evaporated to a small volume, and extracted with ethyl acetate. The extract was washed with water, sodium bicarbonate solution, water, dried over magnesium sulfate, and evaporated to 2.05 g. of a foam that was chromatographed on Florisil (200 g.). Elution with 5.2 l. of 12.5% acetone–87.5% Skellysolve B gave 0.39 g. of oils. Further elution with 2 l. each of 15% acetone–85% Skellylsolve and 20% acetone–80% Skellyslolve B gave 0.90 g. of a gelatinous precipitate. Crystallization of the latter from methanol-water (1:2) gave 0.63 g. of (4), m.p. 206–208° dec. The analytical sample had m.p. 210–211° dec. from methanol water; λ_{max} 241 mμ (ε 16,100).

Anal. Calcd. for $C_{23}H_{31}FO_6 \cdot 0.25H_2O$: C, 64.69; H, 7.44; F, 4.45. Found: C, 64.54; H, 7.68; F, 4.41.

9α,15β-Difluoro-11β,17α,21-trihydroxypregn-4-ene-3,20-dione 21-Acetate (5).—N-Bromoacetamide (0.38 g.) was added to a solution of 0.7 g. (1.65 mmoles) of (4) in 15 ml. of pyridine. The mixture was stirred under an atmosphere of nitrogen for 20 min. at 25°, cooled to 10° in an ice bath, and sulfur dioxide was introduced over the surface for 15 min.[15] The temperature rose rapidly to 20°, then dropped gradually to 10°. The reaction mixture was diluted to 250 ml. with ice water yielding a white precipitate of the 9(11)-dehydro compound, amounting to 0.53 g. after filtration and drying *in vacuo*. The crude 9(11)-olefin (0.53 g.) was dissolved in a mixture of 10 ml. of methylene chloride and 18 ml. of *t*-butyl alcohol; perchloric acid (70%) (0.16 ml.) in 1.25 ml. of water and 0.25 g. of N-bromoacetamide in 2.5 ml. of *t*-butyl alcohol were added. The mixture was stirred for 15 min. at 25–30° and 0.25 g. of sodium sulfite in 2.5 ml. of water was added. Concentration of the solution *in vacuo* and dilution with 100 ml. of ice water gave after filtration and drying 0.55 g. of the bromohydrin which was dissolved in acetone (15 ml.) and heated under reflux with 0.6 g. of potassium acetate for 24 hr. The mixture was evaporated to dryness, the residue partitioned between water and methylene chloride, and the organic extract washed with water and dried over magnesium sulfate. Evaporation of solvent gave 0.486 g. of a yellow foam which was chromatographed on 50 g. of Florisil. Elution with 1 l. of 12.5% acetone–Skellysolve B removed trace of material, further elution with 2.2 l. of the same solvent mixture gave 0.359 g. of crystalline **17α,21-dihydroxy-15β-fluoro-9β,11-epoxypregn-4-ene-3,20-dione 21-acetate,** m.p. 205–207°.

A solution of 0.35 g. of the epoxide in 3.5 ml. of methylene chloride was cooled to −70° and added to a mixture of anhydrous hydrogen fluoride (3.0 g.) and tetrahydrofuran (5.3 ml.) at −20°. The mixture was allowed to stand 16 hr. at −20° and 4 hr. at +5°, then poured into a stirred ice-cold suspension of 21.6 g. of sodium bicarbonate in 160 ml. of water. Extraction with ethyl acetate yielded 0.386 g. of a yellow foam. Two crystallizations from acetone–Skellysolve B gave 110 mg. of (5), m.p. 213–214° dec., λ_{max} 238 mμ (ε 16,000).

Anal. Calcd. for $C_{23}H_{30}F_2O_6$: C, 62.71; H, 6.87; F, 8.63. Found: C, 63.02; H, 6.85, F, 8.12.

9α,15β-Difluoro-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione 21-Acetate (6).—About 300 mg. of crude (5) in 40 ml. of *t*-butyl alcohol containing 0.4 ml. of acetic acid was heated under reflux with 150 mg. of selenium dioxide for 44 hr. An additional 150 mg. of selenium dioxide was added and heating was continued for 19 hr. The mixture was evaporated to dryness, taken up in 150 ml. of ethyl acetate, and washed with cold aqueous sodium bicarbonate, aqueous ammonium polysulfide, dilute ammonium hydroxide, water, dilute hydrochloric acid, and water. Evaporation of the solvent gave 0.326 g. of an orange foam that was chromatographed on 100 g. of Florisil in methylene chloride. Elution with 1.5 l. of 7.5% acetone–92.5% methylene chloride and 750 ml. of 12.5% acetone–87.5% methylene chloride gave 200 mg. of partly crystalline product. Three crystallizations from acetone–Skellysolve B gave 60 mg. of pure (6), m.p. 232–233° dec.; λ_{max} 238 mμ (ε 16,000).

Anal. Calcd. for $C_{23}H_{28}F_2O_6$: C, 63.00; H, 6.44; F, 8.67. Found: C, 63.26; H, 6.24; F, 8.62.

11β,17α,21-Trihydroxy-15β-fluoropregna-1,4-diene-3,20-dione 21-Acetate (7).—A solution of 0.42 g. of (4) in *t*-butyl alcohol (50 ml.) containing 0.5 ml. of acetic acid was dehydrogenated with a total of 0.41 g. of selenium oxide as in the previous example. Chromatography on Florisil (100 g.) using linear gradient elution (2 l. each of 1:9 acetone–Skellysolve B and 4:6 acetone–Skellysolve B) gave 0.358 g. of (7). Crystallization from acetone–Skellysolve B and aqueous acetone gave an analytical sample, m.p. 235–238°; λ_{max} 243 mμ (ε 15,200).

Anal. Calcd. for $C_{23}H_{29}FO_6$: F, 4.52. Found: F, 4.51.

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Steroid Dimethylhydrazones

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Interest in the relative anabolic–androgenic activities[1] of some of the previously unknown steroid dimethylhydrazones prepared in our Laboratories has suggested the desirability of reporting the methods of preparation and physical characterization data for these materials. The properties of the steroid dimethylhydrazones are given in Table I. All were prepared by reaction of the sterol in excess dimethylhydrazine with a trace of acetic acid as a catalyst. The sterols are readily soluble in dimethylhydrazine and the product precipitates on standing or is precipitated by addition of water. In the absence of acetic acid to catalyze the reaction, the steroid is often recovered unchanged. A typical preparation is described in the following paragraph.

Pregnenolone Dimethylhydrazone.—Pregnenolone (1 g.) was heated with 5 ml. of dimethylhydrazine containing 2 drops of acetic acid until a clear solution was obtained. The solution was kept 12 hr. at room temperature. On dilution with 30 ml. of water, a white precipitate was formed. This was collected, washed with water, and recrystallized from methanol. Data characterizing the product are given in Table I.

The biological activity of these compounds is of interest in providing further examples of the observation that the oxygen atom of the three position in steroids is not essential to activity[2] and of the biological activity of the dimethylhydrazones.[3] These hydrazones may be regarded as acyclic derivatives of the steroidal pyrazoles[2,4] some of which have shown enhanced anabolic activity[4] or antiinflammatory activity.[2] In standard assay procedures in rats,[1] the dimethylhydrazones of the five testosterone derivatives (Table I) have shown a considerably reduced anabolic–androgenic activity as compared to the corresponding carbonyl compounds. The 19-nor derivative, however, retains the differentiation between anabolic and androgenic activities observed with other 19-nor types.

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TABLE I

STEROID DIMETHYLHYDRAZONES

Sterol[a]	Yield, %	M.p.,[b] °C.	Solvent[c]	Formula	Analyses[d] Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Pregnenolone	90	166	M	$C_{23}H_{38}N_2O$	77.04	10.68	7.81	76.92	10.74	7.92
Progesterone (bis)	93	148	E	$C_{25}H_{42}N_4$	75.32	10.62	14.06	75.23	10.60	14.52
Testosterone	93	179	M	$C_{21}H_{34}N_2O$	76.31	10.37	8.48	76.03	10.69	8.76
17-α-Methyltestosterone	96	190	M	$C_{22}H_{36}N_2O$	76.69	10.53	8.13	76.60	10.63	8.31
Ethisterone	92	206	M	$C_{23}H_{34}N_2O$	77.92	9.67	7.90	77.62	9.60	8.07
cis-Testosterone[e]	92	200	E	$C_{21}H_{34}N_2O$	76.31	10.37	8.48	75.83	10.35	8.28
Estrone	60	174	E	$C_{20}H_{28}N_2O$	76.88	9.03	8.97	77.12	9.07	8.88
Cortisone (bis)	96	218	M	$C_{25}H_{40}N_4O_3$	67.53	9.07	12.60	67.34	8.99	13.11
Dihydrotestosterone	87	180	M	$C_{21}H_{36}N_2O$	75.85	10.91	8.43	75.08	10.6	8.69
19-Nortestosterone	96	171	M	$C_{20}H_{32}N_2O$	75.90	10.19	8.85	75.33	10.11	8.52
Methyl dehydrocholate[f] (mono)	73	158	C	$C_{25}H_{36}N_2O_4$			6.54			6.23

[a] Commercial materials were used as received. [b] All melting points are with decomposition. [c] Solvent for recrystallization: M, aq. methanol; E, aq. ethanol; C, cyclohexane. [d] Analyses by Micro Tech Laboratories, Skokie, Illinois. [e] Methanol solution used in preparation. [f] Prepared as described in ref. 5.

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The Microbiolgoical Preparation of 17-Deoxytriamcinolone

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The recognition that glucocorticoid activity may be enhanced by the addition of certain functional groups to steroids has motivated the preparation of biologically active compounds that lack one or more of the structural features of the hydrocortisone molecule. An early example is provided by 9α-fluorocorticosterone acetate, which, though lacking the 17α-hydroxy group, displayed a glucocorticoid activity greater than that of hydrocortisone.[1] However, this compound also has powerful sodium retaining properties.[1] It is known that 16α-hydroxylation of 9α-fluorohydrocortisone and 9α-fluoroprednisolone yields derivatives that are lacking in sodium retention properties and yet retain considerable glucocorticoid activity.[2] It was of interest to ascertain whether the analogous 17-deoxysteroids would possess similar biological properties.

Fermentation of 9α-fluorocorticosterone (I) with *Streptomyces roseochromogenes* (ATCC 3347) yielded a product that was identified as 9α-fluoro-11β,16α,21-trihydroxypregn-4-ene-3,20-dione (II). This product was ultraviolet-absorbing and displayed a positive blue tetrazolium reaction, indicating the continued presence of a Δ^4-3-ketone and the α-ketolic side chain, in agreement with infrared absorption data. Elemental analysis supported the presence of one additional hydroxyl group. Appearance of the typical 415 mμ-absorbing chromogen after reaction of II with the Porter–Silber reagent[3] strongly suggested the presence of a hydroxyl group at C-16 or C-17. The rate of formation of the 415 mμ-absorbing chromogen favored C-16 as the hydroxylated position.[4] The ready formation of a diacetate and the separation of II from 9α-fluorohydrocortisone by paper chromatography further confirmed that the hydroxyl was located at C-16. Assignment of the configuration as 16α is based on the known capacity of *Streptomyces roseochromogenes* to 16α-hydroxylate steroids,[5] and on optical rotation data (Table I).

TABLE I

MOLECULAR ROTATION DATA OF SOME C-16 HYDROXYLATED STEROIDS

Steroid	M_D	Solvent	ΔM_D (16α-OH-H)
Progesterone[a]	+629°	$CHCl_3$	
16α-Hydroxyprogesterone[a]	+519°	$CHCl_3$	−110°
16β-Hydroxyprogesterone[a]	+635°	$CHCl_3$	+6°
9α-Fluorocorticosterone (I)	+675°	MeOH	
9α-Fluoro-16α-hydroxy-corticosterone (II)	+483°	MeOH	−192°

[a] See ref. 6.

Fermentation of II with *Nocardia corallina* (ATCC 999) produced 9α-fluoro-11β,16α,21-trihydroxypregna-1,4-diene-3,20-dione (IV) which was isolated from the fermentation mash. Compound IV was ultraviolet absorbing, reduced blue tetrazolium, and gave a positive reaction with the Porter–Silber reagent. The infrared spectrum of IV is in accord with the assignment of a 1,4-diene-3-one system. The bathochromic shift observed in the ultraviolet in proceding from II to IV, together with the characteristic reaction of IV for a 1,4-diene-3-one with isonicotinic acid hydrazide,[7] and phthalic acid *p*-phenylenediamine[8] provide further support for the assigned structure.

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