21-Acetoxy-9α-fluoro-16α-nitromethylpregn-4-ene-3,11,20-trione (XIX).—A suspension of 21-acetoxy-3-ethylenedioxy-9α-fluoro-16α-nitromethylpregn-5-ene-11,20-dione (250 mg., 0.5 mmole) in methanol (6 ml.) containing 8% (v./v.) aqueous sulfuric acid solution (0.6 ml.) was stirred at reflux for 2 hr. The white solid was collected by filtration, washed with methanol and dried *in vacuo* to yield 1.54 mg. (67%) of XIX, m.p. 234–237°. For analysis this material was recrystallized 3 times from methylene chloride-ether to give product with m.p. 239–242°. Physical properties and analytical data are given in Table I.

16-Alkylated Progesterones

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Comparison is made of the progestational activity of some 16-alkylated progesterones. The synthesis of these compounds is described.

Enhancement or transformation of activity by the modification of steroid structures represents a continuing effort on the part of the steroid chemist. Variations in activities, although often predictable, are, however, frequently surprising. In this connection, it seemed of particular interest to examine progesterone derivatives alkylated at carbon 16.

Accordingly, we have prepared: 16-methylene-17α-acetoxyprogesterone (IVa), 16,16-dimethylprogesterone (X), 16β-methyl-17α-acetoxyprogesterone (XVIIa), and 16α-methyl-17α-acetoxyprogesterone (XVIIb). The biological activities of some of them, as well as methods of synthesis, already have been reported. It seemed desirable, however, that the testing and comparison of the progestational activities be carried out by the same laboratory, and our results are presented below. We have included the older compounds, 16α- and 16β-methylprogesterone. In addition, because of certain differences both in procedures employed and in physical constants reported (both for end-products and intermediates[1]) we are presenting our methods of preparation.

(1) See experimental section, compounds XIVa, XVIb, XVIIa, XVIIb.

16-Methylene-17α-acetoxyprogesterone[2] (IVa) was prepared by us as follows: 16β-methyl-16α,17α-oxidoprogesterone (II), obtained from Ia by Oppenauer oxidation[2] or from Ib by *Flavobacterium dehydrogenans*,[3] was treated at approximately 80° with trifluoroacetic anhydride-acetic acid[4] to give IVa directly in 35% yield. No 3-enol acetate formation was observed. This is in contrast with the 17α-hydroxy esterification procedures which utilize acetic anhydride and *p*-toluenesulfonic acid, wherein a complicating side reaction may be the formation of the 3-enol acetate. In the latter case, the 3-keto-Δ^4-system must then be regenerated.

In addition, we also isolated a substance which exhibited absorption maxima at 238 mμ and 308 mμ, and a high specific rotation in dioxane of +475.2°. Based upon the work of Plattner *et al.*,[5] who have reported for 3β-hydroxy-5,14,16-pregnatrien-20-one a specific rotation of + 359° (chloroform) and spectroscopic data of λ_{max} 307 (log ϵ = 4.231), we assigned to our side product the structure of 16-methyl-4,14,16-pregnatriene-3,20-dione (V).[6]

Alternatively, IVa was obtained by the conventional two step process of preparing III[2] initially from II by treatment with hydrogen bromide in acetic acid[2a] and then esterification. Here again, trifluoroacetic anhydride–acetic acid was used as the esterifying agent. In both of these steps some of the triene V also was formed. Although our constants for IVa were in good agreement with the published data, added support for the 16-methylene structure was obtained from the nuclear magnetic resonance spectrum of IVa.[7]

It is interesting to note that the treatment of III (prepared directly from the oxide II) with either hydrogen bromide in acetic acid or trifluoroacetic acid in acetic acid gave a small amount of V (spectroscopic evidence) in addition to starting material. However, similar

(2) (a) D. N. Kirk, V. Petrow, M. Stansfield and D. M. Williamson, *J. Chem. Soc.*, 2385 (1960); (b) K. Syhora, *Tetrahedron Letters*, **17**, 34 (1960); and (c) H. J. Mannhardt, F. v. Werder, K. H. Bork, H. Metz and K. Bruckner, *Tetrahedron Letters*, **16**, 21 (1960).

(3) (a) C. Arnaudi, *Zentr. Parasitenk.*, **105**, 352 (1942); (b) A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore and W. Charney, *J. Am. Chem. Soc.*, **79**, 4814 (1957); see also South African Patent 3462/55.

(4) K. H. Pawlowski and M. Schenck, German Patent 1,013,284, August 8, 1957.

(5) Pl. A. Plattner, H. Heusser, and A. Serge, *Helv. Chim. Acta*, **31**, 249 (1948).

(6) In a related area, G. Nomine, D. Bertin and A. Pierdet [*Tetrahedron*, **8**, 217 (1960)], obtained from the reaction of 16-methyl-3α-acetoxy-17α-hydroxy-15-pregnene-11,20-dione with *p*-toluenesulfonic acid in benzene a substance having physical constants of λ_{max}^{EtOH} 240; 303(E% 54;260); [α] + 345° (C = 1% chloroform) for which the structure assigned was 16-methyl-3α-acetoxy-14,16-pregnadiene-11,20-dione.

(7) We wish to thank Dr. Leon Mandell of Emory University for the n.m.r. evaluation: In $DCCl_3$, $CHCl_3$ as external reference, 62 c.p.s.: C-4 proton (α to carbonyl and on vinyl carbon); multiplet from 69–73 c.p.s.: vinyl protons on methylene at C-16; 206 c.p.s.: side chain acetyl; 208 c.p.s.: C-17-acetate; 243 c.p.s.: C-19 methyl (with 3-keto-Δ^4); 262 c.p.s.: C-18 methyl.

treatment of III (obtained by saponification[2b] of IVa) did not generate the 14,16-diene V.[8]

Compound IVa was dehydrogenated microbiologically with *Bacillus sphaericus*[9] to give in modest yield 16-methylene-17α-hydroxy-1,4-pregnadiene-3,20-dione 17-acetate (1-dehydro-16-methylene-17α-acetoxyprogesterone, VI). In addition IVa was transformed with ethyl orthoformate in dioxane[10] to its 3-ethyl enol ether VII. Supportive evidence that no change had occurred in the D-ring was obtained by regeneration of IVa from the enol ether with aqueous acetic acid.

The one-step ring opening and esterification procedure was used for the formation of 16-methylene-17α-hydroxy-4-pregnene-3,20-dione 17-caproate (IVb). In this instance the esterifying medium was caproic acid–trifluoroacetic anhydride. Here again, the triene V was obtained as a side product.

For the preparation of 16α,16β-dimethyl-4-pregnene-3,20-dione (16α,16β-dimethylprogesterone, X), 16-methyl-3β-acetoxy-5,16-pregnadien-20-one (VIII)[11] was utilized as starting material. The Grignard reaction on the latter compound, employing methylmagnesium iodide in the presence of cuprous chloride, resulted in a good yield of the 16,16-dimethyl product[12] which for ease of isolation was esterified to give IXb. Mild alkaline saponification regenerated the free alcohol IXa. Microbiological oxidation of IXa with *Flavobacterium dehydrogenans* gave 16α,16β-dimethylprogesterone (X).

The assignment of the acetyl side chain as β for IX follows from the rule of the rear addition[13,14] of the proton to the $\Delta^{17(20)}$ enolate ion. Comparison of rotational data of related compounds supports this assignment.

As depicted in Table I, molecular rotational values for IXa and X (No. 9 and 4 respectively in Table I) have been derived by calculating the additive rotational effect of an α-methyl and β-methyl substituent upon the parent steroid. The calculated and experimental values are noted. It would be anticipated that the large levorotatory effect

(8) See D. Taub, R. D. Hoffsommer, H. L. Slates, C. H. Kuo and N. L. Wendler, *J. Am. Chem. Soc.*, **82**, 4012 (1960), for an explanation regarding the generation of the 14,16-diene system.

(9) T. H. Stoudt, W. J. McAleer, J. M. Chemerda, M. A. Kozlowski, R. F. Hirschmann, V. Marlatt and R. Miller, *Arch. Biochem. and Biophys.*, **59**, 304 (1955).

(10) E. P. Oliveto, C. Gerold and E. B. Hershberg, *J. Am. Chem. Soc.*, **74**, 2248 (1952).

(11) (a) A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944); (b) J. Romo, J. Lepe, and M. Romero, *Bol. Inst. Quim. Univ. n. Auton.*, Mex., **4**, 125 (1952).

(12) R. D. Hoffsommer, H. L. Slates, D. Taub and N. L. Wendler, *J. Org. Chem.*, **24**, 1617 (1959), have employed a similar procedure for preparing 16,16-dimethylpregnanes in the 11-oxygenated series.

(13) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948).

(14) T. F. Gallagher and T. H. Kritchevsky, *J. Am. Chem. Soc.*, **72**, 882 (1950).

TABLE I

MOLECULAR ROTATIONAL COMPARISONS OF 16-METHYL STEROIDS

No.	Compound	$[\alpha]_D$	MD	MD differences: Compound	MD differences: Δ^c	MD differences: Calc'd. MD
1	Progesterone	$+202^{a,d}$	+635			
2	16α-methylprogesterone	$+165^{a,e}$	+555	2-1	−80	
3	16β-methylprogesterone	$+133^{a,e}$	+440	3-1	−195	
4	16,16-dimethylproges-terone	$+104^{a}$	+358			635 + (−80) + (−195) = +360
5	16β-methyl-17-isoproges-terone	$+27^{a,e}$	+88.7			
6	Pregnenolone	$+15.9^{b,c,f}$	+49.1			
7	16α-methylpregnenolone	$-1^{b,c,g}$	−3.23	7-6	−52.3	
8	16β-methylpregnenolone	$-13^{b,c}$	−42.0	8-6	−91.1	
9	16α,16β-dimethylpreg-nenolone	-49^{b}	−165			49.1 + (−52.3) + (−91.1) = −94.3
10	16β-methyl-17-isopreg-nenolone	$-117^{a,c}$	−386			

[a] Chloroform; [b] dioxane; [c] rotation in dioxane at Schering; [d] J.-P. Mathieu and A. Petit "Pouvoir Rotatoir Naturel," Paris, France, 1956, p. 36; [e] reference 11b; [f] "Pouvoir Rotatoir" lists $[\alpha]_D$ + 25° (chloroform), p. 46; [g] reference 11b: $[\alpha]_D$ 0° (chloroform).

of an isoacetyl side chain (*cf.* Table I, No. 5 *vs.* 3 and 10 *vs.* 8) would have significantly modified the experimental values for compounds no. 4 and 9 so that the relatively good agreement between these values and the calculated values would not have been observed.

Our 16β-methyl-17α-acetoxyprogesterone[1,15] was prepared as follows: chlorination[16] of 16β - methyl - 3β - acetoxy - 5 - pregnen - 20-one (XIa)[11] gave the 5,6-dichloride XIIa. Introduction of the 17α-hydroxy group was accomplished by the Gallagher procedure of forming the $\Delta^{17(20)}$ enol acetate[17a] with acetic anhydride using *p*-toluenesulfonic acid as catalyst, followed by epoxidation with peracetic acid[17b,c] and then alkaline treatment, giving thereby 5α,6β-dichloro-16β - methyl - 3β,17α - dihydroxypregnan - 20 - one (XIIIa). Esterification of XIIIa with acetic anhydride in pyridine gave the 3-monoacetate (XIX). 16β - Methyl - 5 - pregnene - 3β,17α - diol-20-one (XIVa)[1,15] was obtained directly from XIIIa by eliminating, with zinc and aqueous ethanol, the protective 5,6-dichloro function. Oxidation of the hydroxyl substituent at carbon 3 of XIVa with

(15) R. Sciaky, *Gazz. Chim. Ital.*, **91**, 562 (1961).

(16) The procedure employed is essentially outlined by F. A. Cutler, Jr., L. Mandell, D. Shew, J. F. Fisher, and J. M. Chemerda, *J. Org. Chem.*, **24**, 1621 (1959), for the chlorination of pregnenolone acetate. However, the solvent used by us was carbon tetrachloride rather than chloroform.

(17) (a) T. H. Kritchevsky and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 184 (1951); (b) H. V. Anderson, E. R. Garrett, F. H. Lincoln, Jr., A. H. Nathan and J. A. Hogg, *ibid.*, **76**, 743 (1954); (c) E. P. Oliveto and E. B. Hershberg, *ibid.*, **76**, 5167 (1954).

chromium trioxide in acetone,[18] followed by treatment of the crude 16β - methyl - 17α - hydroxy - 5 - pregnene - 3,20 - dione (XVa) with methanolic perchloric acid gave 16β-methyl-17α-hydroxy-4-pregnene-3,20-dione (XVIa). In addition, another substance was obtained in an impure state which exhibited physical characteristics similar to those reported by Bladon[19] for a steroidal 3-keto-$\Delta^{4,6}$-6-methoxy moiety obtained *via* a similar sequence of reactions.

Esterification of XVIa by the trifluoroacetic anhydride–acetic acid procedure gave 16β-methyl-17α-acetoxyprogesterone (XVIIa). On the basis of the nuclear magnetic resonance spectrum,[20] XVIIa is not a D-homo annulation product which might have resulted from the esterification of XVIa under acid conditions.

The following sequence was employed for the preparation of compound XVIIb:[21] 5α,6β-dichloro-16α-methylallopregnane-3β,17α-dihydroxy-20-one[21a] (XIIIb), obtained from 16α-methyl-3β-acetoxy-5-pregnen-20-one[22] (XIb) by essentially the process outlined for the preparation of XIIIa, was transformed by oxidation with chromium trioxide in aqueous acetic acid to the crude 3-keto analog XVIII. Zinc in aqueous ethanol treatment of XVIII, followed by perchloric acid equilibration of the generated 3-keto-Δ^5 substance XVb gave, in a relatively poor conversion from XIIIb, 16α-methyl-17α-hydroxyprogesterone[1,23] (XVIb). Esterification of XVIb with trifluoroacetic anhydride–acetic acid gave 16α-methyl-17α-acetoxyprogesterone (XVIIb).

In Table II are presented the biological activities of various 16-substituted progesterones. Although 16α-methylprogesterone has an activity equal to progesterone, it is interesting to note the inactivity not only of 16β-methylprogesterone, but also that of the gem-disubstituted compound 16α,16β-dimethylprogesterone. The comparison of activities of the 16-substituted-17α-acetoxy compounds

(18) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946); C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

(19) P. Bladon, *J. Chem. Soc.*, 3723 (1958).

(20) We wish to thank Dr. James N. Shoolery of Varian Associates, of Palo Alto, California, for the interpretation of the spectrum of XVIIa, taken on a A-60 n.m.r. spectrometer, using deuterated chloroform as solvent, with tetramethylsilane as the internal reference. The pertinent absorptions are as follows: 127 c.p.s., methyl protons on C-21 attached to 20-keto group; 118 c.p.s., acetate methyl group; 71 c.p.s., C-19 methyl; 44 c.p.s., C-18 methyl group; 77 and 84 c.p.s., methyl at C-16 split by spin-coupling to the proton on the same carbon atom. There is no evidence of a peak around 80 c.p.s. which would correspond to a methyl group attached to the same carbon atom as the oxygen of an acetate group.

(21) (a) E. Batres, T. Cardenas, J. A. Edwards, G. Monroy, O. Mancera, C. Djerassi and H. J. Ringold, *J. Org. Chem.*, **26**, 871 (1961), and (b) S. Bernstein, E. W. Cantrall, J. P. Dusza, and J. P. Joseph, *Experientia*, **17**, 454 (1961).

(22) R. E. Marker and H. Crooks, *J. Am. Chem. Soc.*, **64**, 1280 (1942).

(23) K. Heusler, J. Kebrle, C. Meystre, H. Ueberwasser, P. Wieland, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, **42**, 2043 (1959).

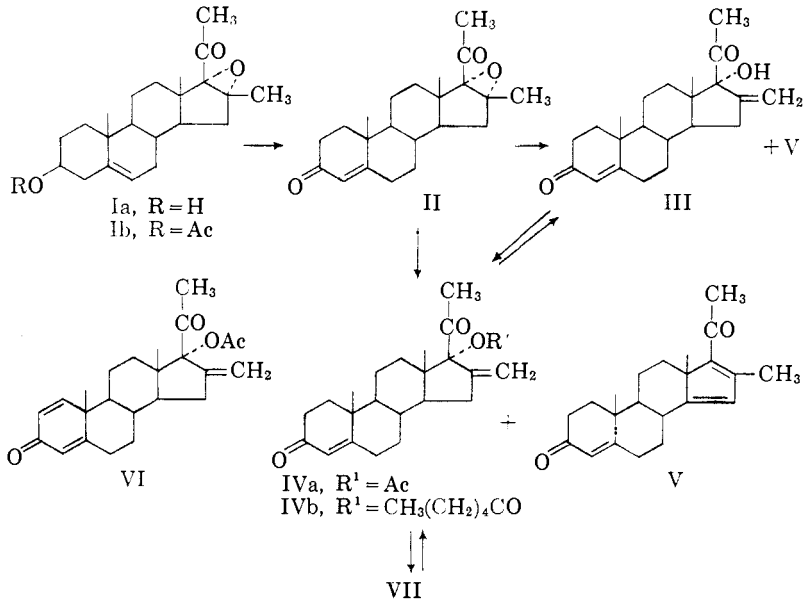
TABLE II

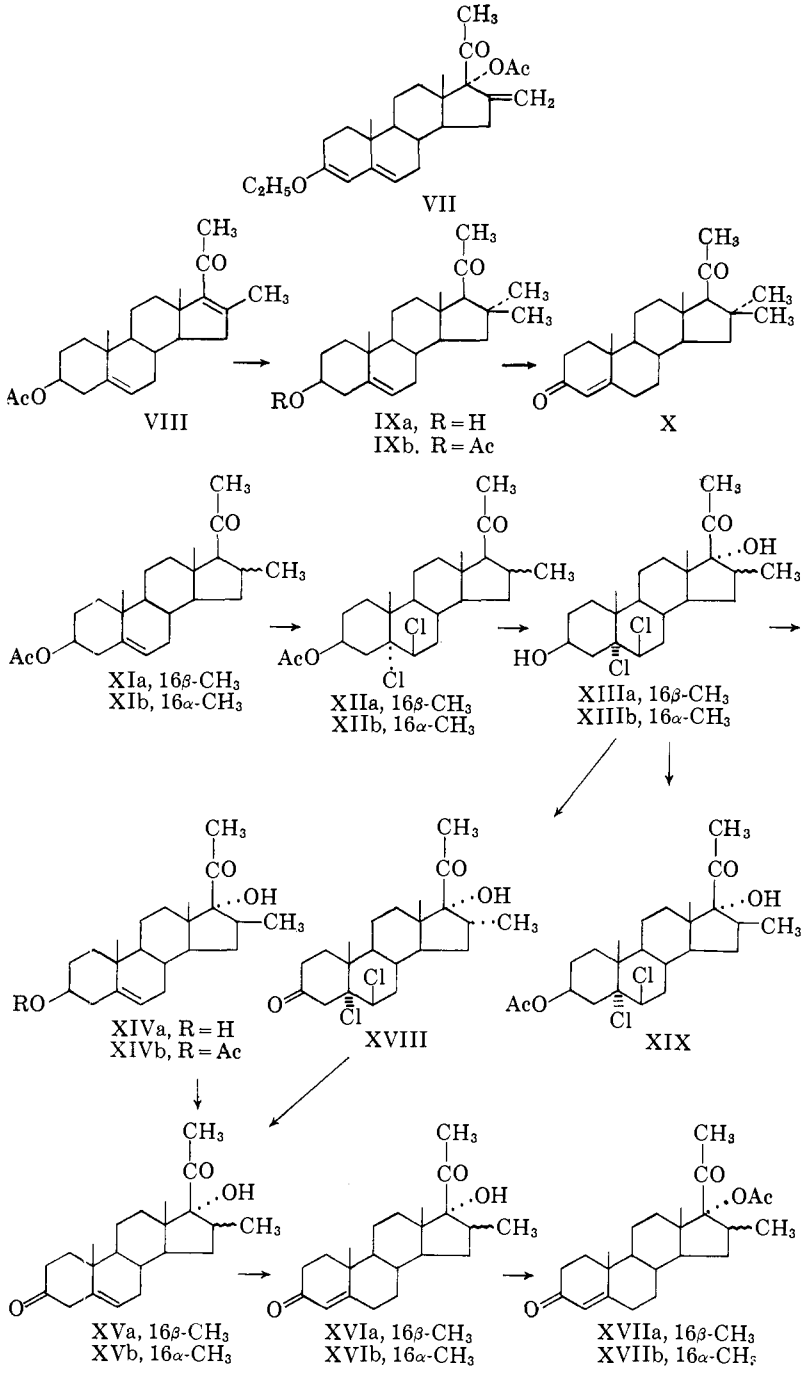
PROGESTATIONAL ACTIVITY OF 16-SUBSTITUTED PROGESTERONES

Compound	Activity[a]
16α-Methyl	~1
16β-Methyl	0
16α,16β-Dimethyl	0
16β-Methyl-16α,17α-oxido	0
16-Methylene-17α-hydroxy	0
16α-Methyl-17α-acetoxy	3.0[c]
16β-Methyl-17α-acetoxy	1.0[d]
16-Methylene-17α-acetoxy	32.0[e]
16-Methylene-17α-acetoxy 3-ethyl-enol ether	0[b]
16-Methylene-17α-caproöxy	3.0

[a] According to the method of M. K. McPhail, *J. Physiol.*, **83**, 145 (1934) in immature rabbits; parenteral, progesterone = 1. [b] Oral activity *vs.* ethisterone. [c] Reported by Bernstein, *et al.*[21b] as having activity orally approximately equal to 17α-acetoxyprogesterone. [d] Reported by Sciaky[15] as having activity orally like progesterone. [e] Reported by Kirk, *et al.*,[2a] as having marked progestational activity orally and by Mannhardt, *et al.*,[2c] as having increased progestational activity.

listed in this Table is most striking. Whereas 16α-methyl-17α-acetoxyprogesterone has an activity factor of 3 and 16β-methyl-17α-acetoxyprogesterone has an activity factor of only 1, when an unsaturation is introduced at C-16 in the form of a methylene func-




VII
VIII
IXa, R = H
IXb. R = Ac
X
XIa, 16β-CH3
XIb, 16α-CH3
XIIa, 16β-CH3
XIIb, 16α-CH3
XIIIa, 16β-CH3
XIIIb, 16α-CH3
XIVa, R = H
XIVb, R = Ac
XVIII
XIX
XVa, 16β-CH3
XVb, 16α-CH3
XVIa, 16β-CH3
XVIb, 16α-CH3
XVIIa, 16β-CH3
XVIIb, 16α-CH3

tion, there is a dramatic change in activity as observed with 16-methylene-17α-acetoxyprogesterone, which has a factor of 32.

Acknowledgment.—We wish to thank Dr. C. H. Robinson for his helpful discussions during the preparation of this manuscript and Mr. Richard Wayne for his infrared evaluations.

Experimental[24]

16β-Methyl-16α,17α-oxido-4-pregnene-3,20-dione (II). **(Microbiologically).**—*Flavobacterium dehydrogenans*[3] was maintained on a 1% yeast extract agar buffered at pH 6.8. Inoculum for the fermentation was prepared by subculturing from an agar slant to a 300 ml. Erlenmeyer flask containing 100 ml. of sterile medium containing 1% Difco yeast extract buffered at pH 6.8. The flask was incubated at 28° on a rotary shaker running at 320 r.p.m. for 48 hr. under constant illumination. A 2% inoculum of the 48 hr. culture was used to inoculate a series of fermentation flasks containing the same medium. After 24 hr. incubation, on the shaking machine, 50 mg. of Ib, dissolved in 1.5 ml. of dimethylformamide was added to each flask (a total of 500 mg.). Incubation was continued on the shaker for 48 hr. The flasks were then pooled and extensively extracted with chloroform. The solvent was removed *in vacuo* to give an oily residue which was placed upon a Florisil column with hexane. The 100% hexane fractions were combined and crystallized from isopropyl ether to give 0.21 g. of II,[2] m.p. 164° confirmed by infrared comparison with II obtained by Oppenauer oxidation of Ia.

16-Methylene-17α-acetoxy-4-pregnene-3,20-dione (**16-Methylene-17α-acetoxyprogesterone, IVa.**)—*A*. To a solution of II (0.93 g.) in 9.3 ml. of acetic acid at room temperature under an atmosphere of argon was added 1.85 ml. of trifluoroacetic anhydride. The mixture was warmed rapidly under anhydrous conditions to approximately 90–95° (4 min.). After 55 min. the dark reaction mixture was cooled to about 30° and added to 15 vol. of ice-water. Extraction with methylene chloride gave, after washing the organic phase with sodium carbonate, then water, and evaporation, 0.97 g. of product. Crystallization from isopropyl ether gave IVa (290 mg.), m.p. 214–219°. Analytical sample, m.p. 224–226°, $[\alpha]_D$ − 51.8°; $[\alpha]_D$ − 66.2° ($CHCl_3$); λ_{max} 240 mμ (ϵ 17,300); λ_{max} 5.76, 5.84, 6.00, 6.20, 7.94, 8.14, 11.05 (methylene) μ; n.m.r. in $DCCl_3$ ($CHCl_3$ as reference).[7] Reported[2a] m.p. was 222–224°; $[\alpha]^{23}$ − 68° ($CHCl_3$).

Purification of the mother liquors by chromatography over Florisil gave V in approximately 10% yield, m.p. 148–149°; λ_{max} 238, 308 mμ (ϵ 18,700; 12,800); $[\alpha]_D$ + 475.2°; λ_{max} 5.98, 6.12, 6.52, 11.62 μ.

Anal. Calcd. for $C_{22}H_{28}O_3$: C, 81.44; H, 8.70. Found: C, 81.69; H, 8.67.

B. As described above, 1.0 g. of III[2a] (obtained from II) was treated with trifluoroacetic anhydride (1.99 ml.) and acetic acid (10 ml.). A total crude of 930 mg. was isolated. Crystallization from isopropyl ether gave IVa (460 mg.),

(24) Melting points were determined on a micro hot stage apparatus. Rotations are in dioxane at 25° at about 1% concentration, infrared spectra are of the solids in Nujol and ultraviolet spectra are of methanolic solutions, unless stated otherwise. Rotational and spectral data were obtained by the Physical Chemistry Department, Schering Corporation. Microanalyses were performed by Mr. E. Conner and Staff (Microanalytical Laboratory, Schering Corporation), Galbraith Laboratories, Knoxville, Tenn.; and the Schwartzkopf Microanalytical Laboratory, Woodside, L. I.

m.p. 222–225°; infrared identical with IVa obtained from the direct conversion of **II**. Spectroscopic analysis of the mother liquor revealed absorption at 308 mμ, indicating the presence of approximately 5% of V.

16-Methylene-17α-hydroxy-4-pregnene-3,20-dione 17-Caproate (IVb).—A solution of 16β-methyl-16α,17α-oxido-4-pregnene-3,20-dione (II) in 10 ml. of caproic acid and 2 ml. of trifluoroacetic anhydride was heated at 90-95° under an atmosphere of argon under anhydrous conditions for 55 min. The reaction solution was cooled and then added to 20 vol. of water. The resulting mixture was extracted with ether. After washing the organic layer with a 3% aqueous sodium hydroxide solution and then water, the dried (magnesium sulfate) organic phase was evaporated to give an oily residue which was dissolved in hexane and chromatographed on Florisil. From the 5% ether in hexane through 15% ether in hexane there was isolated after ether–hexane crystallization 220 mg. of the caproate IVb, m.p. 112–118°. The analytical sample was crystallized from ether–hexane, m.p. 124–127°; $[\alpha]_D$ − 44.9°; λ_{max} 239 mμ (ε 17,300); λ_{max} 5.74, 5.82, 5.95, 6.15, 9.18, 11.04, 11.56 μ.

Anal. Calcd. for $C_{28}H_{40}O_4$: C, 76.32; H, 9.15. Found: C, 76.00; H, 8.87.

The mother liquors exhibited the 308 mμ absorption indicative of the presence of V.

16-Methylene-17α-acetoxyprogesterone 3-ethyl Enol Ether (VII).—16-Methylene-17α-acetoxyprogesterone (IVa) (2 g.) was added to a solution containing ethyl orthoformate (2.14 ml.), absolute ethanol (0.108 ml.), dioxane (23.84 ml.) and concd. sulfuric acid (0.025 ml.). After remaining at 31° for 18 min., pyridine (1.1 ml.) was added and the solution was evaporated under vacuum to near dryness. Methanol (2.0 ml.) was added to effect crystallization. The collected crystalline material was crystallized directly from methanol to give the enol ether VII, 0.73 g., m.p. 150–153°; $[\alpha]_D$ − 236.9°; λ_{max} 240 mμ. (ε 21,250); λ_{max} 5.74, 5.82, 6.02, 6.12, 8.10, 10.92, 11.05 μ.

Anal. Calcd. for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.37; H, 8.90.

Reversal of 16-Methylene-17α-acetoxyprogesterone 3-Ethyl Enol Ether (VII) to 16-Methylene-17α-acetoxyprogesterone (IVa).—A solution of 40 mg. of VII in 1 ml. of 90% aqueous acetic acid was warmed at 90-95° for 15 min. then diluted with 10 ml. of water to effect precipitation. Isolation by decantation followed by drying of the water insoluble material gave IVa identical by infrared comparison with an authentic spectrum.

16-Methylene-17α-acetoxy-1,4-pregnadiene-3,20-dione (VI).—*Bacillus sphaericus* SCC No. 35[9] was maintained on nutrient agar slants. Inoculum for the fermentation was prepared by transferring a loopful of cell material from the slant to three 300 ml. Erlenmeyer flasks containing 100 ml. of 1% yeast extracts at pH 7. The flasks were incubated at 28° on a rotary shaker running at 320 r.p.m. for 24 hr. The flasks were then pooled into a sterile flask with side-arm and used as inoculum for a fermentor containing 10 l. of the same yeast extract medium. Foaming was controlled by the addition of a silicon antifoam G.E. 60. Sterile air was sparged in at the rate of 0.5 vol. air/vol. of medium/min. and agitation speed was 500 r.p.m. The temperature was 29°. After 10 hr. of growth, 1 g. of 16-methylene-17α-acetoxyprogesterone (IVa) in 50 ml. methanol was aseptically added to the fermentor and the run continued for a total fermentation time of 40 hr. The pH rose from 7.0 to a final pH of 7.95. The broth was exhaustively extracted with chloroform and then the organic phase was evaporated to near dryness and chromatographed over Florisil. Collection of the 25/75 ether-

hexane to 50/50 ether-hexane fractions gave the 1-dehydrosubstance VI (0.22 g.), m.p. 218–220°. The analytical sample was obtained from acetone, m.p. 222–223°; $[\alpha]_D$ − 89.8°; λ_{max} 242.5 mμ (ε 16,500); λ_{max} 5.79, 6.04, 6.17, 6.25, 8.08, 9.87, 11.06, 11.35 μ; $\lambda_{max}^{CHCl_3}$ 5.76, 5.84 μ.

Anal. Calcd. for $C_{24}H_{30}O_4$: C, 75.36; H, 7.91. Found: C, 75.57; H, 7.70.

16-Methylene-17α-hydroxy-4-pregnene-3,20-dione (16-Methylene-17α-hydroxyprogesterone, III). A. From II.—To a solution of 6.93 g. of II in 170 ml. of acetic acid maintained at 37° was added 1.7 ml. of a 4 *N* hydrogen bromide in acetic acid solution. The temperature was maintained at 37–41° for 20 min., and then the bluish-green solution was added to 2 l. of water. The resultant precipitate was separated by filtration and crystallized from acetone to give III (4.02 g.), m.p. 220–225°. The analytical sample was crystallized from acetone, m.p. 219–220; $[\alpha]_D$ + 17°; $[\alpha]_D$ − 6.6 ($CHCl_3$), $[\alpha]_D$ + 22.9° (ethanol); λ_{max} 240 mμ (ε 16,600); λ_{max} 2.98, 5.85, 6.02, 6.22, 9.42, 10.84, 11.14, 11.22, 11.52 μ. Reported[2a] m.p. was 208–210°; $[\alpha]_D^{22}$ − 9° ($CHCl_3$).

From the mother liquors was isolated a substance exhibiting spectroscopic maximum at 309 mμ. See experiment describing preparation of IVa.

B. From IVa.—To a refluxing solution of IVa (50 mg.) in 1 ml. of 90% aqueous methanol was added 30 mg. of potassium hydroxide contained in 0.1 ml. of 90% aqueous methanol. After 20 min. at reflux the alkaline reaction solution was cooled and neutralized with 50% glacial acetic acid. Air evaporation afforded a solid residue which was treated with water. The water insoluble material, 34 mg., was the same by infrared comparison as material obtained by procedure A. A sample crystallized from acetone had m.p. 220–225°, $[\alpha]_D$ + 21.3°, λ_{max} 239 mμ (ε 16,750).

Reaction of 16-Methylene-17α-hydroxy-4-pregnene-3,20-dione (III) with Acids.—A. A solution of 100 mg. of III (from IVa) in 9.6 ml. of acetic acid and 2 ml. of trifluoroacetic acid, under an atmosphere of argon, was warmed at 90–93° for 1 hr. under anhydrous conditions. Dilution with ice-water, and collection of insolubles gave 74 mg. Infrared: matches starting material; λ_{max} 240 mμ (ε 16,100) no absorption at 308 mμ indicative of the presence of V.

B. To a solution of 150 mg. of III (from IVa) in 2.46 ml. of acetic acid at 35° was added 0.0246 ml. of a solution containing 10% hydrobromic acid in acetic acid. After 20 min. at 37–41°, the solution was diluted with 25 ml. of water; the insolubles were collected by filtration to give after drying a crude weight of 150 mg. Infrared consistent with starting material, no absorption at 308 mμ, indicative of the presence of V.

C. A solution of 18 mg. of III (from II) in 0.36 ml. trifluoroacetic acid and 1.73 ml. of acetic acid, under argon, was warmed at 90–95° for 1 hr. Dilution with 25 ml. of water gave after collecting the resulting precipitate, 10 mg. Absorption in ultraviolet at 308 mμ, in addition to 240 mμ, was indicative of the generation of a small amount of V.

D. To a solution of 18 mg. of III (from II) in 0.443 ml. of acetic acid at 35° was added 0.01 ml. of a solution containing 10% HBr in acetic acid. After 30 min. at 37–41° the reaction solution was diluted with 3 ml. of water. The resultant precipitate was collected and dried at 60° to yield 11 mg. Absorption at 308 mμ indicated formation of a small amount of V.

16,16-Dimethyl-3β-acetoxy-5-pregnen-20-one (IXb).—A Grignard reagent was prepared in ethyl ether with 28.8 g. of magnesium and 171 g. of methyl iodide, and to this reagent was added 2.38 g. of cuprous chloride. A solution of

37.3 g. of 16-methyl-16-dehydropregnenolone acetate (VIII) in 750 ml. of purified tetrahydrofuran was added to the cold Grignard reagent over a period of 10 min., and then stirred for an additional 15 min. at 25°. The reaction mixture was cooled to 0° and 1 l. of 10% aqueous ammonium chloride solution added very cautiously. The layers were separated, the water layer extracted with ethyl ether, and the combined organic layers were then washed until neutral. The solution was dried with magnesium sulfate, filtered, and evaporated to an oily residue. This residue was acetylated in pyridine–acetic anhydride at 80° for 1.5 hr. and then the excess anhydride destroyed with water. The solution was poured into a mixture of hydrochloric acid in water and filtered. The crude product weighed 45 g. and had a barely noticeable ultraviolet absorption. It was taken up in 3.9 l. of ethyl ether and some insoluble material was removed by filtration. The filtrate was concentrated to about 320 ml., cooled in the ice-box for 3 hr., and filtered to yield 20.70 g., m.p. 176–177°. This product then was crystallized from methyl alcohol to yield 18.30 g. (47.3%), m.p. 179.4–180°; $[\alpha]_D$ − 50.8°. An analytical sample had m.p. 180.6–181.6°; $[\alpha]_D$ − 49.0°; λ_{max} 5.80, 5.86, 5.98, and 8.05 μ.

Anal. Calcd. for $C_{25}H_{38}O_3$: C, 77.67; H, 9.91. Found: C, 77.99; H, 9.70.

16,16-Dimethyl-3β-hydroxy-5-pregnen-20-one (IXa).—A solution of 15.46 g. of IXb in 155 ml. of chloroform and 310 ml. of methyl alcohol was saturated with nitrogen, and a solution of 1.68 g. of sodium hydroxide in 32 ml. of water was added over a period of 15 min. The reaction solution was stirred at 25° for an additional 3 hr., and then neutralized with glacial acetic acid. The organic solvents were removed by steam distillation, the suspension cooled to 20° and filtered to yield 13.78 g., m.p. 184–185°. The product was recrystallized from methyl alcohol to yield 10.76 g. (78%), m.p. 184.2–185.4°; $[\alpha]_D$ − 49.1°; λ_{max} 2.92, 5.90, and 9.35 μ.

Anal. Calcd. for $C_{23}H_{36}O_2$: C, 80.18; H, 10.53. Found: C, 80.46; H, 10.50.

16,16-Dimethyl-4-pregnene-3,20-dione (X).—A solution of 3.0 g. of IXa was submitted to a microbiological oxidation using *Flavobacterium dehydrogenans* by the procedure outlined for the conversion of Ib to II. The crude product was chromatographed on 56 g. of activated Florisil, eluting with ethyl ether–hexane mixtures, to yield a total of 2.25 g. of product. A crystallization from ethyl ether gave 1.59 g. (53%), m.p. 167.8–169.0°; $[\alpha]_D$ + 87.8; $[\alpha]_D$ + 104.4 ($CHCl_3$); λ_{max} 241 mμ (ϵ 16,800); λ_{max} 5.86, 5.94, 6.16, and 11.56 μ.

Anal. Calcd. for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.71; H, 10.06.

16β-Methyl-5α,6β-dichloro-3β-hydroxyallopregnan-20-one 3-Acetate (XIIa).—A solution consisting of 31 g. of 16β-methyl-3β-hydroxy-5-pregnen-20-one 3-acetate, 745 ml. of carbon tetrachloride, and 28 ml. of pyridine was cooled to −23°. To this solution was added dropwise over a 10 min. period 7.0 g. chlorine in 135 ml. carbon tetrachloride. The stirred reaction solution was then allowed to warm to approximately 15°. Methylene chloride (about 350 ml.) then was added and the solution was washed successively with dil. hydrochloric acid, water, aqueous sodium bicarbonate and then water. The dried organic phase was evaporated under vacuum to near dryness. Addition of methanol gave the 5,6-dichloride XIIa (19.6 g.), m.p. 164–168°. The analytical sample was crystallized from methylene chloride–methanol, m.p. 169–170°; $[\alpha]_D$ − 42.2°; λ_{max} 5.76, 5.88, 8.05, 9.71, 15.20 μ.

Anal. Calcd. for $C_{24}H_{36}Cl_2O_3$: C, 65.00; H, 8.18; Cl, 15.99. Found: C, 65.20; H, 8.13; Cl, 15.71.

16β-Methyl-5α,6β-dichloro-3β,17α-dihydroxyallopregnan-20-one (XIIIa). A solution consisting of 5 g. of 16β-methyl-5α,6β-dichloro-3β-hydroxypregnan-20-one 3-acetate (XIIa), 1 g. of *p*-toluenesulfonic acid monohydrate, and 125 ml. of acetic anhydride was refluxed for 6 hr. while maintaining a constant distillation rate so that 100 ml. of distillate was collected. The residual solvent was removed under vacuum at approximately 70–90°. A thick brown syrup was obtained which was cooled to room temperature and added with stirring to 200 ml. of ice water. The resulting mixture was extracted with benzene. The benzene solution was washed with water and then with a 2% sodium acetate solution. The volume of organic phase, after drying over magnesium sulfate, was adjusted by evaporation under vacuum to approximately 80 ml. and then stirred at room temperature for 42 hr. with 20 ml. of commercial 40% peracetic acid which contained 0.56 g. sodium acetate. Excess peracetic acid was destroyed by the dropwise addition of a solution of sodium sulfite, while maintaining the temperature between 10-20°. The benzene layer was separated, washed three times with water and evaporated to an amorphous residue. Methanol (226 ml.), water (22.6 ml.), and potassium bicarbonate (6.9 g.) were added and the reaction solution refluxed for 3 hr. After isolation of the crude product by precipitation with water, crystallization from ethyl acetate gave 1.1 g. of the 17α-hydroxy XIIIa, m.p. 216–218; $[\alpha]_D$ − 19.7°; λ_{max} 2.95, 3.02, 5.92, 15.3 μ.

Anal. Calcd. for $C_{22}H_{34}Cl_2O_3$: C, 63.30; H, 8.21; Cl, 16.98. Found: C, 63.61; H, 8.09; Cl, 16.32.

16β-Methyl-5α,6β-dichloro-3β,17α-dihydroxyallopregnan-20-one 3-Acetate (XIX).—Esterification of 0.5 g. of XIIIa was accomplished at room temperature with an acetic anhydride in pyridine medium to give after crystallization from ether 0.360 g. of XIX, m.p. 175–178° dec.; $[\alpha]_D$ − 25.9°; λ_{max} 2.88, 5.78, 5.86, 7.98, 9.40, 9.68 μ.

Anal. Calcd. for $C_{24}H_{36}Cl_2O_4$: C, 62.73; H, 7.90; Cl, 15.43. Found: C, 62.95; H, 7.69; Cl, 15.28.

16β-Methyl-3β,17α-dihydroxy-5-pregnen-20-one (XIVa).—16β-Methyl-5α,6β-dichloro-3β,17α-dihydroxypregnane-20-one (XIIIa) (2 g.) was added to a stirred refluxing mixture of 536 ml. of ethanol, 99.5 ml. water and 20 g. zinc dust. After 1 hr. the insolubles were removed by filtration. The filtrate was evaporated to near dryness and triturated with water. Decantation of the supernatant and crystallization of the insolubles from acetone gave the crude substance XIVa, 1.05 g., m.p. 220–224°. An analytical sample was obtained from acetone, m.p. 224–226.5°; $[\alpha]_D$ − 12.2° (1% dimethylformamide); λ_{max} 3.00, 5.92, 9.30, 9.50 μ; reported[15] m.p. 230–232°; $[\alpha]_D$ −33° ± 2° (dioxane).

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 75.99; H, 9.96.

Zinc and aqueous ethanol treatment of XIX gave XIVb, the 3-acetate of XIVa, m.p. 168–170°, $[\alpha]_D$ − 17.6°.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.03; H, 9.46.

Saponification of XIVb with dilute aqueous sodium hydroxide gave XIVa.

16β-Methyl-17α-hydroxy-4-pregnene-3,20-dione (16β-Methyl-17α-hydroxyprogesterone, XVIa).—To a stirred solution of 16β-methyl-3β,17α-dihydroxy-5-pregnen-20-one (XIVa) (4.35 g.), dissolved in 3800 ml. acetone and maintained at 5–10°, was added 3.8 ml. of a chromic acid solution (26.72 g. CrO_3, 23 ml. concd. H_2SO_4 diluted with water to 100 ml.). The reaction was held at 5-10° for 1 hr. After the addition of methanol, then water, the mixture was air evaporated to an amorphous residue. Trituration with water gave 4.4 g. of insoluble material

which was dissolved in 135 ml. of 0.27 *N* methanolic perchloric acid (0.5 ml. of 70% $HClO_4$ in 19.5 ml. of CH_3OH) and allowed to remain at room temperature for 19 hr. Dilution with 1500 ml. ice water afforded a precipitate which was filtered, washed with 5% sodium acetate solution, then water. The crude product exhibited significant spectroscopic absorption at 305 mμ, in addition to an absorption at 241 mμ. Chromatography over Florisil and elution with 25% ether-75% hexane and crystallization of the combined portions from ethyl acetate gave 0.21 g. of 16β-methyl-17α-hydroxyprogesterone (XVIa), m.p. 194–195.5°; $[\alpha]D$ + 108.9°; λ_{max} 241 mμ. (ϵ 15,000); λ_{max} 2.88, 3.00, 5.88, 5.98, 6.18 μ.

Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.92, 76.84; H, 9.46, 9.61.

Additional XVIa was present in the mother liquors which were considerably contaminated by another substance which in an impure state exhibited spectroscopic absorptions at 246 and 305 mμ; and at 6.10, 6.13, 6.32 μ.[19]

16β-Methyl-17α-acetoxy-4-pregnen-3-one (XVIIa).—16β-Methyl-17α-hydroxyprogesterone (0.15 g.) was treated with 1.5 ml. of glacial acetic acid and 0.3 ml. of trifluoroacetic anhydride as in the preparation of IVa. Crystallization from ether gave 38 mg. of XVIIa, m.p. 188–195°. See footnote (20) for n.m.r. evaluation. The analytical sample (crystallized from ether) had m.p. 195–198°; $[\alpha]D$ + 90.1°; λ_{max} 240 mμ (ϵ 16,350); λ_{max} 5.78, 5.82, 6.04, 6.22, 8.05 μ; reported[15] m.p. 205–206°; λ_{max} 242 mμ (ϵ 14,500); $[\alpha]D$ +65° ± 2° (dioxane).

Anal. Calcd. for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.80; H, 8.89.

16α-Methyl-5α,6β-dichloro-17α-hydroxyallopregnane-3,20-dione (XVIII).—To a stirred suspension of 16α-methyl-5α,6β-dichloro-3β,17α-dihydroxyallopregnan-20-one[21a] (XIIIb) (m.p. 214–216°) (1.25 g.) and 13.8 ml. of 80% aqueous acetic acid was added a solution of chromium trioxide (0.396 g. in 1.25 ml. water). After stirring at room temperature for 17 hr., sodium bisulfite was added, then water, and the resulting precipitate of dichloride (XVIII) was collected by filtration and dried at 60°; m.p. 165–168° dec.; yield, 1.15 g. Crystallization from ether did not change the m.p.; λ_{max} no absorption; $\lambda_{max.}^{MeOH(KOH)}$ 242 mμ (ϵ 11,000). An acceptable analysis for this compound was not obtained.

16α-Methyl-17α-hydroxy-4-pregnene-3,20-dione (16α-Methyl-17α-hydroxyprogesterone, XVIb).—To a stirred refluxing mixture of 1210 ml. of ethanol, 224 ml. of water and 45 g. of zinc dust was added 4.5 g. of crude 16α-methyl-5α,6β-dichloro-17α-hydroxypregnane-3,20-dione (XVIII). After 1 hr. at reflux the reaction mixture was cooled, the supernatant was separated by filtration and evaporated to near dryness. Addition of water gave a precipitate which was separated by filtration and dried at 60°. It weighed 3.91 g.; negative Beilstein, λ_{max} 236 mμ (ϵ 2,520; calcd. on basis of XVIb).

The impure 3-keto-Δ^5 intermediate (XVb) was dissolved with stirring at room temperature in 105 ml. of 0.27 *N* methanolic perchloric acid and after 23.5 hr. at room temperature the reaction solution was diluted with 10 vol. of water. The resulting precipitate was separated by filtration, washed with a dilute solution of sodium acetate, than water and dried at 60°; wt., 2.73 g. Chromatography over Florisil gave 1.5 g. from 50% ether in hexane. Crystallization from ether gave 660 mg., m.p. 177–179°. An analytical sample, crystallized again from ether, had m.p. 182–185°; $[\alpha]D$ + 81.8°; $[\alpha]D$ + 68.1° ($CHCl_3$); λ_{max} 241 mμ (ϵ 16,280); λ_{max} 2.94, 5.88, 6.02, 6.20 μ. Reported by Heusler, *et al.*[23] m.p. 217,220°; $[\alpha]^{27.5}$ 37.3 ± 2° ($CHCl_3$); λ_{max}^{EtOH} 242 mμ (ϵ 16,600); by Bernstein, *et al.*[21b] 180-183.5°; $[\alpha]D$ + 86° ($CHCl_3$); λ_{max}^{MeOH} 242 mμ (ϵ 17,200).

In a ligroin–propylene glycol descending papergram, overnight development, the R_f for 16α-methyl-17α-hydroxyprogesterone (XVIb) is slightly greater than that of 16β-methyl-17α-hydroxyprogesterone (XVIa).

Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.55; H, 9.32.

Also isolated from this column was a substance identified as 16α-methyl-3β,17α-dihydroxy-5-pregnen-20-one,[21a] which was obtained by incomplete oxidation of XIIIb followed by zinc and ethanol treatment.

16α-Methyl-17α-hydroxy-4-pregnene-3,20-dione 17-Acetate (16α-Methyl-17α-acetoxyprogesterone, XVIIb).—To 0.3 g. of 16α-methyl-17α-hydroxyprogesterone (XVIb) contained in 3 ml. of acetic acid under nitrogen was added 0.6 ml. of trifluoroacetic anhydride. The reation solution was warmed at 90–95° for 1 hr., then diluted with 50 ml. water. The aqueous mixture was extracted with methylene chloride which in turn was washed with 5% aqueous sodium carbonate, then with water. Evaporation of the organic phase to a solid residue afforded, after trituration with hexane, 165 mg. of product, m.p. 213–220°. An analytical sample, crystallized from ether, had m.p. 229–232°, $[\alpha]_D$ + 70.7°; λ_{max} 239 mμ (ϵ 17,050); 5.78, 5.84, 6.04, 6.20, 8.02 μ. Reported by Batres, *et al.*,[21a] m.p. 239–240°, $[\alpha]_D$ + 82° ($CHCl_3$), λ_{max} 240 mμ log ϵ 4.24; and by Bernstein, *et al.*,[21b] m.p. 233–236°; $[\alpha]$ + 80° ($CHCl_3$); λ_{max}^{MeOH} 242 (ϵ 15,200).

Anal. Calcd. for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.76; H, 8.85.

In a ligroin–propylene glycol descending papergram the R_f of the 16α-methyl-17α-acetoxyprogesterone (XVIIb) is slightly less than that of the 16β-methyl-17α-acetoxyprogesterone (XVIIa).

Cardiac Activity of Newer Digitalis Glycosides and Aglycones

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Thirty-one cardiac glycosides or aglycones, isolated from the leaves of *Digitalis lanata* or from the leaves and seeds of *D. purpurea*, or partially synthesized, have been pharmacologically assayed in etherized cats. Certain comparisons were made for structure-activity relationship. Esterification at C_{16} may give rise to aglycones, such as gitaloxigenin and 16-acetylgitoxigenin (oleandrigenin), that have a higher potency than their glycosides. Digipurpurin, a member of the C_{21}-steroids, has no digitalis-like action.

Chemical work during recent years on the leaves and seeds of *Digitalis purpurea* and on the leaves of *D. lanata* has resulted in the isolation of newer glycosides and aglycones and in the partial synthesis of heretofore unknown derivatives. It has been our privilege to evaluate the pharmacological activity of the newer substances