Preparation and Progestational Activity of 6-Trifluoromethyl-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate and Related Compounds

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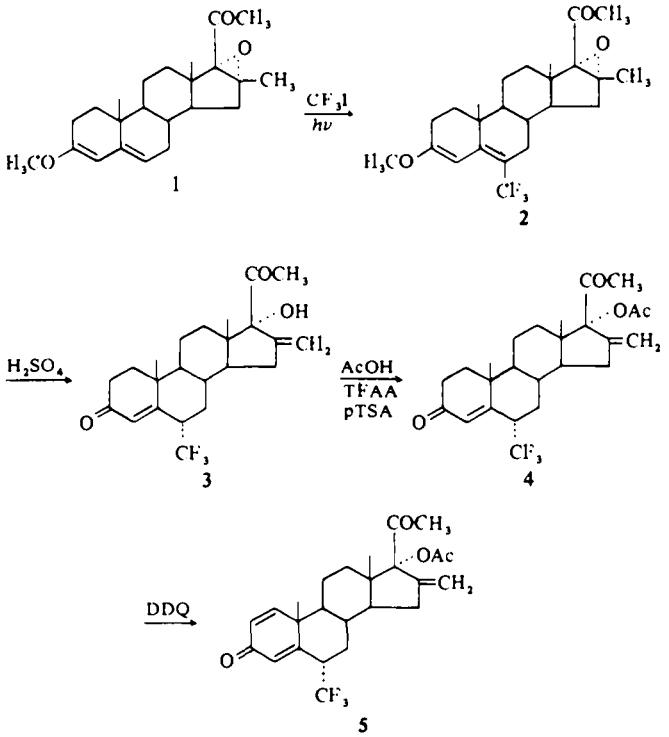
Received January 14, 1972

Several years ago, the synthesis of 6α-trifluoromethyl-17α-acetoxyprogesterone and its Δ^1 and Δ^6 analogs, had been reported and their progestational activities were comparable to those of the corresponding 6-methyl compounds.[1] Our continuing interest in 6-substituted-Δ^6-16-methylene-17-acetoxyprogesterones[2] prompted us to synthesize the corresponding 16-methylene analogs **4**, **5**, and **6**.

Our synthesis was based essentially on the published procedure,[1] in which an enol ether is irradiated with uv light in the presence of CF_3I and a tertiary base as proton acceptor. Since CF_3I reacts with a variety of double bonds,[3] a starting material was desirable which would allow introduction of the 16-methylene-17α-acetoxy substituents at a later stage. For this purpose the 16β-methyl-16,17α-oxido enol ether **1**[4] was a suitable substrate. The synthesis of 6α-trifluoromethyl-16-methylene-17α-acetoxyprogesterone (**4**) and its 1-dehydro analog **5** is described in Scheme I.

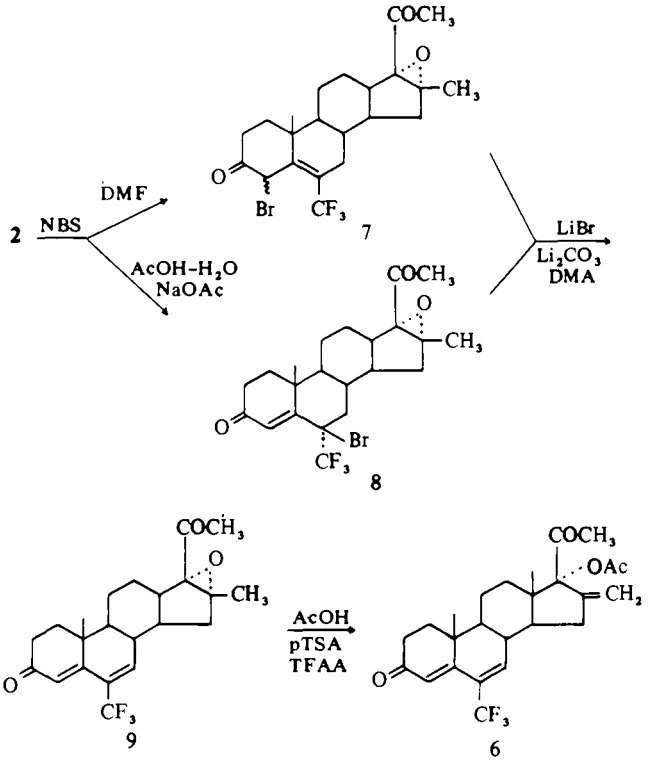
Scheme I



A convenient starting material for the synthesis of the desired 6-trifluoromethyl-Δ^6-16-methylene-17α-acetoxyprogesterone (**6**) seemed to be the product of photolysis, **2**, since an easy method for the dehydrogenation of 3-alkoxy-$\Delta^{3,5}$-steroids to 3-keto-$\Delta^{4,6}$-steroids with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) has been reported.[5] However, as it was observed with a 6-cyano enol ether in our earlier work[2a] the strongly electron-withdrawing trifluoromethyl group at C_6 prevented hydride abstraction at C_7. Thus, exposure of **2**, even to forcing conditions (large excess of DDQ in refluxing dioxane over 48 hr), failed to give any of the desired **6**.

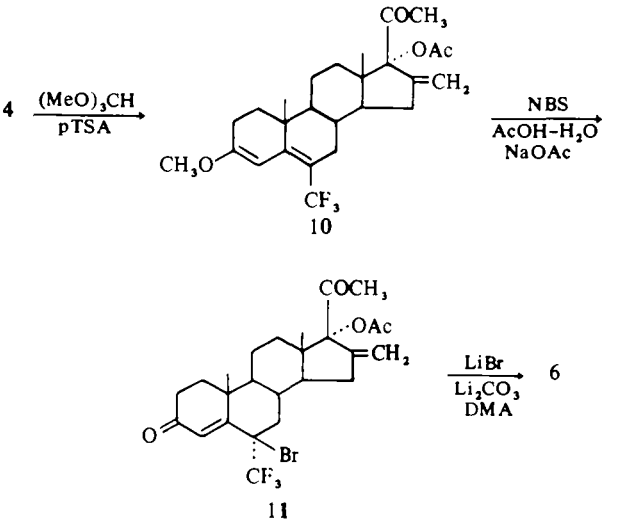
A more successful method for the preparation of **6** is described in Scheme II. Treatment of the enol ether **2** with

Scheme II



NBS[6] resulted in two different bromo compounds **7** and **8**, depending upon the buffer system used. Both the unstable **7** and the somewhat more stable **8** were separately dehydrobrominated with LiBr and Li_2CO_3 in DMA, affording the same dienone **9**. Although **9** was not obtained in crystalline state, it could be converted directly to the desired **6**. For larger scale preparations of **6**, it was decided to introduce the second double bond into **4** which already had the desired substituents in ring D (Scheme III). Treatment of **4**

Scheme III



with methyl orthoformate and pTSA in THF[4] gave the enol ether **10**. Bromination of **10** with NBS in buffered AcOH gave a rather unstable bromo compound (presumably the

Table I

Compound	Progestational activity[a] (Progesterone = 1.0) (im)
4[d]	15.7
5[d]	2.5
6[d]	11.0
12[b]	75
13[c]	91

[a]Progestational activity was determined in immature rabbits by the method of McPhail.[7] The compounds were dissolved in sesame oil for intramuscular administration. The statistical analysis for this assay utilized the randomized Bloch analysis of variance with Dunnett's and Duncan's multiple comparison procedure (see ref 8). [b]6α-Methyl-16-methylene-17α-hydroxy-4-pregnene-3,20-dione 17-acetate; *cf.* ref 9. [c]6-Methyl-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-acetate; *cf.* ref 10. [d]Did not show any antiandrogenic activity. Antiandrogenic activity was determined in immature male Charles River CD rats. The compounds were suspended in an aqueous suspending vehicle (0.4% polysorbate 80, 0.9% NaCl, 0.5% CM-cellulose, and 0.9% benzyl alcohol–ASV) and injected subcutaneously (sc) at 10 and 1 mg/kg daily for 3 weeks. The day following the last injection, the animals were sacrificed and the seminal vesicles, ventral prostates, and testes were removed, freed of extraneous tissue, and weighed and compared to control weights. Control animals received ASV daily for 3 weeks.

6β-bromo **11**) which on dehydrobromination with LiBr and Li_2CO_3 afforded the desired **6**.

Biological Activity. Table I lists the intramuscular (im) progestational activities of compounds **4**, **5**, and **6**. For comparison, the progestational activities of the corresponding 6-methyl compounds **12** and **13** are also listed. Whereas in the 6-trifluoromethyl series the Δ^4-compound **4** was more active than the corresponding $\Delta^{4,6}$-compound **6**, an opposite effect was observed in the 6-methyl series, the $\Delta^{4,6}$ **13** being more active than the Δ^4 **12**. Compounds **4**, **5**, and **6** did not show any antiandrogenic activity.

Experimental Section†

3-Methoxy-6-trifluoromethyl-16,17α-oxido-3,5-pregnadien-20-one (2). 3-Methoxy-16,17α-oxido-16β-methyl-3,5-pregnadien-20-one (**1**)[4] (2.5 g, 7.02 mmoles) in dry pyridine (2.5 ml) was placed in a heavy-walled quartz flask, and the flask was purged with dry N_2 and cooled to −78°. Approx 7.5 ml of CF_3I was condensed into the mixt, the flask was sealed, warmed to room temp, and irradiated in a Rayonet Photochemical Reactor at 2537 Å with magnetic stirring. After 16 hr, it was again cooled to −78°, opened, and excess CF_3I was carefully evapd. The residue was dild with CH_2Cl_2, washed with H_2O, $Na_2S_2O_3$ soln, H_2O, dil HCl, and satd Na_2CO_3 soln, then dried (Na_2SO_4), evapd, and crystd (MeOH), affording 1.95 g (65%) of **2**; mp 132–133°; [α]D −121°; λ_{max} 254 mμ (ε 20,400). *Anal.* ($C_{24}H_{31}F_3O_3$) C, H, F.

6α-Trifluoromethyl-16-methylene-17α-hydroxy-4-pregnene-3,-20-dione (3). Compound **2** (1 g, 2.36 mmoles) was dissolved in dioxane (10 ml), and the soln added to a mixt of dioxane (10 ml) and H_2SO_4 (2 ml) at 0° under N_2, and stirred for 45 min. H_2O was added and the mixt extd with $CHCl_3$. The organic phase was washed (H_2O, satd $NaHCO_3$ soln), dried (Na_2SO_4), and evapd. The crude oil was crystd from Et_2O–C_6H_{14}, affording 727 mg (75%) of **3**; mp 183–184°; [α]D −35°; λ_{max} 235 mμ (ε 15,000). *Anal.* ($C_{23}H_{29}F_3O_3$) C, H, F.

6α-Trifluoromethyl-16-methylene-17α-hydroxy-4-pregnene-3,20-dione 17-Acetate (4). Compound **3** (193 mg, 0.471 mmole) and pTSA (30 mg) were dissolved in AcOH (3.0 ml) and trifluoroacetic anhydride (TFAA) (1.5 ml) was added, and the mixt left for 1 hr at room temp. Ice H_2O was added, and the mixt stirred until the ppt could be filtered. The solids were dissolved in MeOH, decolorized with charcoal, and crystd from MeOH to give 181 mg of **4** (85%); mp 247–249° dec; [α]D −79°; λ_{max} 234 mμ (ε 15,400); nmr, δ 1.23 (10-CH_3), 2.7–3.4 (6β-H), 5.46, 5.60 (16=CH_2), 6.02 (4-H) ppm; ν_{max} 1120 cm^{-1} (CF_3). *Anal.* ($C_{25}H_{31}F_3O_4$) C, H, F.

6α-Trifluoromethyl-16-methylene-17α-hydroxy-1,4-pregnadiene-3,20-one 17-Acetate (5). Compound **4** (470 mg, 1.04 mmoles) and DDQ (510 mg, 2.2 mmoles) were refluxed in dioxane (25 ml) for 21 hr. The mixt was poured onto a short column of neutral Al_2O_3 (Woelm grade I) and eluted with a 1:1 mixt of $CHCl_3$ and MeOH until a yellow band (unreacted DDQ) approached the bottom of the column. The eluate was evapd, the residue treated with activated charcoal in MeOH (10 ml) and, after evapn of the solvent, crystd from EtOAc–C_6H_{14}, yielding 315 mg (67%) of **5**; mp 226.5–228°; [α]D −117.5°; λ_{max} 242 mμ (ε 16,200), nmr, δ 1.30 (10-CH_3), 2.7–3.4 (6β-H), 5.48, 5,60 (16=CH_2), 6.21, 6.38 (4-H, 2-H), 7.08 (1-H, d, J = 10 Hz); ν_{max} 1125 cm^{-1} (CF_3). *Anal.* ($C_{25}H_{29}F_3O_4$) C, H, F.

4ξ-Bromo-6-trifluoromethyl-16,17α-oxido-16β-methyl-5-pregnene-3,20-dione (7). Enol ether **2** (424 mg, 1 mmole) was dissolved in DMF (8 ml) containing H_2O (2%) and cooled to 5°. NBS (440 mg, 2.5 mmoles) was added in one portion and stirred for 5 min at 5°. The mixt was poured into 40 ml of H_2O–ice and the ppt collected by filtration (uv: only end absorption).

6α-Trifluoromethyl-6β-bromo-16,17α-oxido-16β-methyl-4-pregnene-3,20-dione (8). Compound **2** (600 mg, 1.42 mmoles) was dissolved in Me_2CO (10 ml). NaOAc (400 mg) in H_2O (3 ml) was added, the mixt was stirred, cooled to 0°, and NBS (640 mg) added, followed immediately by HOAc (9.0 ml). After 30 min at 0° the mixt was poured into H_2O–ice, the ppt was collected, dried for a short time at room temp *in vacuo* to give 635 mg of **8**; mp 100° dec; λ_{max} 243 mμ (ε 9800); [α]D +7.3°; nmr, δ 1.50 (sharp, 10-CH_3), 6.24 ppm (4-H). *Anal.* ($C_{23}H_{28}BrF_3O_3$) H, F; C: calcd, 56.45; found, 56.00; Br: calcd, 16.33; found, 15.91 (**8** could not be recrystd without decompn, the values were obtained from the crude ppt).

6-Trifluoromethyl-16,17α-oxido-16β-methyl-4,6-pregnadiene-3,20-dione (9). The crude bromide **8** (obtained from 1.33 g, 3.14 mmoles of **2**) was rapidly dissolved in DMA (25 ml) containing H_2O (2%). Li_2CO_3 (1.4 g) and LiBr (0.8 g) were added, and the mixt was heated at 105° for 15 min, cooled rapidly, dild with EtOAc, and filtered. The organic phase, after washing (H_2O, 2 *N* HCl, H_2O, and satd $NaHCO_3$ soln), was dried, evapd, taken up in MeOH, and decolorized with charcoal. Chromatography on silica gel (Woelm, grade V, 50 g) using C_6H_6 containing increasing amounts of CH_2Cl_2 (H_2O satd) gave 460 mg (36%) of noncrystalline **9**, λ_{max} 271 mμ (ε 23,000), which was used without further purification. When **7**, obtained from **2** (424 mg, 1 mmole), was treated in the same manner (7.5 ml of DMA, 500 mg of Li_2CO_3, 200 mg of LiBr) it gave after column chromatography 115 mg of **9** (28%), identical with the material obtained from **8**.

3-Methoxy-6-trifluoromethyl-16-methylene-17α-hydroxy-3,5-pregnadien-20-one 17-Acetate (10). Compound **4** (3 g, 6.64 mmoles) was dissolved in THF (5 ml) and MeOH (0.5 ml). Trimethyl orthoformate (1 ml) and pTSA (30 mg) were added and the mixt was stirred at room temp for 90 min. Pyridine (1 ml) was added, and the mixt was dild with $CHCl_3$ (50 ml), washed with H_2O, dried, and evapd. Crystn from MeOH, containing a trace of pyridine, gave 1.40 g of slightly impure **10** (45%). A sample was recrystd (MeOH); mp 257–257.5°; [α]D −247°; λ_{max} 254 mμ (ε 21,300). *Anal.* ($C_{26}H_{33}F_3O_4$) C, H, F.

6α-Trifluoromethyl-6β-bromo-16-methylene-17α-hydroxy-4-pregnene-3,20-dione 17-Acetate (11). Compound **10** (233 mg, 0.5 mmole) was dissolved in Me_2CO (5 ml). A soln of NaOAc (200 mg) in H_2O (1.5 ml) was added, the mixt was cooled (0°), and NBS (320 mg) added, followed immediately by AcOH (0.25 ml). After 30 min at 0°, the mixt was poured into ice–H_2O, extd rapidly with $CHCl_3$, dried, and evapd *in vacuo* to a residue at low temp, affording 220 mg of **11** (82%); λ_{max} 242 mμ (ε 8300); nmr, δ 5.37 (16=CH_2), 6.00 ppm (4-H).

6-Trifluoromethyl-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate (6). (a) Compound **9** (110 mg, 0.27 mmole) was dissolved in AcOH (2.5 ml) and frozen. TFAA (1.5 ml) and pTSA (40 mg) were added. The soln, which had partially melted, was stirred and allowed to warm to room temp over 1 hr. It then was poured into H_2O and filtered. The solids were taken up in $CHCl_3$, dried, and evapd to a residue. Prep tlc (2 mm silica gel plates in CH_2Cl_2–10% Et_2O) and subsequent crystn from EtOAc gave 74 mg of **6** (61%); mp 246.5–247.5°; λ_{max} 270 mμ (ε 25,300); [α]D −112°; nmr, δ 5.51, 5.64 (16=CH_2), 6.10 (7-H), 6.71 (4-H); ν_{max} 1130 cm^{-1} (CF_3). *Anal.* ($C_{25}H_{31}F_3O_4$) C, H, F.

†Melting points (capillary) are uncorrected. Unless otherwise stated, rotations were measured in $CHCl_3$ solution (0.3%) at 26°, ir spectra in Nujol, uv spectra in MeOH. The nmr spectra were measured on a Varian A 60-A spectrometer in $CDCl_3$ (Me_4Si). Analyses were performed by the Physical Organic Chemistry Department of Schering Corp. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(b) Compound 11 (220 mg, 0.414 mmole) was dissolved in DMF (10 ml) containing H_2O (0.5 ml), Li_2CO_3 (0.55 g), and LiCl (0.35 g), and the mixt was brought quickly to reflux and kept refluxing vigorously for 10 min. After diln with EtOAc, the soln was filtered, and the filtrate washed (H_2O, 2 *N* HCl, and $NaHCO_3$ soln), dried, and evapd to give 100 mg of 6 (54%) (from C_6H_{14}), identical with 6 prepared from 9 in all respects.

Acknowledgments. We are indebted to Mr. E. L. Shapiro for helpful discussions and to Dr. M. D. Yudis and Mr. J. Morton for interpretation of the nmr spectra.

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Specificity in Enzyme Inhibition. 2. α-Aminohydroxamic Acids as Inhibitors of Histidine Decarboxylase and 3,4-Dihydroxyphenylalanine Decarboxylase

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A recent report by Gale, *et al.*,[1] concerning the effects of hydroxamic acids on histidine decarboxylase prompts us to report on our work dealing with this system. This work is part of a continuing study on the design of active-site-directed reversible inhibitors of enzymes utilizing amino acids as substrates.[2] In order to study the specificity of inhibition of histidine decarboxylase and L-aromatic amino acid decarboxylase, derivatives of the α-amino acids phenylalanine, tyrosine, 3,4-dihydroxyphenylalanine (dopa), histidine, and tryptophan were prepared.

On the basis of both enzyme-substrate specificity and the requirement of pyridoxal as a cofactor, the hypothetical receptor site of a decarboxylase enzyme can be pictured as having 2 binding sites; a specific site, which differentiates the amino acid (active-site-directed), and a nonspecific site, which binds the amino group. It is predicted that the carboxyl group would assume little or no role in binding the substrate to the enzyme surface, since it interacts with the active site (Figure 1).

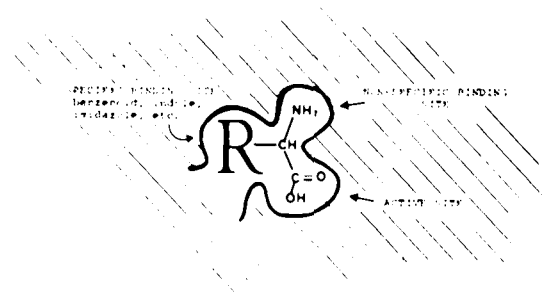

Figure 1.

Based on this model an active-site-directed inhibitor of a decarboxylase enzyme should be able to bind at the specific and the nonspecific site of the enzyme, while being unable to undergo decarboxylation at the active site. The inhibitor should possess the amino acid side chain for active-site-directed binding, along with a basic function for nonspecific binding. The carboxylic acid group should be exchanged for a function which cannot undergo decarboxylation, while still having similar structural and electronic characteristics. The inhibitor would temporarily cover the receptor, blocking the attachment of the substrate.[1] It was assumed that α-aminohydroxamic acids would meet the requirements discussed above.

The syntheses of L-phenylalanine hydroxamic acid (1), L-histidine hydroxamic acid (2), and L-tryptophan hydroxamic acid (3) were based on the method of Cunningham and coworkers.[3] The general procedure involved the neutralization of amino acid ester hydrochlorides with base followed by their treatment with methanolic NH_2OH.

L-Tyrosine methyl ester was insoluble in MeOH under the above experimental conditions, therefore, it was necessary to run the reaction in the presence of excess KOH. DL-3,4-Dihydroxyphenylalanine methyl ester was quite susceptible to air oxidation under basic or neutral conditions, therefore it was necessary to form the hydroxamic acid under acidic conditions.

Table 1

Compound	Histidine decarboxylase: % inhibition at $3 \times 10^{-4} M$	Histidine decarboxylase: % inhibition at $3 \times 10^{-5} M$	Dopa decarboxylase: % inhibition at $3 \times 10^{-4} M$	Dopa decarboxylase: % inhibition at $3 \times 10^{-5} M$
L-Phenylalanine hydroxamic acid (1)	0	6	16	11
L-Tyrosine hydroxamic acid (2)	70	6	5	8
DL-Dihydroxyalanine hydroxamic acid (3)	100	51	89	18
L-Histidine hydroxamic acid (4)	81	31	42	5
L-Tryptophan hydroxamic acid (5)	82	25	13	9
α-Methyl dihydroxyphenylalanine (6)	0	0	100	82
1-(4-Imidazolyl)-2-amino-3-butanone (7)	87	50	0	0

†Taken in part from the dissertation presented by V. D. Warner, Sept 1970, to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Doctor of Philosophy Degree.