between O(2) and H(1) and a H bond type linkage (albeit a strong one) between O(3) and H(1).

The very strong interactions of this arrangement are partially relieved in 2 ways. As in the KHMal, KHClMal, and maleic acid structures the internal angles of the ring system are strained considerably. The two angles O(2)–C(1)–C(2) and O(3)–C(4)–C(3) are not significantly different from each other and average 119° 36′ ± 42′. Similarly the angles C(1)–C(2)–C(3) and C(2)–C(3)–C(4) are insignificantly different and average 130° 24′ ± 24′. These angles are however significantly different from their unstrained counterparts of 121° 18′ and 121° 30′, respectively.[24] A situation closely paralleling this has been found in the 3 structures mentioned above.

The second manner in which the strain is relieved is by rotation of the CO_2H groups around their C–C bonds. The atoms of the C spine here are all within 0.007 Å of the plane with equation $-0.815x + 0.4940y - 0.3027z - 6.0592 = 0$. ($\chi^2 = 3.6$). The O(1), O(2), O(3), and O(4) atoms on the other hand are 0.163(5), −0.156(4), −0.133(4), and 0.126(4) Å from this plane. These figures imply that the torsion angle about C(1)–C(2) for CO_2H 1 is 8° 23′ and that about C(3)–C(4) for CO_2H 2 is 6° 46′ the directions of twist being such as to put O(2) and O(3) on the same side of the plane of the carbons. A view of the ion showing this conformation is included in Figure 5.

A similar situation was found in KHClMal but not in KHMal or maleic acid. At present there seems little reason for O(2) and O(3) being on the same side of the C spine but is is intended to investigate the structures of disodium maleate and maleic acid in the hope of shedding some light on this question, or at least partially delineate its occurrence.

Acknowledgments.—We thank the Schering Corporation for the sample of *dl*-brompheniramine maleate used in this analysis. Doctors A. F. Casy and R. R. Ison of the Department of Pharmacy of this University originally suggested this project to us and have continued to be a source of information regarding it. One of us (G. W.) would like to thank Dr. Ison for a copy of his Doctoral Dissertation. Messrs. L. P. Simonsen and R. J. Ulrich worked on this project as senior undergraduates and we thank them for their help. Financial support from the Medical Research Council of Canada is gratefully acknowledged. The crystallographic computer programs of Dr. F. R. Ahmed, National Research Council of Canada have been invaluable in this work.

(24) S. F. Darlow, *Acta Crystallogr.*, **14**, 1257 (1961).

Steroidal Androgen Biosynthesis Inhibitors

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A screening procedure using a rat testicular microsomal preparation with [21-^{14}C]17α-hydroxyprogesterone as substrate was used to search for inhibitors of 17,20-lyase. A series of 17β-acylaminoandrost-4-en-3-ones and derivatives has been prepared and their syntheses have been described; they are shown to be androgen synthesis inhibitors *in vitro* and *in vivo*. These steroidal androgen synthesis inhibitors are demonstrated to be more specific in their action than the nonsteroidal inhibitors previously known. A mechanism for the action of these compounds is postulated.

Selective control of androgen biosynthesis is of potential interest in the treatment of benign prostatic hypertrophy, hirsutism, acne, and androgen-dependent tumors. Nonsteroidal synthesis inhibitors are known[1] which display varying degrees of selectivity in respect to enzymes involved in the adrenal and testicular synthesis of steroids including androgens.[2]

The major pathways[2,3] which have been established for testicular androgen biosynthesis involve hydroxylation of progesterone and pregnenolone to the 17α-hydroxyl derivatives and cleavage by 17,20-lyase(s) to yield androst-4-ene-3,17-dione and 3β-hydroxyandrost-5-en-17-one, respectively. Testosterone arises by reduction of the former at C-17 and in the latter by the combined action of 3(or 17)β-hydroxy steroid: NAD(P) oxidoreductase, 1.1.1.51, and 3-keto steroid Δ^5,Δ^4-isomerase, 5.3.3.1. The possibility of direct formation of testosterone from progesterone has also been suggested but not conclusively established.[4] Of several possibilities we chose to search for inhibitors of the 17,20-lyase step in androgen synthesis because of its key role in the conversion of C-21 to C-17 steroids and in the hope that the enzyme might show considerable structural specificity with respect to inhibitors. Nonsteroidal inhibitors were not excluded from consideration but steroidal inhibitors were judged to have the best chance to show a selective action.

Assay Method.—An *in vitro* screening procedure was used which involved measurement of [^{14}C]acetate formed in the side-chain cleavage of [21-^{14}C]17α-hydroxyprogesterone[5] by a rat testicular microsomal preparation. (See footnote *a*, Table I for the protocol used.)

Chemistry.—The first significant observation was that testosterone acetate but not testosterone was a potent inhibitor of the 17,20-lyase, and prompted a

(1) R. Neher and F. W. Kahnt, *Experientia*, **21**, 310 (1965).

(2) For an excellent review of this general subject see R. Gaunt, B. G. Steinetz, and J. J. Chart, *Clin. Pharmacol. Ther.*, **9**, 657 (1968).

(3) J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes and Acetogenins," W. A. Benjamin, Inc., New York, N. Y., 1964, pp 351–357.

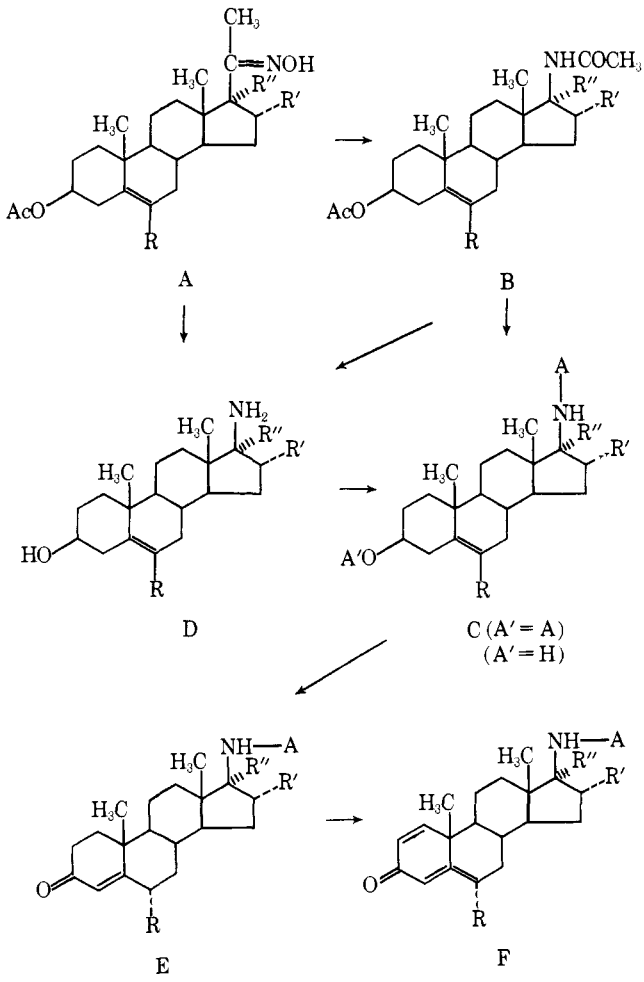
(4) (a) R. I. Dorfman, E. Forchielli, and M. Gut, *Recent Progr. Horm. Res.*, **19**, 14 (1963); (b) R. I. Dorfman and F. Ungar, "Metabolism of Steroid Hormones," Academic Press, New York, N. Y., 1965, p 1127; (c) M. A. Drosdowky, E. Forchielli, and R. I. Dorfman, *J. Eur. Steroides*, **2**, 515 (1967).

(5) Kindly supplied to us by Dr. Robert E. Erickson, formerly of these laboratories.

synthetic program to make related but metabolically stable analogs of testosterone acetate. This was approached by making amide, urea, guanidino, and carbamate substitutions for the 17β-OAc group.

Scheme I summarizes the procedures used to make

SCHEME I



R = H, CH_3; R′ = H, CH_3; R″ = H, CH_3;
A = HCO, CH_3CO, C_2H_5CO, NH_2CO, CH_3NHCO

many of the acyl derivatives and conversion products of 17β-aminoandrost-5-en-3β-ol.[6] A Beckamn rearrangement of the optionally substituted 3β-acetoxypregn-5-en-20-one 20-oxime afforded the corresponding 17β-acetamidoandrost-5-en-3β-yl acetate. This process is one first described by Schmidt-Thome.[6b] In those cases in which substitution other than 17β-CH_3CONH was desired, strong alkaline hydrolysis was employed to afford the 17β-amino derivative which was converted to other 17β-acyl or -ureido functions by standard means. Oppenauer oxidations were used to convert the 3β-hydroxy-5-ene functionality to 3-keto-4-ene derivatives and DDQ oxidations afforded the 3-keto-1,4-diene structures from the latter. Most of these conversions proceeded without difficulty. However, an exception was the Oppenauer oxidation of 17β-ureidoandrost-5-en-3β-ol (**9**) which could only be accomplished in good yield after N^3-acetylation of the 17β-ureido function (see Experimental Section for details).

Because of the unanticipated difficulty encountered in the Oppenauer oxidation of 17β-ureidoandrost-5-en-3β-ol to **7** a second route to the latter was employed using 17β-aminoandrost-5-en-3-one ethylene cyclic acetal[7] which afforded an identical final product. This intermediate also served as a convenient starting material for other derivatives, notably **6**.

The procedure of Joska and Sôrm[8] was the method of choice for the synthesis of **4** from which a derivative **22** was prepared.

The preparation of *N*-methyl-17β-acetamidoandrost-4-en-3-one (**19**) was accomplished by N-methylation of 17β-acetamidoandrost-5-en-3β-yl acetate followed by partial hydrolysis and oxidation. The isomeric 17α-methyl-17β-acetamidoandrost-4-en-3-one (**18**) was prepared from 17α-methylpregnenolone acetate[9] by the standard procedure.

Synthesis of 17β-acetamidoestr-4-en-3-one (**20**) proceeded from 3-methoxyestra-1,3,5(10)-trien-17-one oxime[10] which was reduced with Na in EtOH[9] to the 17β-amino compound. Standard Birch reduction conditions afforded the dihydro derivative which, without purification, was acetylated and then treated with acid affording **20**. To further delineate the relation of structure to activity two 17α-acylamido derivatives **10** and **25** were prepared through a common intermediate 17α-aminoandrost-5-en-3β-ol[11] by methods described in the Experimental Section.

Relationship of Lyase Inhibition with Structure.—It is evident from an examination of the data presented in Table I that high inhibition was associated with androst-4-en-3-ones bearing substituents at C-17β closely related to CH_3CO_2 in size and polarity. Thus **3** (17β-$OCONH_2$), **4** (17β-NHCOH), **5** (17β-$NHCOCH_3$), and **7** (17β-$NHCONH_2$) were all highly active. Larger groups at C-17 were associated with decreased activity as was epimerization at C-17 or by 17α substitution as illustrated by **18** and **23**. The 17β-guanidino analog, (**12**) of androst-5-ene-3β,17β-diol was virtually inactive. Considerable tolerance, however, was permitted in respect to changes around the A ring of the inhibitors. In this *in vitro* system virtual equivalence to the corresponding 4-en-3-one analog was found with **9** and **14** (5-en-3β-ol), **8** and **22** (1,4-dien-3-one), and **13** (4-en-3β-ol) and **15** (4,5-α-dihydro-3-one). Methylation at 6α (**16**) was consistent with good activity but 16α-methylation (**17**) was not. In connection with the latter it is of interest that 16α-methylation also markedly decreases androgenic activity. A 19-nor variant (**20**) was active but this change did not increase potency. An aromatic A ring derivative (**28**) was inactive.

Specificity of Inhibition.—Compounds **4**, **5**, **7**, **9**, **13**, and **24** did not inhibit the *in vitro* conversion of cholesterol to pregnenolone by an acetone preparation from bovine corpora lutea, indicating that these compounds

(6) (a) G. Ehrhart, H. Ruschig, and W. Aumüller, *Angew. Chem.*, **52**, 363 (1939); (b) J. Schmidt-Thomé, *Chem. Ber.*, **88**, 895 (1955).

(7) Von A. Schubert, R. Zepter, H. Greiner, and E. Watzke, *J. Prakt. Chem.*, **26**, 324 (1964).

(8) J. Joska and F. Sôrm, *Collect. Czech. Chem. Commun.*, **21**, 754 (1956); *Chem. Listy*, **49**, 1687 (1955); *Chem. Abstr.*, **56**, 5715.

(9) Pl. A. Plattner, H. Heusser, and P. Th. Herzig, *Helv. Chim. Acta.*, **32**, 270 (1949).

(10) B. M. Regan and F. N. Hayes, *J. Amer. Chem. Soc.*, **78**, 639 (1956).

(11) (a) J. W. Cole, *Chem. Abstr.*, **62**, 5319 (1965); (b) C. H. Robinson and C. Ermann, *Steroids*, **6**, 509 (1965).

TABLE I

ACTIVITIES OF SOME OF THE 17,20-LYASE INHIBITORS

Compd no.	Compd	Method of preparation	% Inhibition of 17,20-lyase[a] Concentration of inhibitor in μg/ml 0.5	0.25	0.125
1	Testosterone		<10		
2	Testosterone acetate		65	60	
3	Testosterone carbamate	*b*	95		85
4	17β-Formamidoandrost-4-en-3-one	*c*	100	90	85
5	17β-Acetamidoandrost-4-en-3-one	*d*	85	80	60
6	17β-Propionamidoandrost-4-en-3-one	*b*	35		
7	17β-Ureidoandrost-4-en-3-one	*f*	90	85	75
8	17β-Ureidoandrosta-1,4-dien-3-one	*b*	85	80	60
9	17β-Ureidoandrost-5-en-3-β-ol	*b*	80	70	60
10	17α-Ureidoandrost-4-en-3-one	*b*	<10		
11	17β-N^3-Acetureidoandrost-4-en-3-one	*b*	<10		
12	17β-Guanidinoandrost-5-en-3β-ol	*b*	<10		
13	17β-Acetamidoandrost-4-en-3β-ol	*b*	90	80	65
14	17β-Acetamidoandrost-5-en-3β-ol	*d*	80	70	60
15	17β-Acetamido-5α-androstan-3-one	*j*	70	60	40
16	17β-Acetamido-6α-methylandrost-4-en-3-one	*b*	70	60	50
17	17β-Acetamido-16α-methylandrost-4-en-3-one	*b,e*	<10		
18	17β-Acetamido-17α-methylandrost-4-en-3-one	*b*	<10		
19	*N*-Methyl-17β-acetamidoandrost-4-en-3-one	*b*	<10		
20	17β-Acetamidoestr-4-en-3-one	*b*		60	
21	17β-Acetamidoandrosta-1,4-dien-3-one	*b,f*	70	50	20
22	17β-Formamidoandrosta-1,4-dien-3-one	*b*	95		70
23	3-(3β-Hydroxy-17β-aminoandrost-5-en-17α-yl)-propionic acid lactam	*g*	<10		
24	17β-Acetamidoandrosta-4,6-dien-3-one	*b*	75	50	20
25	17α-Acetamidoandrost-4-en-3-one	*b*	<10		
26	3-(6-Chloro-3-methyl-2-indenyl)pyridine, SU8000	*h,i*	90	80	70
27	3-(1,2,3,4-Tetrahydro-1-oxo-7-chloro-2-naphthyl)pyridine	*h,i*	90	80	65
28	17β-Acetamido-3-methoxyestra-1,3,5(10)-triene	*b*	<10		

[a] The incubation mixt contained 0.60 mg of testicular microsomal protein prepd from mature Holtzman rats, 5 mg (50,000 dpm) of [21-^{14}C]17α-hydroxy progesterone, 0.74 mg of NADPH, and 20 μl of *i*-PrOH in 2.0 ml of 0.02 *M*, pH 7.0, Tris buffer. The incubation was allowed to proceed at 37° for 30 min, and the [2-^{14}C]AcOH which was formed was sepd from the mixt with the use of a 1.5-ml column of Dowex 1-X8 resin of 200–400 mesh in the incubation buffer. H_2SO_4 (3.0 ml, 0.5 *N*) was used to elute the [2-^{14}C]AcOH. Control incubations produced 30% cleavage. The screening level for inhibitors was set at 0.5 μg/ml. Compds producing <10% inhibition at this concn were considered to be inact. The percentage inhibition is reported to the nearest 5% and is reproducible to ±2.5%. [b] This communication, see Experimental Section. [c] See ref 8. [d] M. F. Murray, U. S. Patent 2,707,189 (1955); *Chem. Abstr.*, **50**, 4246*f* (1956). [e] By Oppenauer oxidn of 16α-methyl-17β-acetamidoandrost-5-en-3β-ol, described by P. DeRuggieri, C. Ferrari, and C. Gandolfi, *Gazz. Chim. Ital.*, **91**, 655 (1961). [f] O. El-Tayeb, S. G. Knight, and C. J. Sih, *Biochem. Biophys. Acta*, **93**, 411 (1964). [g] A. A. Patchett, F. Hoffman, F. F. Giarrusso, H. Schwam, and G. E. Arth, *J. Org. Chem.*, **27**, 3822 (1962). [h] We are indebted to Dr. Emil Schlittler of Ciba Pharmaceutical Company, Summit, N. J., for generous supplies of this compd. [i] Confirmation of the findings of D. C. Sharma, E. Forchielli, and R. I. Tamaoki, *Biochim. Biophys. Acta.*, **105**, 516 (1965), and refs cited. [j] See ref 7.

are not general lyase inhibitors.[12] Further, **5**, **7**, and **9** did not inhibit the *in vitro* conversion of corticosterone to 18-hydroxycorticosterone by crude adrenal mitochondria,[12] whereas in the same assay **26** and **27** did significantly (>20% at 0.01 μg/ml of incubate) inhibit this conversion. It thus appears that the steroidal lyase inhibitors herein described are more specific in their action than the nonsteroidal inhibitors **26** and **27**. Indirect evidence indicating specificity, particularly that 21- and 11β-hydroxylation are not inhibited, was obtained by feeding 8 to male rats at 500 mg/kg of diet for 6 weeks.[13] Adrenal weights of the treated animals were not significantly different from those of controls. Inhibition of 21- and/or 11β-hydroxylases would be expected to result in adrenal hypertrophy.

Testes of the rats treated as above showed a 75–90% decrease in testosterone levels as compared with controls.[14] This is considered to be a qualitative *in vivo* correlation with predictions based on the *in vitro*

(12) We are indebted to Thomas Gutwein for the numerous 17,20- and 20,22-lyase inhibition assays herein reported. We also thank James Fridie for the 18-hydroxylation observations. The method used for measuring the cholesterol to pregnenolone conversion is that of S. Ichii, E. Forichelli, and R. I. Dorfman, *Steroids*, **2**, 631 (1963), and for 18-hydroxylation that of D. C. Sharma and R. I. Dorfman, *Biochemistry*, **5**, 1795 (1966).

(13) D. J. Patanelli, C. Berman, R. Primka, and S. L. Steelman, of these laboratories, unpublished results.

(14) The testosterone content of rat testes was determined as follows. Approximately 20 rat testes were homogenized and spiked with tritrated testosterone (5000 cps). Acetone (10 vol) was added to ppt protein which was sepd by centrifugation. The supernatant was reduced to an aq residue in a rotary evaporator. H_2O was added to a total of 10 ml, and the mixt was extd (3 × 15 ml) with EtOAc. The EtOAc ext was evapd to dryness in a stream of N_2. The residue was partitioned between hexane and a 90% MeOH–10% H_2O mixt. The hexane ext containing nonpolar lipids was discarded. The aq MeOH phase was coned to an aq residue in a stream of N_2; 2 ml of H_2O was added and the mixt was extd with EtOAc (3 × 3 ml), the aq layer containing water-sol impurities being discarded. This ext was adsorbed on a silica gel tlc and developed with 50:50 EtOAc–cyclohexane. The testosterone spot was eluted with EtOAc and evapd. An aliquot of this eluate was applied to a 1.7% S.E. 30 glc column whence the mass of the testosterone was detn. Another aliquot was counted (to detn yield through the process). The androstenone (A) and dehydroisoandrosterone (DHA) spots were also eluted from the tlc plate and their masses detn by glc. Their mass detns were corr using the yield figure from the testosterone isolation. Since the A and DHA content of testes of treated rats were also substantially reduced as compared with controls it is clear that the drug is not merely changing the ratios of the testicular androgens.

results. None of the acylaminoandrostenones is antiandrogenic.[15]

Nature of the Inhibition.—The structures of these inhibitors suggest that they are reversible antagonists which bear a homologous relationship to the lyase substrate 17α-hydroxyprogesterone. This lyase reaction requires O_2 and NADPH and the ensuing reaction bears an apparent formal resemblance to the Baeyer–Villiger oxidation. The expected intermediate based on this analogy, 17α-hydroxy-17β-acetoxyandrost-4-en-3-one, would not be expected to be stable, breaking down to androstenedione and AcOH. This postulated mechanism, which was first proposed by Nakano, *et al.*,[16] was based on the observations of Fonken, *et al.*,[17] Rahim and Sih,[18] and Nakano, *et al.*,[19] that progesterone is aerobically converted directly to testosterone acetate and thence to testosterone by *Cladosporium resinae*. We hypothesize that inhibitors of the *in vitro* rat lyase system such as testosterone acetate and the other analogs described in this paper resemble an intermediate or transition state on the enzyme at which a separation of the C-17,20 carbon atoms occurs. The inhibitory compounds, however, lack a 17α-OH group and therefore there is no pathway to products.

Experimental Section[20]

General Methods. I. **Oximes.**—The general method used for the prepns of compds A (Scheme I) is that described in ref 6 (see footnote *b*, Table I). By this method the following new compds were obtained: **6-methylpregnenolone acetate 20-oxime** (**29**) (from 6-methylpregnenolone acetate[21]), mp 195–200° dec, from MeOH, [α]D −70°, *anal.* ($C_{24}H_{36}NO_3$) C, H, N; **17α-methylpregnenolone acetate 20-oxime** (**30**) (from 17α-methylpregnenolone acetate[9]), mp 194–196°, from MeOH, [α]D −48.3°, *anal.* ($C_{24}H_{37}NO_3$) C, H, N.

II. **Beckman Rearrangement.**—The general method used for the prepn of compds B (Scheme I) is that described in ref 6 (see footnote *b*, Table I). By this method the following new compds were obtained: **6-methyl-17β-acetamidoandrost-5-en-3β-yl acetate** (**31**) (from **29**), mp 204–207°, from Me_2CO, [α]D −113°, *anal.* ($C_{24}H_{37}NO_3$) C, H, N; **17α-methyl-17β-acetamidoandrost-5-en-3β-yl acetate** (**32**) (from **30**), mp 194–196°, from MeOH, *anal.* ($C_{24}H_{36}NO_3$) C, H, N.

III. **O-Deacylation.**—The general method used for the prepn of compds C (A′ = H) (Scheme I) is that described in footnote *d*, Table I. By this method the following new compds were obtained: **6-methyl-17β-acetamidoandrost-5-en-3β-ol** (**33**) (from **31**), mp 255–258°, from MeOH, [α]D −116°, *anal.* ($C_{22}H_{35}NO_2$) C, H, N; **16α-methyl-17β-acetamidoandrost-5-en-3β-ol** (**34**) (footnote *e*, Table I); **17α-methyl-17β-acetamidoanerost-5-en-3β-ol** (**35**) (from **32**), mp 284–286°, from MeOH, *anal.* ($C_{22}H_{33}NO_2$) C, H, N. **17β-*N*-Methylacetamidoandost-5-en-3β-ol** was not characterized but was oxidized to the 3-keto-Δ^4 deriv as described below.

IV. **Oppenauer Oxidn.**—The general method for the prepn of compds D (Scheme I) is that given in footnote *d*, Table I. By this method the following compds were prepd: **6α-methyl-17β-acetamidoandrost-4-en-3-one** (**16**) (from **33**), mp 298–301°, from EtOAc, [α]D +15°, *anal.* ($C_{22}H_{33}NO_2$) C, H, N; **16α-methyl-17β-acetamidoandrost-4-en-3-one** (**17**) (from **34**), mp 311–315°, from MeOH, [α]D +23°, *anal.* ($C_{22}H_{33}NO_2$) C, H, N; **17α-methyl-17β-acetamidoandrost-4-en-3-one** (**18**) (from **35**), mp 232–234°, from MeOH, [α]D +66.6°, *anal.* ($C_{22}H_{33}NO_2$) C, H, N; **17α-acetamidoandrost-4-en-3-one** (**25**) (from 17α-acetamidoandrost-5-en-β-ol[11]), mp 252–254°, from EtOAc, [α]D +139.5°, *anal.* ($C_{21}H_{31}NO_2$) C, H, N; **17β-(N^3-acetureido)-androst-4-en-3-one** (**11**) (from **39**), mp 270–272°, from MeOH, *anal.* ($C_{22}H_{32}N_2O_3$) C, H, N; **17β-*N*-methylacetamidoandrost-4-en-3-one** (**19**) (from 17β-*N*-methylacetamidoandrost-5-en-3β-ol), mp 186–188°, from MeOH, [α]D +61.8°, *anal.* ($C_{22}H_{33}NO_2$) C, H, N.

V. **1,4-Dien-3-ones.**—The general procedure is illustrated by the conversion of 17β-acetamidoandrost-4-en-3-one (**5**) to 17β-acetamidoandrosta-1,4-dien-3-one (**21**) as follows. A mixt of **5** (4.5 g), DDQ, and dioxane (90 ml) was heated at reflux under N_2 for 3 hr. Dissoln of all of the reactants occurred and the color changed to deep red. EtOAc (200 ml) was added to the cooled soln and the insol hydroquinone was sepd by filtration and washed once with EtOAc (50 ml). The filtrate was washed with aq 2.5 *N* NaOH which removed most of the color and then with H_2O to neutrality. The org layer was dried (Na_2SO_4) and evapd under reduced pressure leaving crude dienone which was purified by chromatog on Al_2O_3. The product **21**, 4.1 g, eluted with a 7:3 $CHCl_3$–Et_2O mixt, was recrystd from EtOAc–$CHCl_3$, mp 270–272° (*cf.* footnote *f*, Table I).

Using the above procedure, **4** was converted to **17β-formamidoandrosta-1,4-dien-3-one** (**22**), mp 227–228°, from EtOAc–$CHCl_3$, [α]D +53°. *Anal.* ($C_{20}H_{27}NO_2$) C, H, N. Also **11** was converted to **17β-(N^3-acetureido)androsta-1,4-dien-3-one** (**40**), mp 264–265°, from MeOH, [α]D +92.8°. *Anal.* ($C_{22}H_{30}N_2O_3$) C, H, N.

17β-Aminoandrost-5-en-3β-ol·HCl (**36**) was prepd by a slight modification of the method of Schmidt-Thomé[6b] which, however, gives a superior product. A soln of 45 g of NaOH in 150 ml of H_2O was added to a soln of 5 g of 17β-acetamidoandrost-5-en-3β-yl acetate[20] in 350 ml of EtOH. A clear soln resulted. The reaction mixt was heated at 180° in a stirred autoclave for 8 hr under N_2. The contents of the cooled autoclave were filtered through Supercel and the clear, nearly colorless filtrate was evapd under reduced pressure until a thick syrup remained. H_2O (500 ml) was added, and the mixt was extd with $CHCl_3$ (2 × 300 ml). The combined $CHCl_3$ exts were washed once with H_2O (50 ml) which was discarded. To the $CHCl_3$ was added excess 2.5 *N* aq HCl to ppt the amine·HCl, which was then sepd by filtration, washed once with Me_2CO, and dried in a stream of air, yield 40 g, mp (dec) *ca.* 300°.[22] This colorless product was of excellent quality and was used in subsequent reactions without further purification.

17β-*N*-Methylacetamidoandrost-5-en-3β-yl Acetate (**37**).—To a soln of 1.7 g of 17β-acetamidoandrost-5-en-3β-yl acetate in 35 ml of dry DMF was added 1.2 g of a 54.7% dispersion of NaH in mineral oil, and the resulting mixt was stirred at room temp for 1 hr. The reaction mixt was then cooled to 0° and to this was added in a dropwise manner a soln of freshly distd MeI (20 ml) in 17 ml of dry DMF. The reaction mixt was then permitted to warm to room temp and stirred overnight. After cooling to 0°, H_2O was cautiously added to destroy the remaining NaH, followed by sufficient H_2O to ppt the product which was extd into $CHCl_3$ (100 ml). The $CHCl_3$ ext was thoroughly washed with H_2O (5 × 20 ml), dried, and evapd at reduced pressure, finally at 0.1 mm, to remove the remaining DMF. The residue was adsorbed on a 40:1 alumina column from a C_6H_6 soln. The product **37**, was eluted with increasing concns of $CHCl_3$ in C_6H_6 and crystd from MeOH, yield 1.6 g, mp 199°. *Anal.* ($C_{24}H_{37}NO_3$) C, H.[23]

17β-Ureidoandrost-5-en-3β-ol (**9**).—To a suspension of 5 g of **36** and 50 ml of EtOH was added 7.5 g of KOCN. The mixt was heated for 2–3 min on the steam bath and then treated with 7.5 ml of 2.5 *N* HCl. The mixt was then heated at reflux in the steam bath for 15 min and filtered while hot. H_2O was added to the cooled filtrate until crystn was complete. The product **9** was sepd by filtration and washed well with H_2O. It had mp 320°

(15) A definition of this term is implicit in the method used in its detn. See S. L. Steelman, J. R. Brooks, E. R. Morgan, and D. J. Patanelli, *Steroids*, **14**, 449 (1969).

(16) H. Nakano, H. Ivano, H. Sato, M. Shikita, and B. Tamaoki, *Biochim. Biophys. Acta*, **137**, 335 (1967).

(17) G. S. Fonken, H. C. Murray, and L. M. Reinecke, *J. Amer. Chem. Soc.*, **82**, 5507 (1960).

(18) M. A. Rakim and C. J. Sih, *J. Biol. Chem.*, **241**, 3615 (1966).

(19) H. Nakano, H. Sato, and B. Tamaoki, *Steroids*, **12**, 291 (1968).

(20) Melting points are uncorr and were detd on a Kofler hot stage. Rotations were run in $CHCl_3$ (*c* 1) unless otherwise noted and are expressed as $[\alpha]^{25}D$. Uv spectra were determined in EtOH and all 3-keto-Δ^4 and 3-keto-$\Delta^{1,4}$ compounds absorbed at 240–242 mμ and showed ϵ_{max} *ca.* 16,000. All of the new compds showed ir spectra consistent with the assigned structures. Nmr spectra were run on selected compds and were consistent with the findings of Robinson and Ermann.[10b] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(21) Purchased from com sources.

(22) Swiss Patent 187,936; *Chem. Abstr.*, **31**, 5109 (1937).

(23) Unpublished procedure of Dr. N. G. Steinberg of these laboratories.

dec and was recrystd from MeOH, mp 327° dec, $[\alpha]_D$ −67° (*c* 1, MeOH). *Anal.* ($C_{20}H_{28}N_2O_2 \cdot 0.5H_2O$) C, H, N.

17β-(N^3-Acetureido)androst-5-en-3β-yl Acetate (38).—A stirred mixt of 280 g of **9** and 4 l. of Ac_2O was maintained at 50° for 48 hr. It was then poured into 15 l. of a 50:50 ice–H_2O mixt contg 20 ml of Pyr and was stirred until all of the Ac_2O had been destroyed. The cryst ppt was sepd by filtration and washed with H_2O to remove most of the HOAc. The ppt was then dissolved in $CHCl_3$ (1000 ml) and washed sequentially with H_2O (100 ml), satd aq $NaHCO_3$ until the washings were basic, and again with H_2O (100 ml). The dried soln was evapd to dryness under reduced pressure. The product **38** was recrystd from MeOH, yield 245 g, 69.8%, mp 210°, resolidifies and remelts at 227–230°, $[\alpha]_D$ −18°. *Anal.* ($C_{24}H_{36}N_2O_4$) C, H, N.

17β-(N^3-Acetureido)androst-5-en-3β-ol (39).—To a stirred soln of **38** (245 g) in anhyd MeOH under N_2 at room temp was added NaOMe (33 g). The mixt was stirred under N_2 for 16 hr and was then quenched by the addn of gl HOAc (40 ml). The reaction mixt was concd to about 1 l. under reduced pressure and dild with 4 l. of H_2O. The cryst product **39** was sepd by filtration and washed with H_2O (200 ml). The wet filter cake was dissolved in $CHCl_3$ (500 ml), washed with H_2O (2 × 50 ml), dried, and concd *in vacuo*. The residue was recrystd from MeOH, yield 75.8%, mp 221–224°, $[\alpha]_D$ −31.7° (*Anal.* ($C_{22}H_{34}N_2O_3$) C, H, N.

17β-Ureidoandrosta-1,4-dien-3-one (8).—A mixt of **40** (87 g), MeOH (3200 ml), and 2.5 *N* HCl (800 ml) was heated at reflux for 1 hr. The solvents were removed *in vacuo* in a rotating evaporator at 50°, leaving an oily emulsion which was extd with $CHCl_3$ (3 × 200 ml). The $CHCl_3$ exts were washed with H_2O to neutrality after which the solvent was distd *in vacuo*. The residue **8** was crystd from Me_2CO and had mp 248–249°, $[\alpha]_D$ +47.6°, yield 51.8 g. *Anal.* ($C_{20}H_{28}N_2O_2$) C, H, N.

Using this same procedure **11** was converted to **17β-uriedoandrost-4-en-3-one (7)**, mp 208–209°, from MeOH or EtOAc, $[\alpha]_D$ +74.9. *Anal.* ($C_{20}H_{30}N_2O_2$) C, H, N.

Testosterone Carbamate (3).—The reagents and conditions used are those of Loev and Kormendy.[24] To a stirred suspension of testosterone (1 g), NaOCN (455 mg), and CH_2Cl_2 (10 ml) at room temp was added $(F_3CCO)_2O$ (0.52 ml) in CH_2Cl_2 (1 ml) over 10 min. The resulting mixt was stirred overnight, and then dild to 50 ml with CH_2Cl_2. It was washed with H_2O (15 ml). The dried CH_2Cl_2 soln was evapd *in vacuo* at <50° leaving a residue which afforded 700 mg of cryst **3**, from MeOH. The anal. sample was recrystd successively from MeOH and EtOAc and showed mp 158–160°, $[\alpha]_D$ +109.9°, (*c* 1, MeOH). *Anal.* ($C_{20}H_{29}NO_3$) C, H, N.

17β-Propionamidoandrost-4-en-3-one (6).—To a cooled (0°) soln of 17β-aminoandrost-5-en-3-one cyclic ethylene acetal[7] (331 mg) in Pyr (5 ml) was added *n*-PrCl (0.25 ml). The mixt was stirred at room temp overnight. H_2O was added to the homogeneous reaction mixt and the ppt which formed was sepd by filtration, washed well with H_2O, and dried. Two recrystns from MeOH afforded the anal. sample of **17β-propionamidoandrost-5-en-3-one cyclic ethylene acetal (42).** *Anal.* ($C_{24}H_{37}NO_4$) C, H, N. The above dioxolane (250 mg) was dissolved in MeOH (7 ml) and treated with *p*-TsOH (300 mg) and H_2O (1 ml). The stirred soln was maintained at room temp overnight and then dild with H_2O until no further pptn occurred. The product was collected by filtration, washed with H_2O, and dried, yield 160 mg. Crude **6** was recrystd from MeOH–EtOAc for anal., mp 230–231°, $[\alpha]_D$ +55°. *Anal.* ($C_{22}H_{33}NO_2$) C, H, N.

Compd 7 from 17β-Aminoandrost-5-en-3-one Cyclic Ethylene Acetal.—To a hot soln of the dioxolane (6.7 g) in EtOH (65 ml) was added a soln of KOCN (9.5 g) in H_2O (13 ml). Heating was contd until a clear soln resulted. Aq 2.5 *N* HCl (40 ml) was then added to the hot soln at such a rate that a pH >7 was maintained. The mixt was heated at reflux for 15 min after addn of the acid. A ppt formed. For cooling to room temp H_2O was added to ppt the product, **17β-ureidoandrost-5-en-3-one cyclic ethylene ketal**, which was washed well with H_2O, dried, and recrystd from MeOH to which a drop of Pyr had been added, mp 320° dec. *Anal.* ($C_{22}H_{34}N_2O_3$) C, H, N. This compd was converted to **7** by the method used in the conversion of **42** to **6**.

(24) B. Loev and M. F. Kormendy, *J. Org. Chem.*, **28**, 4322 (1963).

17β-Amino-3-methoxy-1,3,5(10)-estratriene (43).—A soln of 3-methoxyestra-1,3,5(10)-trien-17-one oxime[10] (9.0 g) in EtOH (1600 ml) was heated to boiling. Pieces of Na (total 185 g) were added until solids began to separate. EtOH (200 ml) was added to dissolve the solid. The mixt was poured into H_2O (1 l.) and the solvents were distd *in vacuo* to a dry residue. The solids were suspended in H_2O and extd with Et_2O (3 × 200 ml). The Et_2O exts were washed with H_2O, dried, and evapd leaving a residue which crystd from Et_2O. The product **43**, 5.8 g, had mp 110–120° raised to 119–121° by recrystn from CH_2Cl_2–hexane. *Anal.* ($C_{19}H_{27}NO$) C, H, N.

17β-Acetamido-3-methoxy-1,3,5(10)-estratriene (28).—A soln of **43** (250 mg) was acetylated with Pyr–Ac_2O. After the usual work-up there was obtained **28** (260 mg), mp 243–244° (from MeOH), $[\alpha]_D$ −26°. *Anal.* ($C_{21}H_{29}NO_2$) C, H, N.

17β-Acetamidoestr-4-en-3-one (20).—A sample of 17β-amino-3-methoxy-1,3,5(10)-estratriene (**43**) (2.0 g) was reduced with Li–NH_3 (standard Birch reducing conditions) to 17β-amino-3-methoxy-estra-2,5(10)-diene, which was not purified. The crude diene was acetylated with Pyr–Ac_2O by the usual methods, worked up in an unexceptional manner, and then treated in aq MeOH with 2.5 *N* HCl on the steam bath for 15 min. The reaction mixt was dild with H_2O and extrd with $CHCl_3$. The ext was washed, dried, and evapd leaving an oil which was chromatographed on alumina, the product being eluted with Et_2O–$CHCl_3$ mixts. The combined eluates were recrystd from EtOAc and then from MeOH yielding **20**, mp 236–238°, $[\alpha]_D$ −6.1°. *Anal.* ($C_{21}H_{31}NO_2$) C, H, N.

17β-Acetamidoandrost-4-en-3β-ol (13).—To a soln of **5** (0.5 g) in 20 ml of THF was added 1.0 g of LiAl(O-*tert*-Bu)$_3$H. After stirring at room temp for 48 hr the reaction mixt was poured into ice–H_2O satd with NaH_2PO_4. The product was extd into $CHCl_3$ which was washed with H_2O, dried, and evapd. The residue was crystd twice from $CHCl_3$ affording **13**, mp 236–238°, $[\alpha]_D$ +11.4°. *Anal.* ($C_{21}H_{33}NO_2$) C, H, N.

17β-Acetamidoandrosta-4,6-dien-3-one (24).—Using the chloranil procedure essentially as described by Patchett, *et al.* [footnote *g*, Table I], **5** was converted to **24**, mp 250–252°, from EtOAc, $[\alpha]_D$ −26.2°. *Anal.* ($C_{21}H_{29}NO_2$) C, H, N.

17β-Acetamido-5α-androstan-3-one (15).—To a suspension of 17β-acetamido-5α-androstan-3β-ol[6b] (300 mg) in 15 ml of Me_2CO, cooled and stirred in an ice-bath, was added dropwise Jones reagent (0.67 ml). After 10 min H_2O was added until no more pptn occurred. The product was extd with $CHCl_3$, washed with H_2O, dried, and evapd leaving an oil which was chromatographed on alumina. Et_2O eluted the product (130 mg) **15**, mp 228–230°, from EtOAc.

3β-Hydroxyandrost-5-en-17β-yl Guanidine Hydroacetate Monohydrate (12).—A soln of methylisothiourea·HCl (6.7 g) in 30 ml of H_2O was added to a stirred suspension of **34** (10.0 g) in 200 ml of *N*-methylpyrrolidinone. The stirred mixt was treated with KOAc (12 g) and heated to reflux which effected dissoln of the components and was accompanied by evoln of CH_3SH. After 6 hr at reflux, the cooled reaction mixt was poured into cold H_2O. The ppt which formed was sepd by filtration, washed successively with H_2O, MeOH, and Et_2O, and dried *in vacuo*. The crude product weighed 8.5 g and was recrystd from gl HOAc affording 7.17 g of **12**, mp >300°. This product gave a positive Sakaguchi test for guanidines. Repeated elemental analyses failed to give satisfactory values for this prepn. In order to obtain a satisfactory analysis, **12** was converted to 17β-(N^2,N^3-diacetylguanidino)androst-5-en-3β-yl acetate (**45**) as follows. A suspension of **12** (250 mg) in DMF (5 ml) was treated with Et_3N (2.5 ml) and Ac_2O (1.5 ml) and the mixt was heated in a steam bath until the steroid dissolved (*ca.* 15 min). The cooled soln was dild with ice–H_2O, and, after the excess Ac_2O had reacted, was extd with EtOAc (3 × 10 ml). The combined exts were washed successively with H_2O, 10% aq $NaHCO_3$, and satd aq NaCl. The solvents from the dried exts were removed *in vacuo* leaving a residue which was adsorbed from C_6H_6 on alumina. The product **45** (90 mg) was eluted with C_6H_6 and crystd from EtOAc, mp 177–178°. *Anal.* ($C_{26}H_{39}N_3O_2$) C, H, N.

17α-Ureidoandrost-5-en-3β-ol (10) was prepd from **17α**-aminoandrost-5-en-3β-ol by the method described above for the prepn of **9**, mp >300° dec, $[\alpha]_D$ −72° (*c* 0.5, MeOH). *Anal.* ($C_{20}H_{32}N_2O_2$) C, H, N.