lack of growth. The yeast and fungus tube-dilution assays were performed by incorporating the compound and inoculum into sensitivity agar instead of broth. Visible inhibition of growth on the surface of the agar after 24 hr was the criterion of activity in these cases.

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Synthesis and Activity of Some Nitro Steroids[1]

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The synthesis of several 3- and 20-nitro-5α-androstane and -pregnene derivatives was undertaken by oxidation of the corresponding oximes. Improved conditions (irradiation and oxygenation) were developed for this technique. Biological evaluation of the final derivatives for anabolic and progestational activities indicated that the replacement of a carbonyl oxygen by a nitro group in these compounds leads to weakly active or inactive products.

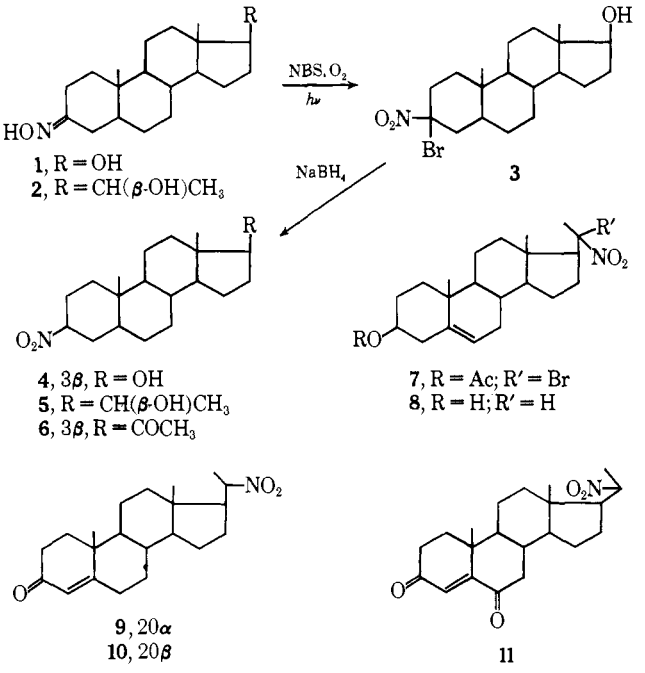
All steroid hormones except estradiol and testosterone possess a carbonyl group at C-20 and all but estradiol have a keto group at C-3. In work directed at defining the function of this moiety in eliciting biological responses,[2] we speculated that a combination of high electron density and hydrogen-bond acceptance might be key factors in the importance of these ketones. In the present work, we have examined this possibility by determining if a nitro group can be substituted in the region of a carbonyl function with retention of activity.

Nitro steroids have been prepared by nitration of unsaturated steroids with nitric acid[3a-c] or nitrogen tetroxide,[3d] by condensation of steroidal aldehydes with nitromethane,[3e] by nitration of oximes,[3f] by oxidation of oximes with a peracid,[3g] from reactions of steroids with nitrosyl chloride,[3h] and by displacement of alkyl nitrates,[3i] but these methods appeared too drastic or otherwise unsuitable for unsaturated steroids. Attempts to displace steroidal 3-tosylates with sodium nitrite[4] failed. Finally we employed and modified the mild oxidation of oximes[5] which had been used for the preparation of 17-nitro steroids.[6]

Treatment of **1** with N-bromosuccinimide (NBS) in dioxane–water solution, followed by stirring and exposure to air for 48 hr and final $NaBH_4$ reduction gave a mixture of the nitro compound **4** (27%) and androstane-3β,17β-diol (Scheme I). It was thus apparent that two competing reaction sequences occur during the NBS reaction: (a) formation of a *gem*-bromonitroso compound followed by air oxidation to a *gem*-bromonitro compound, and (b) hydrolysis of the oxime to the parent ketone, and subsequent reduction to the corresponding alcohol by the borohydride. It was clear that b could be minimized by accelerating the steps in a. This was done by bubbling oxygen through the mixture rather than relying on atmospheric air, and by irradiating with ultraviolet light. We reasoned that the irradiation would generate bromine radicals, thus facilitating the bromination and, second, would convert molecular oxygen, a sluggish oxidizing agent, to atomic oxygen, a much better oxidizing agent. As a result of these modifications, the yield was increased to about 50%. The assignment of the configuration of the nitro group in **4** was based on the broad multiplet exhibited in the nmr spectrum of the 3α-proton; this is due to axial–axial splittings and is compatible only with an axial proton at C-3.

In extending the method to C-20 oximes, a complex mixture of epimeric C-20 nitro compounds and alcohols was obtained, as shown by glpc. Therefore, the intermediate bromonitro compound **7** was isolated and freed

SCHEME I



(1) This investigation was supported in part by a PHS research grant (AM-05016) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

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of C-20 ketone by treatment with Girard's reagent. Reduction and concomitant hydrolysis gave **8**.

Attempts to oxidize **8** by the Oppenauer method gave only a complex mixture. Use of chromic acid in acetone at 0° gave the 3,6-dione **11**, but at −70° the desired 3-ketones were secured. These were isomerized to the conjugated products **9** and **10** with oxalic acid. The structure of **11** was readily deduced from the nmr spectrum. A sharp singlet at 6.2 ppm was produced by the C-4 proton, whereas normally the resonance due to the C-4 proton in a 3-keto-Δ^4 steroid is broadened by allylic coupling to the protons at C-6. The configuration of the nitro groups in **9**, **10**, and **11** was assigned using known nmr relationships.[7] In the 20α isomer the C-18 resonance is shifted upfield and the C-21 resonance is shifted downfield relative to the positions of these peaks in the spectra of the 20β isomers.

Biological Testing.[8]—Compounds **4**, **6**, **9**, and **10** were evaluated in Eisenberg–Gordan–(Hershberger) and Clauberg-type tests[2] (Tables I and II). Compound **4** showed about 10% of the androgenic activity of testosterone but **6**, **9**, and **10** were inactive as progestational substances. It was concluded that the nitro group cannot assume the role of the carbonyl group when substituted in this manner.

TABLE I
RESULTS OF ANABOLIC–ANDROGENIC TESTING[a]

Treatment (total dose, mg)	Wt of ventral prostate ± SE, mg	Wt of seminal vesicles ± SE, mg	Wt of levator ani ± SE, mg
Control	14.3 ± 1.13	12.0 ± 0.73	25.9 ± 1.12
Testosterone (0.5)	37.9 ± 4.59	18.1 ± 0.54	35.1 ± 1.01
4 (3.0)	35.8 ± 5.72	20.1 ± 2.32	30.7 ± 2.84
P	<0.01	0.01	<0.02

[a] Groups of six castrate rats.

TABLE II
RESULTS OF PROGESTATIONAL TEST[a]

Treatment (total dose/rabbit, mg)	Av ovarian wt, mg	Av uterine wt, mg	Av response
Progesterone (0.2)	31.0	1.28	1.0
6 (2.0)	47.6	0.97	0.0
9 (2.0)	29.4	1.50	0.0
10 (2.0)	38.5	0.94	0.0

[a] These values are averages from only two animals at each dose level.

Experimental Section[9]

3β-Nitro-5α-androstan-17β-ol (4) A. NBS Reaction.—To a stirred suspension of 5.42 g (0.034 mole) of NBS in a mixture of 17 ml of H_2O and 17 ml of dioxane there was added a solution of 3.0 g of $KHCO_3$ in 17 ml of H_2O and then a solution of 3.05 g (0.01 mole) of 3-oximinoandrostan-17β-ol[10] in 70 ml of dioxane. The resulting mixture was irradiated (Hanovia 450-W mercury arc, medium pressure) while O_2 was bubbled through. After 24 hr, the color of the mixture had changed from blue to light green. It was diluted with H_2O and the product was extracted (Et_2O). The ether was washed with 5% $FeSO_4$ solution, dried (Na_2SO_4), and evaporated.

B. Borohydride Reduction.—The residue was dissolved in a mixture of 12 ml of H_2O and 70 ml of THF, and 1.5 g of $NaBH_4$ was added portionwise during 1.5 hr. After 2.5 hr, the mixture was acidified with 6.9 g of $NH_2OH \cdot HCl$ in 35 ml of H_2O and the product was extracted (Et_2O). Evaporation of the washed and dried ether solution and trituration of the residue with ether gave 0.67 g (23%) of 3β,17β-dihydroxy-5α-androstane. The mother liquor was evaporated and the residue was treated with acetone and triturated with H_2O giving 1.28 g (40%) of yellow solid. Recrystallization from MeOH–H_2O gave yellow crystals: mp 165–167°; ν_{max}^{KBr} 3508, 1538 cm^{-1}; nmr, 4.50 (broad multiplet) (3α-H), 3.75 (17α-H), 0.92 (19-H_3), 0.77 (18-H_3). The material contained about 5% of the 3α-nitro isomer as judged by nmr. *Anal.* ($C_{19}H_{31}NO_3$) C, H, N.

3-Bromo-3-nitro-5α-pregnan-20β-ol (3) was obtained as a mixture of C-3 epimers from 3-oximino-5α-pregnan-20β-ol[11] by NBS bromination in a manner similar to that used in part A of the preparation of **4**. It had mp 169–170° after recrystallization from MeOH. *Anal.* ($C_{21}H_{34}BrNO_3$) C, H, N.

3-Nitro-5α-pregnan-20β-ol (5) was obtained from **2** after NBS treatment and borohydride reduction in a manner similar to that used in the preparation of **4**. Recrystallization from EtOH–H_2O gave the mixed C-3 epimers as colorless crystals, mp 180–186°. *Anal.* ($C_{19}H_{35}NO_3$) C, H, N.

3β-Nitro-5α-pregnan-20-one (6).—A solution of 0.24 g of **5** in 50 ml of Me_2CO was treated dropwise with 8 *N* CrO_3 at 27° for 15 min. The excess CrO_3 was destroyed by addition of *i*-PrOH and the product was isolated by ether extraction. Recrystallization from EtOH gave colorless needles, mp 163–165° dec. The material contained about 10% of the 3α isomer. *Anal.* ($C_{21}H_{33}NO_3$) C, H, N.

3β-Hydroxy-20-oximinopregn-5-ene 3-Acetate.—A mixture of 7.16 g of 3β-hydroxypregn-5-en-20-one acetate, 7 g of $NH_2OH \cdot HCl$, and 15 ml of pyridine in 150 ml of EtOH was heated under reflux for 11 hr, diluted (H_2O), and refrigerated. The resulting precipitate was recrystallized from EtOH giving 5.35 g (72%) of colorless crystals, mp 195–197°, $[\alpha]^{20}D$ −57° (*c* 1, $CHCl_3$). *Anal.* ($C_{23}H_{35}NO_3$) C, H, N.

20-Bromo-3β-hydroxy-20-nitropregn-5-ene Acetate (7). Treatment of 3.73 g of 3β-hydroxy-20-oximinopregn-5-ene 3-acetate as described in the first part of the preparation of **4** gave a crude product, from which ketonic material was removed by heating with 3.3 g of Girard's reagent T in 100 ml of absolute EtOH. Most of the solvent was evaporated and the residue was diluted with 200 ml of 5% $NaHCO_3$ solution and extracted (Et_2O). Recrystallization from MeOH of the residue obtained from evaporation of the ether gave a mixture of C-20 epimers as colorless crystals (0.27 g), mp 182–183°. *Anal.* ($C_{23}H_{34}BrNO_4$) H, Br, N; C: calcd, 58.96; found, 59.45.

3β-Hydroxy-20-nitropregn-5-ene (8).—Reduction of **7** with $NaBH_4$ as described under the preparation of **4** gave 0.25 g of white solid which was purified by chromatography on alumina. Recrystallization from MeOH gave a mixture of C-20 epimers (nmr), mp 153–183°. *Anal.* ($C_{21}H_{33}NO_3$) C, H, N.

20α-Nitropregn-4-en-3-one (9).—A solution of 1.00 g of **8** in 375 ml of Me_2CO was allowed to react with excess 8 *N* CrO_3 at −70° for 3 hr. After addition of *i*-PrOH, the product was isolated with ether and was found to contain unconjugated ketone. This was isomerized by heating the material for 4 hr in 250 ml of EtOH containing 2 g of oxalic acid. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ and filtered to remove oxalic acid. Evaporation of the filtrate and purification by preparative tlc gave the product from the second band. Recrystallization from MeOH–H_2O gave 10 mg of yellow needles: mp 214–216°; nmr, 5.76 (4-H), 4.60 (20-H), 1.54, 1.44 (d), (21-H_3), 1.18 (19-H_3), 0.84 (18-H_3). *Anal.* ($C_{21}H_{31}NO_3$) C, H, N.

20β-Nitropregn-4-en-3-one (10).—A slower moving fraction from the isolation of **9** was recrystallized from ether–petroleum ether (bp 30–60°) giving 40 mg of yellow needles: mp 206–207°;

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(9) Melting points were determined with a Thomas–Hoover apparatus equipped with a corrected thermometer. Ir spectra were obtained with a Beckman IR-8 or Perkin-Elmer 337 instrument. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Nmr spectra were obtained at a field strength of 60 Mc/sec on samples in $CDCl_3$ solutions on a Varian A-60A instrument, using TMS as internal standard. When only small amounts of sample were available, a Varian C-1024 computer was used for time averaging. Optical rotations were obtained in a 0.5-dm tube with a Rudolph photoelectric polarimeter. Gas chromatography was carried out using a Barber-Coleman Model 5000 system employing 1.83-m U-tube columns of 2% SE-30 on Gas Chrom Q or Z, He carrier, H_2 flame detection, column temperatures of 220–240°, and detector temperatures of 250°.

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nmr, 5.77 (4-H), 4.55 (20-H), 1.63, 1.53 (d, 21-H_3), 1.22 (19-H_3), 0.79 (18-H_3). *Anal.* ($C_{21}H_{31}NO_3$) C, H, N.

20β-Nitropregn-4-ene-3,6-dione (11).—An ice-cold solution of 0.2 g of **8** in 75 ml of acetone was allowed to react with excess 8 *N* CrO_3 solution for 45 min. The excess CrO_3 was destroyed by addition of *i*-PrOH, and the product was isolated by ether extraction. Purification by preparative tlc followed by recrystallization from MeOH gave 9 mg of yellow powder: mp 213–215°; nmr, 6.19 (4-H), 4.56 (20-H), 1.55, 1.45 (d, 21-H_3), 1.17 (19-H_3), 0.84 (18-H_3). *Anal.* ($C_{21}H_{29}NO_4$) C, H, N.

The Solvolysis of 19-Hydroxy Steroid Derivatives[1]

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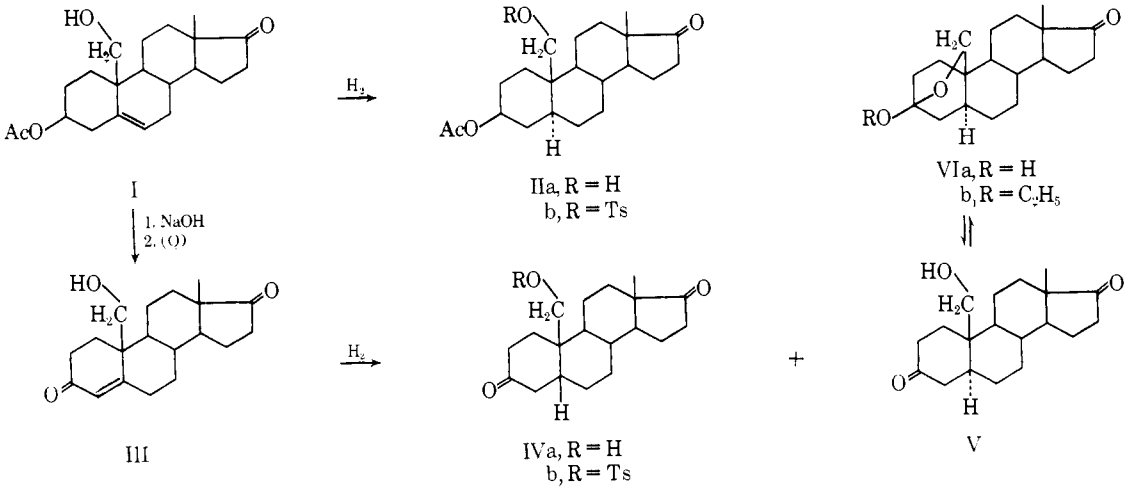
The 5α and 5β isomers IIa and IVa of 19-hydroxy steroids were prepared and their related *p*-toluenesulfonyl esters were solvolyzed in buffered acetic acid. In both series the predominant product was a $\Delta^{1(10)}$-19-nor-A-homoandrostene derivative VIIa or XIII. The structures of these solvolysis products were established by degradation.

The acetolysis of both *cis*- and *trans*-9-decalylcarbinyl *p*-toluenesulfonates[3,4] afforded mixtures of $\Delta^{1(7)}$-bicyclo[5.4.0]undecene and Δ^{1}- and/or $\Delta^{1(11)}$-bicyclo[5.4.0]undecene in a ratio of about 7:3. These results, the absence of any bicyclo[4.4.1]undecane products, and a rate of reaction approximately equal to the rate of the similar neopentyl derivative can be rationalized by consideration of the formation of a classical carbonium ion followed by rearrangement to the most stable carbonium ion, the stability of which is related to the products.[5] The solvolysis results are to be contrasted with the results of the deamination of the corresponding *cis*- and *trans*-9-decalylcarbinylamines where bicyclo[4.4.1]undecane derivatives and tricyclo[4.4.1.-$0^{1,6}$]undecane were obtained.[4,6] These latter results have led to the suggestion that in the deamination reaction the geometry of the transition state closely resembles the conformation of the starting material and it is the steric arrangement of this latter species which controls the migratory aptitude of the groupings.

Since the products from the acetolysis of the decalylcarbinyl system appeared to be dependent upon the relative stability of the carbonium ions, it was of interest to know whether in an unsymmetrically substituted decalylcarbinyl system, where the conformational energies of the products would be different, the acetolysis would favor certain products over others. The recent availability of 19-hydroxy steroids (which are precursors of 19-nor steroids[7]) made this series of materials an attractive group of unsymmetrical decalylcarbinyl systems to study both from the viewpoint of solvolysis mechanisms and of the potential preparation of modified steroidal derivatives of the 19-nor-A- and the 19-nor-B-homo series.

The A/B-*cis* and A/B-*trans* isomers IVa and IIa, respectively, were prepared by slight modifications of published procedures,[8-11] and the synthetic sequences are outlined below. In the hydrogenation of 19-hydroxy-Δ^4-androstene-3,17-dione (III) it had been reported[11] that the steric course of the reaction was



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