Hieber.[12] Alumina used for chromatography was Merck acid washed aluminum oxide.

Acetylergosterol Iron Tricarbonyl (II).—Acetylergosterol (0.9 g., 2.0 mmoles) and triiron dodecacarbonyl (1.1 g., 2.1 mmoles) were heated at reflux under nitrogen in 10 ml. of benzene for 18 hr. The black precipitates[13] formed in the reaction were removed by filtration and the deep brown filtrate was evaporated *in vacuo* to give a yellowish brown solid. Separation of products was carried out by alumina chromatography. From one fraction, III (0.02 g.) was isolated. The other fraction, a yellow solid, was further purified by digestion with methanol. The methanol-insoluble portion was found to be the starting material. The yellow methanol-soluble portion crystallized on concentration and standing. This material was recrystallized from methanol to give pure II (0.2 g.), m.p. 95–99° (sintering at 90°).

Anal. Calcd. for $C_{33}H_{46}FeO_5$: C, 68.50; H, 8.02; Fe, 9.65. Found: C, 69.00; H, 8.08; Fe, 10.97.

Reaction of Ergosterol with Iron Carbonyls.—Ergosterol (1.0 g., 2.2 mmoles) was treated with triiron dodecacarbonyl (0.3 g., 0.8 mmole) in 20 ml. of benzene under nitrogen by heating at 80–90° for 18 hr. The color changed to deep brownish red. Evaporation of the reaction mixture gave a mixture of colorless and brown solids. These solids were soluble in benzene. Chromatographic separation of the solids caused decomposition of the brown compound on the column and only ergosterol was recovered as colorless crystals. An attempt was made to recrystallize the brown solid from benzene–methanol solution by evaporation *in vacuo*. However, crystalline products were not obtained and the brown solid (m.p. 230°, dec. at 200–220°) obtained was found to contain iron on burning and on dissolution in dilute hydrochloric acid. A similar reaction using iron pentacarbonyl and ergosterol with irradiation by an ultraviolet lamp under nitrogen gave a brown solid which had properties similar to that obtained by the thermal reaction. These brown solids could not be purified to a well defined compound. The infrared spectrum of the solids revealed the presence of a very small amount of π-complex as evidenced by absorption near 2000 cm.$^{-1}$.

Irradiation of Acetylergosterol Iron Tricarbonyl (II).—A solution of 0.1 g. of II in benzene was irradiated by a 100-w. ultraviolet lamp for 18 hr. under nitrogen. The color changed to brown and a brown semisolid was obtained on evaporation of the solvent. The infrared spectrum showed essentially no change occurred during the irradiation.

Acknowledgment.—We gratefully acknowledge financial support from the National Institutes of Health, United States Public Health Service, Grant RG-8921.

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(13) The precipitates ignited spontaneously in air and gave ferric oxide.

Synthesis of 16-Chlorinated Pregnenes[1]

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Substitution at the 16-position of steroids often results in significant enhancement of biological activities. Among the groups introduced at position 16 which produce favorable effects are the methyl,[2] hydroxy,[3] fluoro,[4] 16β-chloro,[5] and methoxy[6] groups. Most of the known 16-substituted steroids are in the glucocorticoid class. It is, therefore, of considerable interest to investigate the effect of 16-substitution on the progestational activity of known precursors.

Recently, Hoffman, *et al.*,[7] reported that 16-substituted progesterone derivatives such as 16α-methylthioprogesterone and 16α-methylsulfinylprogesterone appear to be less active than progesterone on subcutaneous assay by the Clauberg–McPhail procedure.[8] We have also found that 16α-chloro-6α-methylprogesterone (IIb) is approximately equivalent to progesterone at a total dose level of 1 mg. per rabbit.[9] At the same levels, 16α-chloroprogesterone (IIa) and 16α-chloro-6-methyl-6-dehydroprogesterone (III) had about one-sixth the activity of progesterone as shown in Table I.

TABLE I

Compound	Total dose, mg./rabbit	Response
16α-Chloroprogesterone (IIa)	1.0	+0.5
	10.0	+3.0
16α-Chloro-6α-methylprogesterone (IIb)	1.0	+2.8
	10.0	+4.0
16α-Chloro-6-methyl-6-dehydroprogesterone (III)	1.0	+0.5
	10.0	+3.8
Progesterone	0.8	+2.0
	1.0	+3.0
	1.5	+4.0

The addition of hydrogen chloride (Chart I) to 16-dehydroprogesterone (Ia) and 6α-methyl-16-dehydroprogesterone (Ib) furnished the corresponding 16α-chloro derivatives. The α-configuration of 16-chlorine has been assigned on the grounds previously reviewed by Gould and co-workers[10]; namely by the negative rotatory contribution.[7]

6-Methyl-16α,17α-epoxypregnenolone acetate (IV) was converted to the chlorohydrin V by opening the epoxide with hydrochloric acid.[11] Saponification of V to 6-methyl-16β-chloro-5-pregnene-3β,17α-diol-20-one (VI) and subsequent oxidation gave rise to 6α-methyl-16β-chloro-4-pregnen-17α-ol-3,20-dione (VII).

The mass spectroscopy studies[12] carried out in these Laboratories on compounds Ia, Ib, IIa, IIb, IV, V, VI, and VII support the specific structures herein pre-

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(9) Subcutaneous (diluent sesame oil) Clauberg–McPhail assay, carried out by Endocrine Laboratories, Madison, Wisconsin.

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(12) The author is grateful to Dr. L. E. Peterson and Mr. L. G. Hickson for the measurement and interpretation of mass spectra. See also L. E. Peterson, *Anal. Chem.*, **34**, 1781 (1962).

sented. 16α-Chloroprogesterone, for example, showed a distinct parent peak, $M - HCl$, $M - 42$ which is characteristic of C ring cleavage, $M - [HCl + CH_3]$, $M - [HCl + CH_3CO]$, and $M - [HCl + CH_3CO + 42]$. The 6α-methyl-16α-chloroprogesterone, on the other hand, showed the peaks due to the loss of CH_3 (weak), HCl (strong), 42 from A ring, $[HCl + CH_3]$ (316), $[HCl + CH_3 + H_2O]$ (weak), $[HCl + CH_3CO]$ (strong). The B ring cleavage of progesterone gives rise to a 138 peak in this 6-methylated compound. Further details of the mass spectroscopic studies will be published elsewhere.

Experimental[13]

16α-Chloroprogesterone (IIa).[14]—16-Dehydroprogesterone (Ia) (3 g.) was dissolved in 200 ml. of chloroform (containing 0.75% ethanol), 0.4 ml. of absolute ethanol was added, the mixture cooled to 0°, and anhydrous hydrogen chloride gas was bubbled in for 2 hr. The reaction mixture was then washed with saturated sodium bicarbonate and then sodium chloride solution to neutrality. The chloroform layer was separated, dried, and concentrated to give IIa (3.343 g.), m.p. 198.5–200.5°. Recrystallization from hexane–acetone gave 3.215 g. (96%) of crystals, m.p. 200.5–201.5° dec., $[\alpha]_D$ +142.9° (*c* 1, $CHCl_3$), $\lambda_{max}^{alcohol}$ 240 mμ, (ε 16,800), ν^{CS_2} 746, 707 cm.$^{-1}$. Mass spectrum agreed with the assigned structure.

Anal. Calcd. for $C_{21}H_{29}ClO_2$: C, 72.29; H, 8.38; Cl, 10.16. Found: C, 72.27; H, 8.44; Cl, 10.13.

6α-Methyl-16α-chloroprogesterone (IIb).—6α-Methyl-16-dehydroprogesterone (Ib) (500 mg.) was dissolved in 100 ml. of chloroform. Absolute ethanol (0.2 ml.) was added, the mixture was cooled to 0°, and then anhydrous hydrogen chloride was bubbled in for 1 hr. After washing with saturated sodium bicarbonate solution and sodium chloride solution to neutrality, the chloroform layer was dried over magnesium sulfate and concentrated to give 549 mg. (99%) of crystals, m.p. 158–161° dec. Two recrystallizations from hexane–acetone gave 407 mg. (73%) of crystals, m.p. 162–163° dec.; λ^{CS_2}; 13.37, 14.13, 5.82, 5.94, 6.19 μ; $\lambda_{max}^{alcohol}$ 240 mμ (ε 16,000); $[\alpha]_D$ +131° (*c* 1, $CHCl_3$). The mass spectrum agreed with the assigned structure.

Anal. Calcd. for $C_{22}H_{31}ClO_2$: C, 72.80; H, 8.61; Cl, 9.77. Found: C, 72.64; H, 8.82; Cl, 9.94.

6-Methyl-6-dehydro-16α-chloroprogesterone (III).—A mixture of 6α-methyl-16α-chloroprogesterone (IIb) (363 mg.) in *t*-butyl alcohol, and 12 ml. of glacial acetic acid, and 500 mg. of chloranil was heated under reflux for 22 hr. under a nitrogen atmosphere. After work-up, the crude product, recrystallized from hexane–acetone, gave crude III (360 mg.), m.p. 134–142°, which was adsorbed on a 20-g. Florisil column. The elutions with 20% ether in methylene chloride gave 180 mg. of crystals, m.p. 148–151° dec., which was recrystallized from hexane–acetone to give 140 mg. of crystals, m.p. 149–151° dec., λ^{KBr} 5.88, 6.02, 6.18, 6.39, 13.48, 14.09 μ, $\lambda_{max}^{alcohol}$ 288 mμ (ε 21,800), $[\alpha]_D$ +114.6° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{22}H_{29}ClO_2$: C, 73.21; H, 8.10; Cl, 9.83. Found: C, 72.92; H, 8.31; Cl, 10.12.

6-Methyl-16β-chloro-5-pregnene-3β,17α-diol-20-one 3-Acetate (V).—6-Methyl-6α,17α-epoxypregnenolone acetate (IV) (670 mg.) in 100 ml. of chloroform was treated with anhydrous hydrogen chloride at room temperature for 3 hr.[11] The organic layer was washed with saturated sodium bicarbonate and sodium chloride solution to neutrality. During the extraction, an additional 100 ml. of chloroform was added, the chloroform phase dried and concentrated to give 650 mg. of crystals, m.p. 111–114°. Repeated recrystallization from hexane–acetone furnished pure V (302 mg.), m.p. 195–196° dec., $[\alpha]_D$ −29° (*c* 1, $CHCl_3$), λ^{KBr} 5.80, 5.84, 8.02, 13.3, and 14.1 μ.

Anal. Calcd. for $C_{24}H_{35}ClO_4$: C, 68.15; H, 8.34; Cl, 8.38. Found: C, 68.02; H, 8.30; Cl, 8.43.

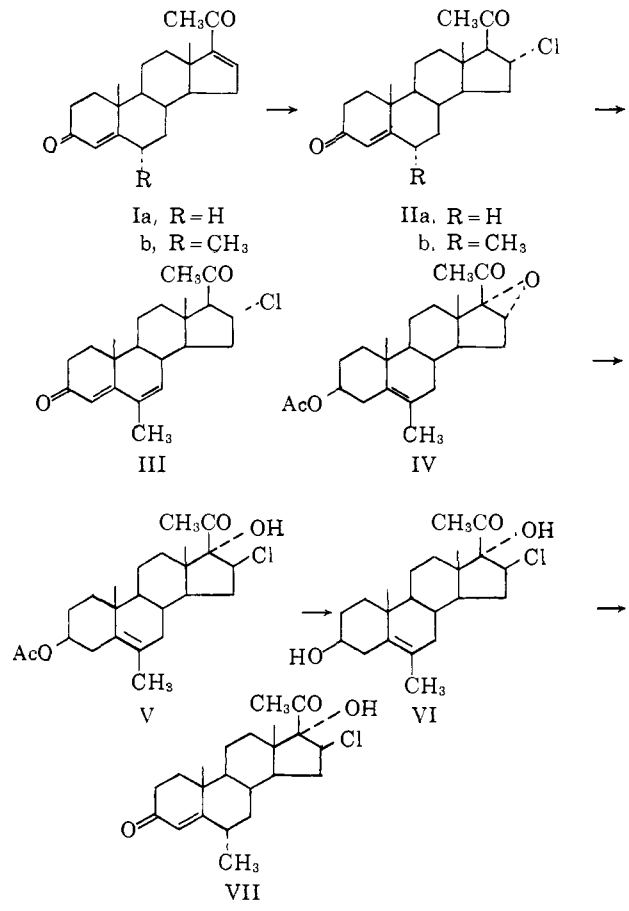
6-Methyl-16β-chloro-5-pregnene-3β,17α-diol-20-one (VI).—Compound V (600 mg.) was dissolved in 10 ml. of methanol containing 0.8 ml. of concentrated hydrochloric acid and the mixture was allowed to stand at room temperature overnight. After work-up in the usual manner, there was obtained the corresponding crude 3β,17α-diol (VI) (590 mg.), m.p. 105–107°. Repeated recrystallizations from aqueous methanol gave pure VI (410 mg.), m.p. 203–204° dec., $[\alpha]_D$ −12° (*c* 1, $CHCl_3$), λ^{KBr} 2.95, 5.84, and 13.62 μ.

Anal. Calcd. for $C_{22}H_{33}ClO_3$: C, 69.36; H, 8.73; Cl, 9.31. Found: C, 69.40; H, 8.77; Cl, 9.34.

6α-Methyl-16β-chloro-4-pregnen-17α-ol-3,20-dione (VII).—The diol VI (590 mg.) was dissolved in 120 ml. of acetone and cooled to 10°. Then 1 ml. of approximately 8 *N* chromium trioxide reagent[15] was added in one portion under nitrogen atmosphere with stirring. After 3 min. the reaction mixture was diluted with 2 l. of water, extracted with methylene chloride, and the extracts were washed with sodium bisulfite and sodium chloride solution, dried, and concentrated to dryness. The residue was redissolved in 20 ml. of methanol and heated to reflux, 0.2 ml. of 6 *N* sulfuric acid was added and the mixture refluxed for 3 min., diluted with water, and extracted with methylene chloride. The extracts were dried and concentrated to give 377 mg. of crystals, m.p. 97–103.5°, which were purified by chromatography on 20 g. of silica gel. The elutions with 10% ethyl acetate in benzene gave 286 mg. of crystals, m.p. 232–235°. Recrystallization from aqueous methanol furnished pure VII, m.p. 235–236° dec., $[\alpha]_D$ +54°, $\lambda_{max}^{alcohol}$ 241 mμ (ε 16,200), λ^{KBr} 2.95, 5.79, 5.84, 6.01, and 6.14 μ.

Anal. Calcd. for $C_{22}H_{31}ClO_3$: C, 69.73; H, 8.25; Cl, 9.36. Found: C, 69.69; H, 8.22; Cl, 9.38.

CHART I



(13) All melting points are capillary melting points and are corrected.

(14) (a) This compound has been prepared previously by a different method: R. M. Dodson and P. B. Sollman, U. S. Patent 2,708,201 (May 10, 1955); m.p. 197–199° dec., λ 240 mμ (ε 15,500); (b) A. S. Hoffman, H. M. Kissman, and M. J. Weiss,[7] reported m.p. 191–200° dec., $[\alpha]_D$ +150° ($CHCl_3$), λ^{MeOH} 240 mμ (ε 16,200).

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