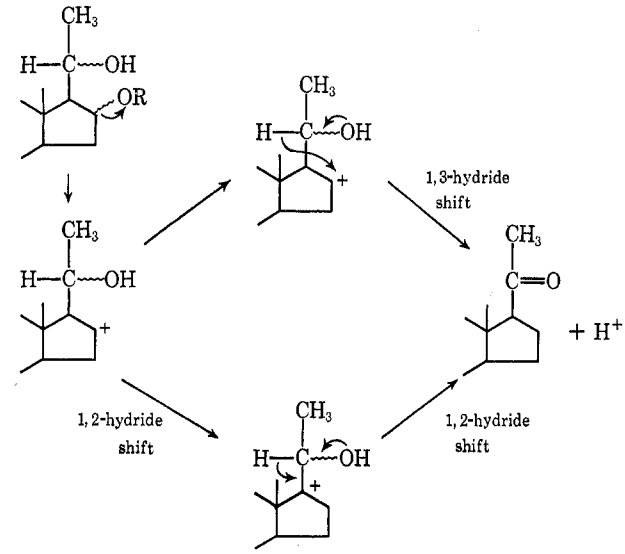


Figure 2.

Experimental Section[14]

Pregn-4-ene-3β,16α,20β-triol 16-Tosylate (2a).—A solution of 1.0 g of 16α-hydroxyprogesterone and 1.9 g of *p*-toluenesulfonyl chloride in 30 ml of pyridine was stored at 5° for 3 days. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with water, dilute hydrochloric acid, water, dilute sodium carbonate, and water. It was dried over sodium sulfate and the solvent evaporated to give 1.4 g of crystals. This was dissolved in 30 ml of methanol–tetrahydrofuran (1:1) and 2 g of sodium borohydride was added during 20 min at 5°. The mixture was stored overnight at 5° and then poured into water. The precipitate was collected and recrystallized from acetone to give 900 mg of needles, mp 114–116°. An additional 173 mg, mp 112–114°, was obtained from the mother liquor. The analytical sample of pregn-4-ene-3β,16α,20β-triol 16-tosylate (**2a**) melted at 115–117°: $[\alpha]_D$ −22.7°; ir 3390, 3040, 1665, 1190, 1175 cm^{-1}; nmr 5.29 (bs), 4.68 (m), 4.02 (m), 3.77 (m), 2.48 (s), 1.08 (d, J = 6 cps), 1.03 (s), 0.78 (s).

Anal. Calcd for $C_{28}H_{40}O_5S$: C, 68.82; H, 8.25; S, 6.56. Found: C, 68.50; H, 8.30; S, 6.66.

Pregn-4-ene-3β,16α,20β-triol 16-Mesylate (2b).—To a cold solution of 1.0 g of 1 in 15 ml of pyridine was added 0.9 ml of methanesulfonyl chloride. The mixture was stored at 5° overnight and worked up as above. It was reduced with 2.5 g of sodium borohydride in 25 ml of methanol–tetrahydrofuran (1:1) for 5 hr at 5°. The product was recrystallized from acetone–ether to yield 690 mg of needles, mp 106–109°. Recrystallization from acetone gave the analytical sample of pregn-4-ene-3β,16α,-20β-triol 16-mesylate (**2b**): mp 110–113°; $[\alpha]_D$ −31.6°; ir 3540 (sh), 3360, 3030, 1658, 1170 cm^{-1}; nmr (DMSO-d_6 and $CDCl_3$, 1:2) 0.80 (s), 1.03 (s), 1.23 (d, J = 6 cps), 3.00 (s), 5.25 (bs).

Anal. Calcd for $C_{22}H_{36}O_5S \cdot {}^1/_2CH_3COCH_3$: C, 63.92; H, 8.90; S, 7.09. Found: C, 63.81; H, 8.97; S, 6.77.

Sodium 3β,20β-Dihydroxypregn-4-en-16α-yl Sulfate (2c).—A solution of 100 mg of 1 and 160 mg of trimethylamine–sulfur trioxide complex[15] in 4 ml of pyridine was stored overnight at 5°. The solvent was removed from the jellylike mixture under reduced pressure and the residue purified by preparative tlc with solvent F. A portion of the chromotagram was sprayed with methylene blue reagent and the steroid sulfate area eluted to give 136 mg of amorphous powder. A solution of 82 mg of this powder and 300 mg of sodium borohydride in 5 ml of methanol was stored overnight at 5°. The reduction product was separated by preparative tlc with solvent C to give 80 mg of amorphous powder. Purification from methanol–ether gave 57 mg of amorphous sodium 3β,20β-dihydroxypregn-4-en-16α-yl sulfate (**2c**): $[\alpha]_D$ −43° (methanol); ir 3450, 3210 (sh), 1260, 1205 cm^{-1}; nmr (DMSO-d_6) 0.72 (s), 0.98 (s), 1.15 (d, J = 6 cps), 5.17 (bs).

Anal. Calcd for $C_{21}H_{33}O_5SNa \cdot 3H_2O$: C, 51.40; H, 8.01; S, 6.53. Found: C, 51.12; H, 7.41; S, 6.28.

5α-Pregnane-3β,16β,20β-triol 3-Acetate 16-Mesylate (7a) and Its 20α Epimer 7b.—3β-Acetoxy-16β-hydroxy-5α-pregnan-20-one[16] (186 mg) was treated with 1 ml of methanesulfonyl chloride and the product reduced with 300 mg of sodium borohydride as above. The reduction product (237 mg) was separated by preparative tlc with solvent B. The faster moving component (R_f 0.30, 86 mg) was recrystallized from acetone–petroleum ether (bp 30–60°) to afford 51 mg of 5α-pregnane-3β,16β,20β-triol 3-acetate 16-mesylate (**7a**): mp 132–134° dec; $[\alpha]_D$ +12.9°; ir 3550, 3490 (sh), 1730, 1365, 1255, 1175, 1168, 993 cm^{-1}; nmr 5.15 (m), 4.70 (m), 4.01 (m), 2.97 (s), 1.32 (d, J = 6 cps), 0.97 (s), 0.83 (s).

Anal. Calcd for $C_{24}H_{40}O_6S$: S, 7.02. Found: S, 6.78.

The more slowly moving one (R_f 0.25, 60 mg) was recrystallized from acetone–petroleum ether to give 41 mg of 5α-pregnane-3β,16β,20α-triol 3-acetate 16-mesylate (**7b**): mp 128–129° dec; $[\alpha]_D$ +18.5°; ir 3530, 3430 (sh), 1703, 1355, 1260, 1178, 1168, 890 cm^{-1}; nmr 5.22 (m), 4.70 (m), 4.01 (m), 3.03 (s), 1.27 (d, J = 6 cps), 0.83 (s).

Anal. Calcd for $C_{24}H_{40}O_6S$: S, 7.02. Found: S, 6.75.

Androsta-4,16-dien-3β-ol (3).—A solution of androsta-4,16-dien-3-one, 200 mg in 4 ml of methanol-tetrahydrofuran (1:1), and 200 mg of sodium borohydride was stored at 5° for 2 hr. The reaction mixture was worked up in the usual manner to give 198 mg of crystals. Purification by tlc with solvent A and recrystallization from hexane afforded 122 mg of androsta-4,16-dien-3β-ol (**3**): mp 116–118°; $[\alpha]_D$ +59.3°; ir 3360, 3300 (sh), 3045, 1663, 1588, 716, 711 cm^{-1}; nmr 5.70 (bm), 5.30 (bs), 4.15 (m), 1.08 (s), 0.78 (s).

Anal. Calcd for $C_{19}H_{28}O$: C, 83.77; H, 10.36. Found: C, 83.95; H, 10.50.

Fragmentation of Pregn-4-ene-3β,16α,20β-triol 16-Tosylate (2a).—A mixture of 900 mg of pregn-4-ene-3β,16α,20β-triol 16-tosylate (**2a**) and 900 mg of potassium *t*-butoxide in 45 ml of *t*-butyl alcohol was refluxed for 1 hr. The mixture was poured

(10) V. Prelog and L. Ruzicka, *Helv. Chim. Acta.*, **27**, 61 (1944).

(11) B. W. L. Brooksbanks and G. A. D. Haslewood, *Biochem. J.*, **47**, 36 (1950).

(12) D. B. Gower and N. Ahmad, *ibid.*, **104**, 550 (1967).

(13) N. Ahmad and D. B. Gower, *ibid.*, **108**, 233 (1968).

(14) All melting points were determined on a micro hot stage and were corrected. Thin layer chromatography (tlc) was carried out on silica gel GF. The following solvent systems were used: solvent A, benzene–acetone (9:1); solvent B, chloroform–acetone (93:7); solvent C, chloroform–methanol (5:2); solvent D, cyclohexane–ethyl acetate (7:3); solvent E, cyclohexane–ethyl acetate (1:1); and solvent F, ethyl acetate–methanol (7:3). Nuclear magnetic resonance (nmr) spectra were determined in deuteriochloroform with tetramethylsilane as internal standard on a Varian A-60 spectrometer. Values are given as δ in parts per million (ppm); s = singlet, bs = broad singlet, d = doublet, q = quartet, m = multiplet, nm = narrow multiplet, bm = broad multiplet. Infrared (ir) spectra were obtained from potassium bromide dispersions on a Beckman IR-9 spectrophotometer; sh = shoulder. Optical rotations were determined in chloroform at 24° (*c* 0.4%) unless otherwise stated.

(15) J. P. Dusza, J. P. Joseph, and S. Bernstein, *Steroids*, **12**, 49 (1968).

(16) R. Neher, Ch. Meystre, and A. Wettstein, *Helv. Chim. Acta*, **42**, 132 (1959).

into ice water and extracted with ethyl acetate. The organic layer was washed with water and dried, and the solvent evaporated to give a yellow syrup. This was separated by preparative tlc with solvent D. Five fractions were obtained: fraction 1, R_f 0.38, 148 mg; fraction 2, R_f 0.27, 190 mg; fraction 3, R_f 0.20, 90 mg; fraction 4, R_f 0.14, 100 mg; and fraction 5, R_f 0.05, 80 mg.

Fraction 1 was recrystallized from hexane to give 121 mg of **androsta-4,16-dien-3β-ol** (**3**), mp 115–117°. There was no depression of melting point on mixture with an authentic sample of **3** and the ir and nmr spectra were identical with those of the authentic sample.

Fraction 2 was acetylated with 0.5 ml of acetic anhydride and 1 ml of pyridine at room temperature overnight. The product (200 mg) was purified by preparative tlc with solvent D. The material with R_f 0.50 (140 mg) was recrystallized from acetone–water and acetone to give 81 mg of **16β,20β-epoxypregn-4-en-3β-ol 3-acetate** (**4b**): $[\alpha]_D$ +1.2°; ir 1730, 1665, 1240 cm^{-1}; nmr 5.25 (bs), 5.20 (m), 2.90 (q, J = 10, 5 cps), 1.27 (d, J = 6 cps), 1.07 (s), 0.80 (s).

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.81; H, 9.65.

Fraction 3 was separated by preparative tlc with solvent B. The material (70 mg) with R_f 0.35 was eluted and recrystallized from methanol to give 46 mg of **3β-hydroxypregn-4-en-20-one** (**5**): mp 157–163°; $[\alpha]_D$ +136°; ir 3508, 1695, 1655, 1040 cm^{-1}; nmr 5.30 (bs), 4.13 (m), 2.10 (s), 1.05 (s), 0.63 (s) (lit.[17] mp 155–161° and $[\alpha]^{26}D$ +135° for this compound). Oxidation of 8 mg of **5** with 9 mg of dichlorodicyanobenzoquinone in 1 ml of dioxane for 24 hr gave 4 mg of progesterone, mp 119–122°.

Fraction 4 was purified by preparative tlc with solvent B. The material with R_f 0.32 was eluted to give 85 mg of crystalline material, mp 152–175°. Recrystallization from acetone afforded 58 mg of **pregna-4,16-diene-3β,20β-diol** (**6**): mp 162–179°; $[\alpha]_D$ +54°; ir 3330, 3050, 3010, 1663, 1625 cm^{-1}; nmr 5.60 (nm), 5.30 (bs), 4.27 (bm), 1.18 (d, J = 6 cps), 1.08 (s), 0.87 (s).

Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.86; H, 10.26.

Oxidation of 14 mg of **6** with chromium trioxide–pyridine complex at room temperature for 2 hr gave 14 mg of pregna-4,16-diene-3,20-dione, mp 186–190°. Recrystallization from acetone–petroleum ether gave mp 192–197°; the infrared spectrum was identical with that of an authentic sample of pregna-4,16-diene-3,20-dione.

Fragmentation of Pregn-4-ene-3β,16α,20β-triol 16-Mesylate (**2b**).—A mixture of 200 mg of pregn-4-ene-3β,16α,20β-triol 16-mesylate and 200 mg of potassium *t*-butoxide in 10 ml of *t*-butyl alcohol was refluxed. The reaction mixture was worked up in the same manner as in the fragmentation of **2a**. The reaction products were the same and characterized by comparison with the products obtained from **2a**. The yields were as follows: androstadienol **3** (19 mg), 16β,20β-epoxypregnenol acetate **4b** (10 mg), pregn-4-enolone **5** (8 mg), and pregnadienediol **6** (28 mg).

Fragmentation of 5α-Pregnane-3β,16β,20β-triol 3-Acetate 16-Mesylate (**7a**).—A mixture of 60 mg of 5α-pregnane-3β,16β,20β-triol 3-acetate 16-mesylate (**7a**) and 70 mg of potassium *t*-butoxide in 3 ml of *t*-butyl alcohol was refluxed for 1 hr and the reaction mixture worked up as before to give 46 mg of syrup. This was separated by preparative tlc with solvent D to afford six fractions: fraction 1, R_f 0.37, 1.4 mg; fraction 2, R_f 0.28, 2 mg; fraction 3, R_f 0.26, 5 mg; fraction 4, R_f 0.22, 13 mg; fraction 5, R_f 0.15, 14 mg; and fraction 6, R_f 0.08, 6 mg.

Fraction 1 was recrystallized from aqueous methanol to give 0.5 mg of **5α-androst-16-en-3β-ol** (**8**), mp 105–117°; the infrared spectrum was identical with that of an authentic sample, mp 125–127°.

Fraction 4 was recrystallized from methanol to yield 6 mg of **3β-hydroxy-5α-pregnan-20-one** (**9**), mp 190–196°; the infrared spectrum was identical with that of an authentic sample, mp 192–196°.

Fraction 6 was recrystallized from acetone to give 8 mg of **5α-pregn-16-ene-3β,20β-diol** (**10a**): mp 193–197°, $[\alpha]_D$ + 21.3°; ir 3430, 3370, 3280, 3050, 1620 cm^{-1}; nmr 5.63 (m), 4.38 (m), 3.58 (m), 1.33 (d, J = 6 cps), 0.83 (s).

(17) M. Gut, *J. Org. Chem.*, **21**, 1327 (1956).

Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.18; H, 10.91. Found: C, 78.96; H, 10.51.

5α-Pregn-16-ene-3β,20β-diol (**10a**, 5 mg) was oxidized with 50 mg of chromium trioxide in 0.5 ml of pyridine at room temperature. Purification by tlc with solvent E and elution of the material with R_f 0.53 afforded 4.3 mg. Recrystallization from acetone yielded 5α-pregn-16-ene-3,20-dione, mp 212–218°; the infrared spectrum was identical with that of the authentic sample and there was no depression of the melting point on mixture with the authentic sample, mp 204–215°. No identifiable material was obtained from the other fractions.

Fragmentation of 5α-Pregnane-3β,16β,20α-triol 3-Acetate 16-Mesylate (**7b**).—A mixture of 40 mg of 5α-pregnane-3β,16β,20α-triol 3-acetate 16-mesylate (**7b**) and 50 mg of potassium *t*-butoxide and 2 ml of *t*-butyl alcohol was refluxed for 1 hr and the reaction mixture worked up as before to give 32 mg of syrup. This was separated by tlc with solvent D to afford six fractions: fraction 1, R_f 0.35, 2 mg; fraction 2, R_f 0.27, 2 mg; fraction 3, R_f 0.22, 7 mg; fraction 4, R_f 0.20, 5 mg; fraction 5, R_f 0.15, 7.5 mg; and fraction 6, R_f 0.08, 4 mg.

Fraction 1 was recrystallized from aqueous methanol to give 1 mg of **5α-androst-16-en-3β-ol** (**8**), mp 115–122°. Fraction 3 was recrystallized from methanol to give 3 mg of **3β-hydroxy-5α-pregnan-20-one** (**9**), mp 190–196°. Fraction 5 was recrystallized from acetone to give 3.5 mg of **5α-pregn-16-ene-3β,20α-diol** (**10b**), mp 168–181°. The analytical sample of **10b** melted at 180–184°: $[\alpha]_D$ −5°, ir 3400 (sh), 3270, 3040, 1625 cm^{-1}; nmr 5.62 (m), 4.30 (m), 3.58 (m), 1.30 (d, J = 6 cps), 0.87 (s), 0.83 (s) (lit.[18] mp 181–182°, $[\alpha]_D$ −14°).

Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.18; H, 10.91. Found: C, 79.32; H, 10.82.

Oxidation of 6 mg of pregn-16-enediol **10b** with chromium trioxide–pyridine complex afforded 4 mg of 5α-pregn-16-ene-3,20-dione, mp 211–218°; the infrared spectrum was identical with that of an authentic sample, mp 204–215°.

Labeled Steroids.—16α-Hydroxyprogesterone-4-^{14}C was prepared by incubation of progesterone-4-^{14}C with a strain of *Streptomyces roseochromogenus*[19] and purified by thin layer chromatography on silica gel GF with ethyl acetate. 16α-Hydroxyprogesterone-4-^{14}C 16-mesylate and 16-sulfate were prepared from 16α-hydroxyprogesterone-^{14}C with methanesulfonyl chloride and trimethylamine sulfur trioxide as described in the "cold" synthesis. The incubation of these substrates was carried out according to the procedure of Gower and Ahmad[12] using 1.4 g of boar testes homogenate and approximately 1.4 × 10^6 cpm of substrates. The incubation extracts were separated by thin layer chromatography with solvent D and the radioactivity in the Δ^{16}-steroid areas, R_f 0.4–0.6, examined and counted. There were insignificant amounts of radioactivity in these areas and no further studies were carried out.

Registry No.—**2a**, 23061-85-0; **2b**, 23061-86-1; **2c**, 23102-70-7; **3**, 23062-06-8; **4b**, 23061-87-2; **5**, 566-66-5; **6**, 23061-89-4; **7a**, 23061-90-7; **7b**, 23061-91-8; **8**, 7148-51-8; **9**, 516-55-2; **10a**, 23061-94-1; **10b**, 23061-95-2.

Acknowledgments.—The authors gratefully acknowledge the interest and support of Dr. T. F. Gallagher throughout this study. We also thank Beatrice S. Gallagher and Julia S. Liang for determination and interpretation of infrared and nmr spectra. We are also grateful to Dr. Patrick A. Diassi, The Squibb Institute, for a generous supply of 16α-hydroxyprogesterone and to Dr. R. Neher, Ciba Ltd, Basle, for samples of 3β-acetoxy-16β-hydroxy-5α-pregnan-20-one, 5α-pregnane-3β,16β,20β-triol triacetate, and 3β-acetoxy-16β,17β-oxido-5α-pregnan-20-one.

(18) M. Nussim, Y. Mazur, and F. Sondheimer, *ibid.*, **29**, 1120 (1964).

(19) U. S. Patent 3,169,978 (1965); J. L. Ruse and S. Solomon, *Biochemistry*, **5**, 1065 (1966).