Synthesis of 6-Chloro-16-methylene-17α-hydroxy-21-fluoro-4,6-pregnadiene-3,20-dione 17-Acetate, a Potent Progestational Agent

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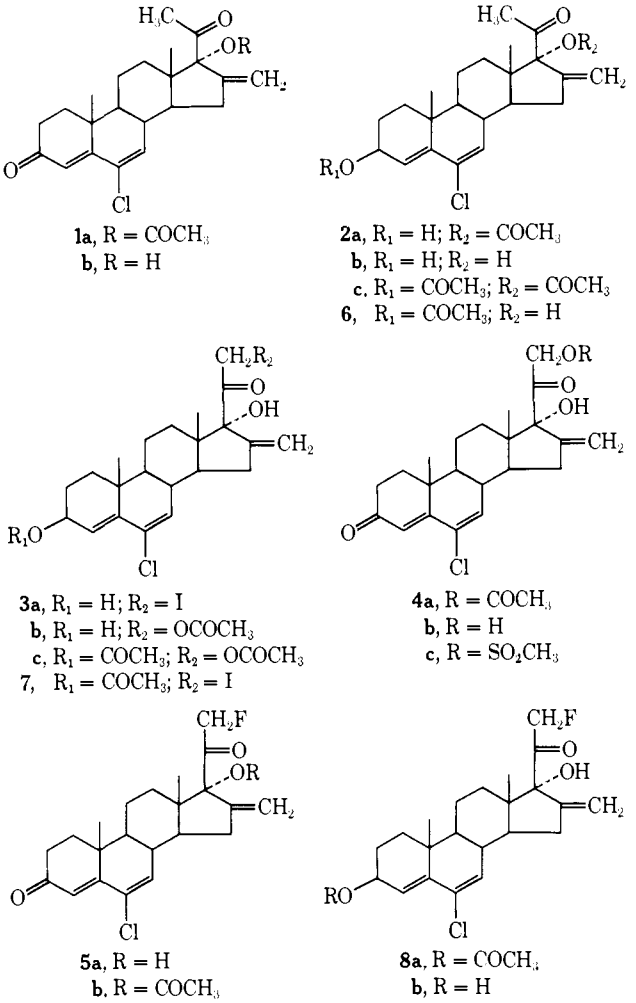
The synthesis of the title compound **5b**, a potent progestational agent, is reported. The intermediates **2a** and **2c** also possessed high progestational activities when tested intramuscularly or orally in the rabbit.

The progestational potentiating effect of the 21-fluoro group in 17α-acetoxyprogesterone derivatives has been described.[1] It was reported recently that 6-chloro-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-acetate (**1a**) has approximately twice the progestational activity of the corresponding 16-unsubstituted compound.[2] We now report the synthesis of 6-chloro-16-methylene-17α-hydroxy-21-fluoro-4,6-pregnadiene-3,20-dione 17-acetate (**5b**) in which both of the structural features known to increase progestational activity are combined in the same molecule.

A straightforward sequence for the preparation of the desired 21-fluoro compounds **5a** and **5b** was the utilization of the 21-bromo and/or-iodo derivatives of **1b** or **1a** for conversion into the corresponding 21-fluoro steroid by known methods. However, all efforts to brominate or iodinate **1b** or its 1-dehydro derivative[3] selectively at C_{21} failed, halogenation at C_2 always preceded halogenation at C_{21}.

A second approach to the desired **5b** involved protection of the 3-ketone by reduction, to avoid halogenation of ring A. This method seemed to be quite attractive since the mesylate **4c** would be an equally suitable precursor for the 21-chloro and 21-bromo analogs of **5b**, also of biological interest. Reduction of **1a** with $NaBH_4$ in cold CH_2Cl_2–MeOH gave in high yield the 3β-hydroxy **2a**, which was hydrolyzed with KOH in MeOH–H_2O affording the diol **2b**. Brief exposure of **2b** to I_2, CaO, and azobis-2-methylpropionitrile[4] gave the impure 21-iodo compound **3a**. Short reaction time in the 21-iodination step was essential to minimize the unwanted oxidation by I_2 of the 3β-hydroxy-$\Delta^{4,6}$-system. Even after brief exposure, however, some oxidation did occur, and the resulting $\Delta^{4,6}$-3-one rapidly underwent iodination at C_2 followed by elimination of HI in the basic medium, resulting in the formation of the undesired $\Delta^{1,4,6}$-3-one. The reaction mixture, without purification, was treated with Et_3N in refluxing AcOH in order to convert the 21-iodo **3a** into the 21-acetoxy **3b**. The crude reaction product was treated with activated MnO_2 in $CHCl_3$ to regenerate the 4,6-dienone system. Chromatography of the MnO_2 oxidation product over Florisil afforded crystalline **4a** in about 25% yield from **2b**.



Hydrolysis of **4a** with KOH in MeOH–H_2O resulted in the diol **4b**, which on treatment with MsCl in pyridine at 0° gave the chromatographically homogeneous mesylate **4c**. When **4c** was treated with anhydrous KF in DMSO at 110°,[5] none of the desired **5a** could be isolated from the reaction mixture, under these conditions only decomposition of **4c** occurred, while milder conditions resulted in the recovery of starting material.

In order to overcome the difficulties in the replacement of the mesylate with KF, the reaction sequence

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was somewhat modified. Acetylation of **2b** with Ac_2O in pyridine in order to minimize the oxidation of the 3β-hydroxy group during the iodination step gave the 3-monoacetate **6**. Brief treatment of **6** with 2 equiv of I_2 in the presence of CaO and azobis-2-methylpropionitrile gave a mixture of products of which the 21-iodo compounds **7** and **3a** predominated. This mixture, without purification was treated with 50% aq AgF in MeCN at reflux[6] and the resulting product, predominantly a mixture of **8a** and **8b**, was treated with KOH in MeOH-H_2O and after chromatography over neutral alumina the desired **8b** was obtained in an overall yield of 20–25% from **6**. Dehydrogenation of **8b** with activated MnO_2 in DMF afforded **5a** which was acetylated with AcOH, trifluoroacetic anhydride (TFAA) and *p*-TSA·H_2O[7] affording the desired **5b**.

Since 6-chloro-3β,17α-dihydroxy-4,6-pregnadien-20-one diacetate was reported to be a potent progestational agent,[8] the 16-methylene analog **2c** was prepared by acetylation of **2a** with Ac_2O in pyridine.

Biological Activity.—Table I lists the intramuscular (im) and oral progestational activities of **1a**, **2a**, **2c** and **5b**. As expected, introduction of the 21-fluoro substituent increased the im progestational activity of **1a** about 1.4-fold.

TABLE I

PROGESTATIONAL ACTIVITY[a]

Compd	Route of administration[b] Im	Oral
9[c]		2.8
1a	77	55
2a	69.8	13.7
2c	34.5	47
5b	105.4	32.5

[a] Progesterone (im) = 1. [b] Progestational activity was determined in immature rabbits by the method of M. K. McPhail, *J. Physiol. (London)*, **83**, 145 (1934). The compounds were dissolved in sesame oil for im administration or suspended in an aq medium (0.9% NaCl, 0.5% CM-cellulose, 0.4% polysorbate 80, and 0.9% $PhCH_2OH$) for oral administration. Progesterone in sesame oil was always given im. The statistical analysis for the progestational assays utilized the randomized Bloch analysis of variance with Dunnett's and Duncan's multiple comparison procedure (see G. Miller, Jr., "Simultaneous Statistical Interference," McGraw-Hill Book Co., Inc., New York, N. Y., 1967). [c] 17α-Ethinyl-19-nortestosterone.

Experimental Section[9]

6-Chloro-16-methylene-3β,17α-dihydroxy-4,6-pregnadien-20-one 17-Acetate (2a).—A soln of 6-chloro-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-acetate (**1a**) (8.6 g) in MeOH (40 ml) and CH_2Cl_2 (40 ml) was stirred with $NaBH_4$ (3.5 g) and H_2O (10 ml) at 0° under N_2 for 20 min. The solution was neutralized with dil AcOH and extracted with CH_2Cl_2. After drying it was evaporated to a residue. The residue was dissolved in 500 ml of MeOH and a 100-ml aliquot of this soln was concd to low vol when crystn occurred yielding 1.10 g of **2a**: mp 220–223°; $[\alpha]_D$ − 215°; λ_{max} 236 mμ (ε 20,900), 243 (24,200), 251 (16,300); δ 4.30 ($C_{3\alpha}$-H, m, 20 Hz wide) ppm. *Anal.* ($C_{24}H_{31}O_4Cl$) C, H, Cl.

6-Chloro-16-methylene-3β,17α-dihydroxy-4,6-pregnadien-20-one (2b).—A soln of **2a** (500 mg) in MeOH (30 ml) was stirred with 1 *N* KOH (5 ml) under N_2 for 20 hr. The soln was neutralized with AcOH and dild with H_2O and the ppt collected by filtration. Crystn from Me_2CO-*i*-Pr_2O gave 242 mg of **2b**: mp 163–165°; $[\alpha]_D$ − 130°; λ_{max} 235 mμ (ε 20,320), 241 (22,700), 250 (15,750). *Anal.* ($C_{22}H_{29}O_3Cl$) C, H, Cl.

6-Chloro-16-methylene-3β,17α-dihydroxy-4,6-pregnadien-20-one 3-Acetate (6).—A soln of **2b** (1.15 g) in C_5H_5N (4 ml) was allowed to stand with Ac_2O (4 ml) at 20° for 3 hr, followed then for 10 min on the steam bath. The reaction mixture was added to ice-H_2O, the ppt collected and dried. Crystn from MeOH–*i*-Pr_2O-C_6H_{14} yielded 779 mg of **6**: mp 162–163°; $[\alpha]_D$ − 136°; λ_{max} 235 mμ (ε 20,400), 240 (22,050), 249 (15,350). *Anal.* ($C_{24}H_{31}O_4Cl$) C, H, Cl.

6-Chloro-16-methylene-3β,17α-dihydroxy-4,6-pregnadien-20-one 3,17-Diacetate (2c).—A C_5H_5N soln (2 ml) of the crude product of **2a** (1 g) was allowed to stand with Ac_2O (3 ml) for 60 hr. The reaction mixture was added to ice-H_2O, the ppt collected and dried. Crystallization from Et_2O-C_6H_{14} gave 767 mg of **2c**: mp 194–196° dec; $[\alpha]_D$ − 215°; λ_{max} 236 mμ (ε 21,300), 243 (24,000), 251 (16,150). *Anal.* ($C_{26}H_{33}O_5Cl$) C, H, Cl.

6-Chloro-16-methylene-17α,21-dihydroxy-4,6-pregnadiene-3,-20-dione 21-Acetate (4a).—To a soln of **2b** (7.2 g, 19 mmoles) in THF (75 ml, passed through act. I. Alumina) and MeOH (35 ml) under N_2 was added freshly fused CaO (12 g), azobis-2-methylpropionitrile (750 mg) and I_2 (8.52 g, 33.5 mmoles). The reaction mixture was stirred for 7 min. The solids were removed by filtration, the filtrate added to H_2O containing $Na_2S_2O_3$. The ppt was collected, dried, and dissolved in Me_2CO (120 ml). A 100-ml part of this soln was heated at reflux with Et_3N (60 ml) and AcOH (40 ml) for 1 hr. The reaction mixture was cooled and added to ice-H_2O. The ppt was collected, dried, dissolved in $CHCl_3$ (500 ml), and stirred with activated MnO_2 (15 g) for 1.5 hr. The solids were removed by filtration and the filtrate concentrated *in vacuo* to a residue which was chromatographed over Florisil (27 × 4 cm). Elution with C_6H_6–CH_2Cl_2 (1:1) yielded, after crystn from Et_2O, 1.71 g of **4a**: mp 229–234° dec; $[\alpha]_D$ + 6°; λ_{max} 284 mμ (ε 20,500); nmr, δ 0.79 (C_{13}-CH_3), 1.14 (C_{10}-CH_3), 2.15 (C_{21}-OCOCH_3), 4.92 and 5.10 (C_{20}-CH_2O, J_{gem} = 18 Hz), 5.13 and 5.28 (C_{16}-=CH_2), 6.27 (C_7-H), and 6.30 (C_4-H) ppm. *Anal.* ($C_{24}H_{29}O_5Cl$) C, H, Cl.

6-Chloro-16-methylene-17α,21-dihydroxy-4,6-pregnadiene-3,-20-dione (4b).—A soln of **4a** (1 g) in MeOH (10 ml) was allowed to stand with 1 *N* aq KOH (3 ml) under N_2 at 20° for 2 hr. After neutralization with AcOH the soln was added to H_2O. The ppt was collected, dried, and chromatographed over silica gel (Baker, act. V, 56 × 2.5 cm). Elution with CH_2Cl_2–Et_2O (9:1) gave 373 mg of **4b** which was crystd from MeOH–Et_2O: mp 154–155°; $[\alpha]_D$ −9°; λ_{max} 283 mμ (ε 21,400). *Anal.* ($C_{22}H_{27}O_4Cl$) C, H, Cl.

Attempted Conversion of 4b into 5a.—A soln of **4b** (200 mg) in C_5H_5N (4 ml) and MsCl (0.3 ml) was allowed to stand at 0° for 2.5 hr. The soln was added to H_2O, the ppt collected and dried. The chromatographically homogeneous mesylate **4c** [ν_{max} 1738, 1665, 1355, 1170 cm^{-1}; nmr, δ 3.20 (C_{21}-OSO_2CH_3), 5.11 and 5.39 (C_{20}-CH_2O, J_{gem} = 18.5 Hz), 5.14 and 5.27 (C_{16}-=CH_2) ppm] was added to DMSO (15 ml) containing KF (500 mg, freshly fused) and kept at 110° for 16 hr. The dark soln was cooled, dild with H_2O, and extd successively with CH_2Cl_2 and EtOAc. The organic solns were combined, dried, and evaporated to a residue *in vacuo*. No **5a** could be detected, apparently complete decompn of **4c** occurred, as evidenced by tlc.

6-Chloro-16-methylene-3β,17α-dihydroxy-21-fluoro-4,6-pregnadien-20-one (8b).—To a soln of **6** (11.168 g, 26.7 mmoles) in THF (120 ml, passed through act. I. Alumina) and MeOH (60 ml) under N_2 was added freshly fused CaO (23 g), azobis-2-methylpropionitrile (700 mg) and I_2 (13.6 g, 53.4 mmoles). The reaction mixture was stirred for 10 min. The solids were removed by filtration, the filtrate added to H_2O containing $Na_2S_2O_3$ and extd with CH_2Cl_2. Evaporation of the solvent gave an oily residue which was dissolved in MeCN (250 ml) and heated at reflux with 50% aq AgF soln (18 ml) for 9 hr. The solids were removed by filtration, the filtrate concd to a residue *in vacuo* which was dissolved in Me_2CO (50 ml) and added to H_2O. The ppt was collected, washed, dissolved in MeOH (100 ml), and

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irred with 1 *N* aq KOH (40 ml) under N_2 for 75 min. After eutralization with AcOH the soln was added to H_2O. The ppt as collected, dried, and chromatographed over neutral alumina Voelm, act. V, 40 × 4 cm). Elution with CH_2Cl_2 gave, after ystn from Me_2CO–Et_2O, 1.30 g of **8b**: mp 178–180°; $[\alpha]_D$ 93°; λ_{max} 237 mμ (ε 18,650), 244 (21,800), 252 (14,900); nr, δ 0.84 (C_{13}-CH_3), 1.09 (C_{10}-CH_3), 5.14 and 5.31 (C_{16}-=CH_2), 13 and 5.35 (C_{20}-CH_2F, J_{HF} = 48 Hz), 5.83 (C_7-H), and 6.10 C_4-H) ppm. *Anal.* ($C_{22}H_{28}O_3ClF$) C, H, Cl; F: calcd, 4.81; und, 4.39.

The mother liquor and the later fractions were rechromatoaphed over neutral alumina (Woelm, act. V, 20 × 4 cm). lution with C_6H_6 gave an additional 793 mg of **8b**.

6-Chloro-16-methylene-17α-hydroxy-21-fluoro-4,6-pregnadiıe-3,20-dione (5a).—A soln of **8b** (1.3 g) in DMF (20 ml) was irred with activated MnO_2 (2.6 g) for 1.5 hr. The solids were moved by filtration, and the filtrate added to ice–H_2O. The ɔt was collected, dried, and crystd from CH_2Cl_2–*i*-Pr_2O afrding 764 mg of **5a**: mp 223–225° dec; $[\alpha]_D$ −8°; λ_{max} 285 mμ (ε 22,200). *Anal.* ($C_{22}H_{26}O_3ClF$) C, H, Cl; F: calcd, 4.83; found, 4.39.

6-Chloro-16-methylene-17α-hydroxy-21-fluoro-4,6-pregnadiene-3,20-dione 17-Acetate (5b).—Trifluoroacetic anhydride (4 ml) was added to a soln of **5a** (607 mg) and *p*-TSA·H_2O (60 mg) in AcOH (6 ml) dropwise at 10° in a period of 10 min under N_2. The reaction mixture was allowed to warm up to 20° and stirred 5 hr. The soln was added to H_2O, the ppt collected, washed, and dried. Crystn from CH_2Cl_2–*i*-Pr_2O gave 384 mg of **5b**: mp 234–238° dec; $[\alpha]_D$ −147°; λ_{max} 284 mμ (ε 22,500); ν_{max} 1747 (sh), 1670, 1607, 1245 cm^{-1}; nmr, δ 0.82 (C_{13}-CH_3), 1.15 (C_{10}-CH_3), 2.20 (C_{17}-$OCOCH_3$), 4.98 and 5.06 (C_{20}-CH_2F, J_{H_1F} = 47 Hz, J_{H_2F} = 47.5 Hz), 5.47 and 5.58 (C_{16}-=CH_2), 6.26 (C_7-H), and 6.30 (C_4-H) ppm. *Anal.* ($C_{24}H_{28}O_4ClF$) C, H, Cl, F.

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Glyoxalase Inhibitors. A Possible Approach to Anticancer Agents[1]

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Some glyoxalase I enzyme inhibitors were assayed for cytotoxic activity against L1210 leukemia and KB cells in tissue culture. Sublethal concentrations of glyoxalase inhibitor caused a 14- to 18-fold increase in methylglyoxal toxicity in L1210 cells. A probable mechanism for the cytotoxic activity of methylglyoxal is discussed.

In a preliminary account of our work we presented ıe proposal that selective inhibition of the enzyme, yoxalase I, may provide carcinostatic activity by preenting the metabolism of the cytotoxic ketoaldehyde, ethylglyoxal, in tumor cells.[3] The carcinostatic acvity of α-ketoaldehydes, including methylglyoxal, was st reported by French and Freedlander.[4] However, ıese agents are metabolized to the corresponding α-hyoxy acids by the glyoxalase system, thus obviating ıeir use as effective anticancer agents. It has been ell established that the cytotoxic methylglyoxal is the ıbstrate for the glyoxalase enzymes and is converted to ıe nontoxic lactic acid in the presence of a cofactor, utathione (GSH).[5] These facts, along with the obrvation by Stern[6] that the GSH concentration in cells rapidly increased just prior to cell division, suggests ıat the glyoxalase system may be involved in the regution of cell growth by maintaining a proper concentraon of methylglyoxal.[3] The high concentration of lac acid[7] and the deficiency of methylglyoxal[8] in cancer lls further suggests that such cells, having lost the abily to maintain a proper balance of methylglyoxal, continue to grow at an uncontrolled rate. Recent interest in the possible role of methylglyoxal in cancer chemotherapy[9] has been concentrated on the preparation of ketoaldehydes. Since the toxicity of ketoaldehydes alone does not demonstrate the involvement of the glyoxalase system, we would like to report some results of our approach to this problem.

The glyoxalase system is widely distributed in cells of all forms of life[10,11] and comprises two enzymes which catalyze the reaction:

$$CH_3COCHO + GSH \xrightarrow{\text{glyoxalase I}} CH_3CHOHCOSG \xrightarrow{\text{glyoxalase II}} CH_3CHOHCOOH + GSH$$

Since the inhibition of glyoxalase I may result in a buildup of methylglyoxal in cancer cells, our efforts have been concentrated on the preparation of inhibitors of this enzyme. Preliminary investigations demonstrated that S-alkyl derivatives of GSH cause potent competitive inhibition of glyoxalase I by taking advantage of a nonpolar region adjacent to the binding region of the enzyme.[3] A wide variety of S-alkyl and S-aryl glutathiones was prepared to investigate further the binding requirements of these inhibitors to the enzyme[12] and to provide for greater penetration of the cell membrane. The compounds were then investigated for cell kill of L1210 leukemia and KB cell cultures.[13] Since the S-alkyl derivatives seem to have difficulty in penetrating the cell membrane, only those S-aryl glutathiones

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