17β-Isoxazolyl and 17β-Pyrazolyl Steroids from 3β-Hydroxy-21-formylpregn-5-en-20-one. Structural Assignments[1,2]

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3β-Hydroxy-21-formylpregn-5-en-20-one (**3**) exists mainly in the 21-hydroxymethylene tautomeric form. When **3** was allowed to react with hydroxylamine hydrochloride in acetic acid, a mixture of 17β-(3-isoxazolyl)-5-androsten-3β-ol acetate (**4**) and 17β-(5-isoxazolyl-5-androsten-3β-ol acetate (**5**) was obtained; treatment with base allowed the isolation of 17β-(3-isoxazolyl)-5-androsten-3β-ol (**6**). Reaction of both **3** and 3β-hydroxy-21-formylpregn-5-en-20-one sodium enolate (**2**) with hydroxylamine hydrochloride in sodium acetate buffer gave exclusively 17β-(5-isoxazolyl)-5-androsten-3β-ol (**8**). The reaction of **2** or **3** with methylhydrazine gave a mixture of two isomeric 17β-N-methylpyrazolyl derivatives. When compounds **2** and **3** were treated with hydrazine and phenylhydrazine, only one 17β-3(5)-pyrazolyl and one 17β-N-phenylpyrazolyl steroid were obtained. Chemical evidence has been adduced for the 17β configuration of the pyrazole rings, which supports the nmr and molecular rotation differences data.

The Claisen condensation of 3β-hydroxypregn-5-en-20-one (**1**) or its 3β acetate with ethyl formate proceeds *via* the ketone–enolate anion.[3] The 20-keto group in **1** enolizes toward either C-17 or C-21,[4,5] which theoretically could lead to formylation at either position. The reaction, carried out in benzene, gave a product which separated from the reaction mixture. As could be anticipated[6] this was the sodium enolate of the 21-formyl derivative. Because of the greater solubility of 3β-acetoxypregn-5-en-20-one in benzene,[7] this starting material was preferred to 3β-hydroxypregn-5-en-20-one. Regardless of the starting material, the same sodium enolate (**2**) was obtained as determined by the infrared spectrum.

The free formyl derivative (**3**) was obtained by acidification of the aqueous solution of **2** with acetic acid, followed by extraction with ether or benzene. In some instances, the benzene extract of **3** was used without further purification to carry out reactions; this was done in order to avoid large losses which arose in the purification step.

The infrared spectrum of **3** had the characteristic broad band for a β-dicarbonyl function,[8] but it did not show the absorption typical of a chelated hydroxyl group, as reported for related compounds.[9]

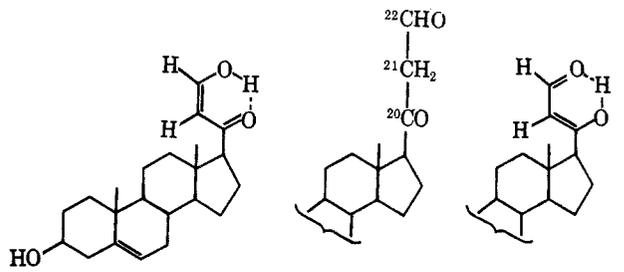
The ultraviolet spectrum of **3** had λ_{max}^{EtOH} 272 mμ (ε 8700) shifted to 291 mμ (ε 17,600) with base, and to 237 mμ (ε 5200) with acid, in good agreement with reported results.[10]

Attempts to prepare a pure sample of **3** for analysis were unsuccessful. However, the cupric chelate derivative of **3**, which was obtained from the sodium enolate **2**, gave the correct analysis.

Both the 21-formyl derivative **3** and its sodium salt **2** gave a deep red color on reaction with ferric chloride.

When **3** was chromatographed by thin layer chromatography, a long streak was observed, suggesting that transformations were occurring on the adsorbent surface. When **3** was chromatographed through neutral alumina, cleavage of the β-ketoaldehyde system occurred and pregnenolone was obtained as the sole product.[11]

On theoretical grounds, **3** could exist in any of the tautomeric forms **3a**, **3b**, or **3c** or as some equilibrium mixture of these. The nmr spectrum in deuteriochloroform demonstrated that **3a** was the main form present in this solvent; there was no signal corresponding to an aldehydic proton[12] but two doublets were observed, centered at δ 7.93 (J = 4.5 cps) and 5.53 (J = 4.5 cps), corresponding to one proton each. The δ 7.93 signal is due to C_{22}–H and the one at 5.53 to C_{21}–H.[13]



3a **3b** **3c**

In acidic media, **3b** is in dynamic equilibrium with **3a** as suggested by the nmr spectrum carried out in acetic acid-d_4. Using this deuterium-exchange solvent, the nmr spectrum showed, immediately after solution, that the two doublet signals, shifted now to

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(2) This work was supported by a grant (CA-04132) from the U. S. Public Health Service and by a fellowship from the National Institutes of Health (1-F3-AM-19,669-01) to one of us (L. M.).

(3) C. R. Hauser, F. W. Swamer, and J. T. Adams, *Org. Reactions*, **8**, 62 (1954).

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(7) "The Merck Index," 7th ed, Merck and Co., Inc., Rahway, N. J., 1960, p 851.

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TABLE I

NUCLEAR MAGNETIC RESONANCE DATA OF 17β-ISOXAZOLYL AND 17β-PYRAZOLYL STEROIDS[a]

Compound	Chemical shift, ppm: C_{19}–H	C_{18}–H	N–CH_3	N–C_6H_5	C_3–H	C_4–H	C_5–H	Coupling constants, cps
17β-(3-Isoxazolyl)-5-androsten-3β-ol acetate (**4**)	1.03	0.58				6.21 d	8.35 d	J_{4-5} = 1.5
17β-(5-Isoxazolyl)-5-androsten-3β-ol acetate (**5**)	1.03	0.56			8.17 d	6.01 d		J_{3-4} = 1.5
17β-(1-Methyl-3-pyrazolyl)-5-androsten-3β-ol acetate (**12**)	1.03	0.55	3.86			6.05 d	7.26 d	J_{4-5} = 2.0
17β-(1-Methyl-5-pyrazolyl)-5-androsten-3β-ol acetate (**13**)	1.02	0.64	3.83		7.42 d	6.08 d		J_{3-4} = 2.0
17β-(1-Phenyl-5-pyrazolyl)-5-androsten-3β-ol acetate (**23**)	0.96	0.68		7.42	7.63 d	6.28 d		J_{3-4} = 2.0

[a] Measured on a Varian A-60 spectrometer, using deuteriochloroform as solvent and tetramethylsilane (TMS) as internal reference. Chemical shifts are given in δ units (*i.e.*, TMS = 0) and coupling constants J in cps (d = doublet).

δ 7.97 and 5.59,[14] began to lose intensity with the simultaneous appearance of a new signal at 7.97. The almost complete disappearance of the doublet signals took about 0.5 hr, leaving as the only detectable peak a singlet at δ 7.97. The exchange of deuterium for the C_{21}–H eliminated the spin–spin coupling,[15] permitting the appearance of the C_{22}–H as a singlet. Deuterium exchange at C-21 could only occur through the free β-ketoaldehydic form, this being evidence for an equilibrium mixture of species **3a** and **3b** in acetic acid medium.

Isoxazolyl Steroids. A. Configuration at C-17.—The β-keto aldehyde **3** was treated with hydroxylamine under a variety of experimental conditions. The products of these reactions were 17β-isoxazolyl steroids without any evidence of formation of 17α isomers. The possible formation of 17α-isoxazolyl steroids was anticipated in view of the fact that **1** partially epimerizes to isopregnenolone in the presence of base.[16] Thus, **3** might have been contaminated with 21-formylisopregnenolone.

The 17β configuration of the isolated isoxazolyl derivatives was established by nmr spectra (Table I) and molecular rotation differences. The nmr data are in good agreement with those reported by Wechter and Murray[17] for the C-13 methyl hydrogens in 17β-acetyl steroidal derivatives (δ 0.60–0.67 downfield from tetramethylsilane). By this technique they are distinguishable from the isomeric 17α-acetyl derivatives (δ 0.80–0.84).

The molecular rotation differences show contributions of negative sign as would be expected from 17β derivatives[18] (see Table II).

B. Characterization of the Isomeric Isoxazolyl Derivatives.—When **3** was treated with hydroxylamine hydrochloride in acetic acid,[19] a mixture of the isoxazoles **4** and **5** was obtained. Acid-catalyzed acetylation of the 3β-hydroxy function occurred. The mixture of isomeric isoxazoles showed in the infrared spectrum sharp absorption bands of medium intensity at 6.28 and 6.40 μ. The isomers **4** and **5** were barely separable on thin layer (tlc) and vapor phase chromatography (vpc) (see the Experimental Section); however, attempts to separate them by preparative chromatography or fractional crystallization failed.

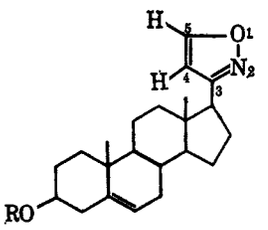
TABLE II

MOLECULAR ROTATION DIFFERENCES (MRD)[a] SHOWING THE NEGATIVE CONTRIBUTIONS IN 17β-ISXOAZOLYL- AND 17β-PYRAZOLYL-SUBSTITUTED STEROIDS

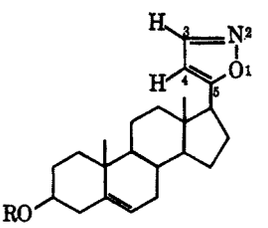
Compound	$[M]^{25}D$	MRD
3β-Hydroxypregn-5-en-20-one	+79	
3β-Hydroxypregn-5-en-20-one 3-acetate	+54	
Pregn-4-en-3,20-dione	+635	
17β-(3-Isoxazolyl)-5-androsten-3β-ol (**6**)	−143	−222
17β-(3-Isoxazolyl)-5-androsten-3β-ol acetate (**4**)	−176	−230
17β-(5-Isoxazolyl)-5-androsten-3β-ol (**8**)	−140	−219
17β-(5-Isoxazolyl)-5-androsten-3β-ol acetate (**5**)	−153	−207
17β-(5-Isoxazolyl)-4-androsten-3-one (**9**)	+485	−140
17β-(1-Phenyl-5-pyrazolyl)-5-androsten-3β-ol (**15**)	−462	−541
17β-(1-Phenyl-5-pyrazolyl)-5-androsten-3β-ol acetate (**23**)	−504	−558
17β-(1-Phenyl-5-pyrazolyl)-4-androsten-3-one (**17**)	+41	−594
17β-(1-Methyl-3-pyrazolyl)-5-androsten-3β-ol (**10**)	−188	−267
17β-(1-Methyl-3-pyrazolyl)-5-androsten-3β-ol acetate (**12**)	−218	−272
17β-(1-Methyl-5-pyrazolyl)-5-androsten-3β-ol (**11**)	−230	−309
17β-(1-Methyl-5-pyrazolyl)-5-androsten-3β-ol acetate (**13**)	−266	−280
17β-[3(5)-Pyrazolyl]-5-androsten-3β-ol (**18**)	−187	−266
17β-[3(5)-Pyrazolyl]-5-androsten-3β-ol acetate (**24**)	−153	−207

[a] See the Experimental Section for specific rotations.

5-Monosubstituted isoxazoles readily form α-cyano ketone enolates upon treatment with strong base, at room temperature.[20] The 3-substituted isoxazoles are unchanged under these conditions. Thus, treatment of the mixture of isomeric isoxazoles **4** and **5**



4, R = CH_3CO
6, R = H



5, R = CH_3CO
8, R = H

with sodium methoxide yielded 17β-(3-isoxazolyl)-5-androsten-3β-ol (**6**) and 3β-hydroxy-21-cyanopregn-5-en-20-one sodium enolate (**7**). The latter absorbed in the ultraviolet region at λ_{max} 264 mμ, with the maximum intensity (ε 13,000) being reached after

(14) (a) Solvent changes may produce signal displacements: N. S. Bhacca and D. H. Williams, "Applications of N.M.R. Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 159; (b) 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt was used as internal standard when acetic acid-d_4 was used as a solvent.

(15) See ref 12b, p 77.

(16) M. B. Rubin, *Steroids*, **2**, 561 (1963).

(17) W. J. Wechter and H. C. Murray, *J. Org. Chem.*, **28**, 755 (1963).

(18) D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951).

(19) W. S. Johnson and W. E. Shelberg, *ibid.*, **67**, 1749 (1945).

(20) For a recent review on isoxazole chemistry, see N. K. Kochetkov and S. D. Sokalov, "Advances in Heterocyclic Chemistry," Vol. 2, A. R. Katritzky, Ed., Academic Press Inc., New York, N. Y., 1963, p 365.

a reaction time of 90 min. After acidification it gave 3β-hydroxy-21-cyanopregn-5-en-20-one.

Isoxazole **6** showed an absorption peak in the infrared spectrum at 6.40, but not at 6.28 μ.

The reaction of **3** with hydroxylamine hydrochloride in glacial acetic acid, buffered with sodium acetate, yielded only 17β-(5-isoxazolyl)-5-androsten-3β-ol (**8**). This was demonstrated by its ready conversion to the cyano ketone enolate **7** on treatment with base, by the 6.28-μ band in the infrared spectrum and by tlc and vpc. Compound **8** was obtained also, as the sole product, by reaction of **2** with hydroxylamine hydrochloride in a solvent mixture of water, ethanol, and acetic acid; here, sodium acetate formed in the reaction acted as the buffering agent. Evidently, the pH of the reaction media has great influence in determining the products formed.

The Oppenauer oxidation of isoxazole **8** gave 17β-(5-isoxazolyl)-4-androsten-3-one (**9**).

The isomeric 3- and 5-alkyl monosubstituted isoxazoles can be differentiated by means which are nonchemically destructive. When the pair of isomers is available, nmr data can be used for this purpose. As can be seen from Table I, isoxazole **5** shows the resonance signals corresponding to C_3–H and C_4–H at a higher field than the corresponding C_5–H and C_4–H in isoxazole **4**.

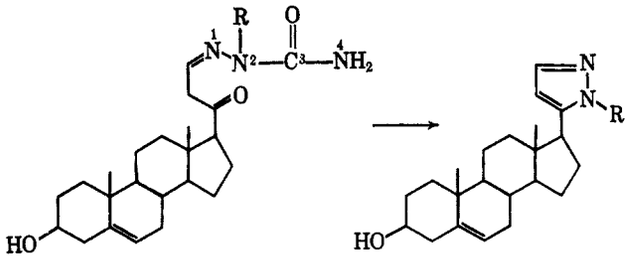
When only one of the isomeric 3- and 5-alkyl-substituted isoxazoles is available, the criteria for determining the location of the substituent can be infrared or ultraviolet spectra. A sharp band at 6.40 μ would indicate the 3-alkyl-substituted isoxazole, while one at 6.28 μ would correspond to the 5-alkyl-substituted isoxazole.

In the electronic spectra we find that the 5-alkyl-isoxazoles absorb at 217 mμ (ε 7600), while the 3-alkyl-isoxazoles have terminal absorption.

Pyrazolyl Steroids. A. Location of the N-Methyl and N-Phenyl Substituents.—The reaction of compounds **2** and **3** with methylhydrazine under a variety of experimental conditions afforded mixtures of 17β-(1-methyl-3-pyrazolyl)-5-androsten-3β-ol (**10**) and 17β-(1-methyl-5-pyrazolyl)-5-androsten-3β-ol (**11**), as shown by tlc and vpc. The mixture of compounds **10** and **11**, as the 3β-acetates, 17β-(1-methyl-3-pyrazolyl)-5-androsten-3β-ol acetate (**12**), and 17β-(1-methyl-5-pyrazolyl)-5-androsten-3β-ol acetate (**13**), was separated by fractional crystallization or by preparative tlc.

We showed earlier that the reaction of β-ketoaldehyde **3** with hydroxylamine hydrochloride in acetate-buffered medium yields exclusively 17β-(5-isoxazolyl)-5-androsten-3β-ol. This clearly indicates that the aldehydic carbon (C_{22}) is more susceptible to nucleophilic attack than the ketonic carbon (C_{20}), under the experiment conditions. Use of this property was made to determine the location of the N-methyl substituents in the pyrazoles **10** and **11**.

The reaction of the sodium enolate **2** with 2-methylsemicarbazide[21] in acetic acid solution yielded the 22-(2-methylsemicarbazone) steroid (**14**), which on pyrolysis[22] afforded the expected pyrazole **11**, exclusively.



14, R = CH_3
16, R = C_6H_5

11, R = CH_3
15, R = C_6H_5

Consequently the position of the N-methyl group in **10** was also established.

In a recent paper, Albright and Goldman[23] differenciated the isomeric N-methyl pyrazoles **12** and **13** by nmr spectroscopy; they confirmed our earlier results[1] (see Table I).

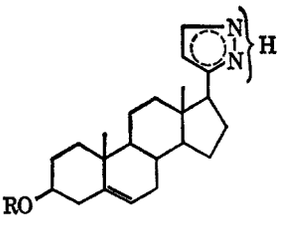
We also found that the isomeric N-methyl 3- and 5-monoalkyl substituted pyrazoles can be differentiated by their ultraviolet spectra. Pyrazoles **10** and **12** have λ_{max} 221 mμ and **11** and **13** show λ_{max} 214 mμ.

The reaction of the β-ketoaldehyde **3** or its sodium enolate **2** with phenylhydrazine gave only one N-phenylpyrazolyl derivative. It is known that the β-nitrogen atom of phenylhydrazine is the most nucleophilic[24] and that the aldehydic carbon atom in a β-ketoaldehyde is the most susceptible to nucleophilic attack.[25] Therefore, the isolated compound was expected to be 17β-(1-phenyl-5-pyrazolyl)-5-androsten-3β-ol (**15**). The assignment of this structure was confirmed when the β-ketoaldehyde **3** was treated with 2-phenylsemicarbazide[26] in an acetate buffered medium to give the 22-(2-phenylsemicarbazone) derivative (**16**); pyrolysis of **16** gave a N-phenylpyrazole as the sole product, which proved to be identical with **15**.

The N-phenylpyrazole **15** forms a very stable solvate with benzene, in a ratio of 2:1, as was demonstrated by the nmr spectrum (see the Experimental Section).

17β-(1-phenyl-5-pyrazolyl)-4-androsten-3-one (**17**) was prepared by the Oppenauer oxidation of **15**.

The reaction of the β-ketoaldehyde **3** with hydrazine gave 17β-[3(5)-pyrazolyl]-5-androsten-3β-ol (**18**). The mobility of the imino hydrogen between the nitrogen atoms in N-unsubstituted pyrazoles has been reported.[27a] Nevertheless, it is possible that one of the isomeric forms predominates.[27b] At this time, we cannot assign the proton to a single structure.



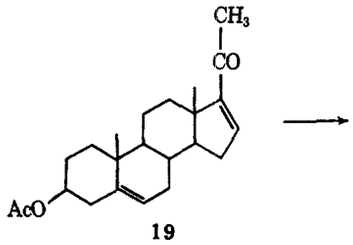
18, R = H
24, R = CH_3CO

(21) G. Bruning, *Ann.*, **253**, 11 (1889).
(22) T. L. Jacobs, in "Heterocyclic Compounds," Vol. 5, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, p 53.
(23) J. D. Albright and L. Goldman, *J. Org. Chem.*, **31**, 273 (1966).
(24) See ref 22, p 50.
(25) R. A. Barnes, ref 22, p 454.
(26) R. C. Goodwin, J. R. Bailey, *J. Am. Chem. Soc.*, **46**, 2887 (1924).
(27) (a) See ref 22, p 91; (b) C. L. Habraken and J. A. Moore, *J. Org. Chem.*, **30**, 1892 (1965).

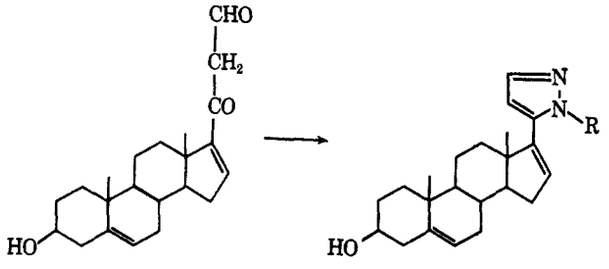
For reasons explained previously, it was necessary to confirm the 17β configuration of the pyrazole rings. The nmr data of the C_{18} hydrogens (see Table I) favored this assignment.[17] Molecular rotation differences also supported the 17β configuration, because of the negative contributions[18] (see Table II).

B. Chemical Proof for the 17β-Pyrazolyl Configuration.—Chemical evidence for the 17β configuration of the pyrazole derivatives was sought. The reaction of pregna-5,16-dien-20-one 3β-acetate (**19**) with ethyl formate under basic conditions[3] gave 3β-hydroxy-21-formylpregna-5,16-dien-20-one[28] (**20**).

The β-ketoaldehyde **20** was treated with hydrazine, methylhydrazine, and phenylhydrazine to give in each reaction only one $\Delta^{5,16}$-17-pyrazolyl derivative. These last results show that the C_{22} is more prone to nucleophilic attack in compound **20** than is the case in



19



20 **21**, R = C_6H_5
26, R = CH_3
25, R = H

compound **3**; this may be due to the fact that the C_{20} carbonyl in compound **20** is conjugated with the Δ^{16} double bond.

It is known that the catalytic hydrogenation of the Δ^{16} double bond in $\Delta^{5,16}$-20-keto steroids is stereospecific and yields Δ^{5}-17β derivatives.[29] We expected that, similarly, the stereoselective hydrogenation of $\Delta^{5,16}$-17-pyrazolyl steroids would lead predominantly, if not exclusively, to the Δ^{5}-17β-pyrazolyl derivatives. Hydrogenation of 17-(1-phenyl-5-pyrazolyl)-5,16-androstadien-3β-ol (**21**) and 17-(1-methyl-5-pyrazolyl)-5,16-androstadien-3β-ol acetate (**22**) yielded exclusively **15** and **13**. The identity of these compounds was established by melting points and spectral and chromatographic data.

The identification of the hydrogenation products established unequivocally the 17β configuration of the pyrazolyl rings in **13** and **15** as well as the locations of the N-phenyl and N-methyl substituents in pyrazoles **21** and **22**.

The 3β-acetates from the pyrazolyl steroids were prepared (see the Experimental Section).

(28) This compound **20** was used in the same benzene solution obtained during its synthesis. The estimated yield was very low, as judged by the amount of $\Delta^{5,16}$-17-pyrazolyl steroids obtained on reaction with hydrazines. The low yield of the β-ketoaldehyde **20** is attributed to the known side reactions of **19** under the basic conditions used in this synthesis (see ref 18).

(29) (a) H. H. Inhoffen and O. Hess, German Patent 876,407 (May 11, 1953); *Chem. Abstr.*, **52**, 8221c (1958); (b) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948); (c) T. F. Gallagher and T. H. Kritchevsky, *J. Am. Chem. Soc.*, **72**, 882 (1950).

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Ultraviolet spectra were obtained with a Beckman Model DB spectrophotometer using 95% ethanol solutions, unless otherwise indicated. Infrared spectra were obtained on a Perkin-Elmer Infracord spectrophotometer using chloroform as the solvent unless otherwise noted. Optical rotations were determined in chloroform (*c* 1.0) at 25° using a Rudolph polarimeter. Elemental analyses were obtained from Weiler and Strauss, Oxford, England. Woelm alumina (neutral, activity grade III) with a ratio of alumina to steroid of 30:1, was used for chromatographic separations.

Thin layer chromatography was carried out using 0.25-mm layers of silica gel G. The samples and cholesterol (reference compound) were developed on the same plates usiug a mixture of benzene–ethyl acetate (1:1) and the relative R_f values ($R_{cholesterol}$) were determined.[30]

Vapor Phase Chromatography.—An E.I.R. instrument was used, with an argon ionization detector (^{90}Sr). The column was 1% SE-30 on 100–120 mesh silanized Gas-Chrom P. The glass column was 6 ft long, 4 mm in i.d. Operating conditions were column temperature, 235°; injector block, 300°; detector, 250°. Argon flow rate was 40 ml/min at outlet (inlet pressure 30 psi). Retention times and relative retention times (cholesterol = 1.0) were determined.

3β-Hydroxy-21-formylpregn-5-en-20-one Sodium Enolate (2). A.—To a suspension of sodium methoxide (43.2 g) in benzene (200 ml) was added a solution of pregnenolone acetate (143.2 g) in benzene (600 ml), while stirring and cooling (ice–water). After 10 min, a mixture of ethyl formate (59.2 g) in benzene (200 ml) was added dropwise, over a 30-min period. Stirring was continued for 2 hr and the resultant suspension was left overnight at room temperature. After filtration, the insoluble sodium–enolate **2** was washed successively with benzene, ether, isopropyl alcohol, and ether. After drying, 140.0 g of crude **2** was obtained: mp 269–280°; λ_{max} 291 mμ (ε 17,000); λ_{max}^{KBr} 2.85 (s, 3β-OH), 6.18 (m), and 6.82 μ (m).

B.—A solution of 3β-hydroxypregn-5-en-20-one (15.8 g) in benzene (1900 ml) was added, with stirring, to a suspension of sodium methoxide (13.6 g) in benzene (250 ml). After 30 min, ethyl formate (14.8 g) was added dropwise. The resulting suspension was worked out as outlined above to give **2** (15.5 g).

3β-Hydroxy-21-formylpregn-5-en-20-one (3). A.—To a solution of **2** (12.0 g) in ice-cold water (1000 ml) was added acetic acid (3 ml); compound **3** separated and was extracted with ether (four 500-ml portions). The combined ether extracts were washed with water, dried over anhydrous sodium sulfate, and concentrated to a final volume of about 250 ml when solid **3** separated. On filtration, 8.2 g of **3** was obtained: mp 111–115°; $[\alpha]_D$ +37°; λ_{max} 272 mμ (ε 8700); $\lambda_{max}^{EtOH,\ HCl}$ 237 mμ (ε 5200);[31] $\lambda_{max}^{EtOH,\ NaOH}$ 291 mμ (ε 17,600);[32] λ_{max} 2.72 (w, 3β-OH), 5.89 (w, C=O) and 6.11–6.25 μ (s, broad) (enolic β-ketoaldehyde).

B.—A suspension of **2** (70.0 g) in benzene (500 ml) was acidified with acetic acid, washed with water, and dried over anhydrous sodium sulfate. The resulting solution of **3** in benzene was used in some of the reactions described below.

Cupric Derivative of 3β-Hydroxy-21-formylpregn-5-en-20-one.—A solution of cupric acetate (1.5 g) in a mixture of water (100 ml) and glacial acetic acid (140 ml) was added to a solution of **2** (3.0 g) in a mixture of water (500 ml) and ethanol (350 ml). A gelatinous precipitate formed immediately. The consistency of this precipitate changed sufficiently for filtration after 1 hr. Three recrystallizations from benzene yielded the analytical sample: mp 180–190° dec; λ_{max} 244 mμ (ε 12,300) and 302 mμ (ε 19,200); λ_{max}^{KBr} 2.86 (m, 3β-OH), 6.27 (m), 6.63 (m), and 6.86 μ (m).

(30) K. Randerath, "Thin-Layer Chromatography," Academic Press Inc., New York, N. Y., 1963, p 5.

(31) Obtained in 95% ethanol containing 2% 1 *N* hydrochloric acid solution.

(32) Obtained in 95% ethanol containing 2% 1 *N* sodium hydroxide solution.

Anal. Calcd. for $C_{44}H_{62}CuO_6$: C, 70.41; H, 8.83. Found: C, 70.89; H, 8.48.

Chromatographic Properties of 3.—Tailing was observed when **3** was subjected to tlc using variable ratios of benzene and ethyl acetate as eluents. When **3** (250 mg), dissolved in benzene (3 ml), was chromatographed through a column of neutral alumina activity grade I, it yielded 3β-hydroxypregn-5-en-20-one (200 mg, mp 190–192°) as the only product isolated.

17β-(3-Isoxazolyl)-5-androsten-3β-ol (6).—A mixture of **3** (4.0 g), hydroxylamine hydrochloride (1.6 g), and glacial acetic acid (100 ml) was stirred for 27 hr at room temperature[19] and then poured into ice-cold water. The solid which separated was filtered and dissolved in ether, and the ether extract was washed with water, dried, and evaporated to dryness to give a product, 4.0 g, mp 150–161°. This product was shown by tlc and vpc to be a mixture of **4** and **5**. In the course of the reaction acid-catalyzed acetylation at 3β-OH occurred. The infrared spectrum showed λ_{max} 5.75 (s) and 7.95 (s, 3β-acetate), 6.28 (m, 5-alkyl monosubstituted isoxazole), and 6.40 μ (m, 3-alkyl monosubstituted isoxazole).

A portion of this product (1.5 g) dissolved in ether (20 ml), was treated with a solution of sodium methoxide (1.0 g) in methanol (10 ml). In 20 min crystals began to separate. After separation was complete, the crystals were filtered, dissolved in benzene, and chromatographed on alumina using benzene–ethyl acetate (8:2) as the eluent. After one crystallization from ether, analytical **6** (500 mg) was obtained: mp 205.5–207°; $[\alpha]_D$ −42°; λ_{max} terminal absorption; λ_{max} 2.75 (w, free 3β-OH), 2.89 (w, broad, bonded 3β-OH), and 6.40 μ (m, 3-alkyl monosubstituted isoxazole); $R_{cholesterol}$, 0.78; relative retention time, 0.69.

Anal. Calcd for $C_{22}H_{31}NO_2$: C, 77.37; H, 9.15; N, 4.10. Found: C, 77.72; H, 8.89; N, 4.12.

The remaining filtrate was acidified with diluted hydrochloric acid, dried, and evaporated to dryness. The infrared spectrum showed a band at 4.39 (vw, C≡N) and another at 5.75 μ (s, C=O). The electronic spectrum had $\lambda_{max}^{EtOH, NaOH}$ 264 mμ (ε 12,000).[32] This compound was therefore identified as 3β-hydroxy-21-cyanopregn-5-en-20-one.[20]

17β-(3-Isoxazolyl)-5-androsten-3β-ol Acetate (4).—A solution of **6** (700 mg) in pyridine (30 ml) and acetic anhydride (30 ml) was left at room temperature overnight. The residue obtained by evaporation *in vacuo* was chromatographed on alumina using benzene as eluent to give **4**: 500 mg; mp 169.5–171.5°; $[\alpha]_D$ −46°; λ_{max} terminal absorption; λ_{max} 5.76 (s), 7.96 (s, 3β-acetate), and 6.40 μ (m, 3-alkyl monosubstituted isoxazole); $R_{cholesterol}$, 1.23; relative retention time, 0.98.

Anal. Calcd for $C_{24}H_{33}NO_3$: C, 75.16; H, 8.67; N, 3.65. Found: C, 74.93; H, 8.19; N, 3.76.

17β-(5-Isoxazolyl)-5-androsten-3β-ol (8). A.—To the benzene solution of **3** (100 ml; see above) were added glacial acetic acid (100 ml) and a solution of hydroxylamine hydrochloride (2.0 g) and sodium acetate (2.0 g) in water (10 ml). This mixture was made homogeneous by the addition of ethanol (55 ml) and then refluxed for 2 hr. After evaporation to dryness, *in vacuo* the residue was taken up with water and chloroform. The chloroform layer was washed with water, dried, and evaporated to dryness to give the product (it showed one spot on tlc). This product was chromatographed on alumina using a mixture of benzene and chloroform (7:3) as eluent. On recrystallization from benzene or ethanol, the isoxazole **8** had mp 198–202°. When ether was used for recrystallization, the melting point was lower (187.5–193°). Compound **8** had $[\alpha]_D$ −41°; λ_{max} 217 mμ (ε 7600); $\lambda_{max}^{MeOH, NaOMe}$ 264 mμ (ε 13,000); λ_{max} 2.73 (w), 2.90 (w, broad, 3β-OH), 6.28 μ (m, 5-alkyl monosubstituted isoxazole); $R_{cholesterol}$, 0.83; relative retention time, 0.70.

Anal. Calcd for $C_{22}H_{31}NO_2$: C, 77.37; H, 9.15; N, 4.10. Found: C, 76.82; H, 9.18; N, 4.26.

B.—A solution of hydroxylamine hydrochloride (5.0 g) in 15% acetic acid (120 ml) was added to a solution of **2** (10.0 g) in ethanol (230 ml) and the mixture was refluxed for 5 hr. After evaporation to dryness *in vacuo*, the compound was isolated as described above to give **8** (5.2 g), mp 198–202° (from ethanol).

17β-(5-Isoxazolyl)-5-androsten-3β-ol Acetate (5).—Isoxazole **8** (2.0 g) was dissolved in a 1:1 mixture of pyridine–acetic anhydride (100 ml). After 15 hr at room temperature, the mixture was worked up in the same way as **4**, to give **5**: 1.5 g; mp 182.5–184.5°; $[\alpha]_D$ −40°; λ_{max} 217 mμ (ε 9000): λ_{max} 5.75 (s), 7.94 (s, 3β-acetate), and 6.28 μ (m, 5-alkyl monosubstituted isoxazole); $R_{cholesterol}$], 1.46; relative retention time, 1.01.

Anal. Calcd for $C_{24}H_3NO_3$: C, 75.16; H, 8.67; N, 3.65. Found: C, 74.85; H, 8.83; N, 3.80.

17β-(5-Isoxazolyl)-4-androsten-3-one (9).—A mixtute of isoxazole **8** (2.0 g), aluminum isopropoxide (3.0 g), toluene (30 ml), and cyclohexanone (30 ml) was refluxed for 1 hr and cooled, and then a saturated solution of potassium bitartrate (100 ml) was added. The organic layer was separated, washed with water, dried, and evaporated *in vacuo*. When the residue was triturated with petroleum ether (bp 30–60°; 20 ml) the crystalline product **9** separated. This compound was chromatographed on alumina and recrystallized from 95% ethanol to give **9**: 1.0 g; mp 173.5–174°; $[\alpha]_D$ +143°; λ_{max} 235 mμ (ε 15,600); λ_{max} 6.00 (s, C=O), 6.18 (m, C=C), and 6.28 μ (m, 5-alkyl monosubstituted isoxazole); $R_{cholesterol}$, 1.23; relative retention time, 0.93.

Anal. Calcd for $C_{22}H_{29}NO_2$: C, 77.84; H, 8.61; N, 4.13. Found: C, 77.67; H, 8.66; N, 4.30.

17β-(1-Methyl-3-pyrazolyl)-5-androsten-3β-ol (10).—A mixture of **2** (5.5 g), methylhydrazine sulfate (4.5 g), ethanol (125 ml), acetic acid (12 ml), and water (25 ml) was refluxed for 1 hr., cooled, and poured into ice-cold water. A solid formed and it was separated by filtration, washed with water, and dried to yield a product, mp 210–232°. Tlc indicated the presence of two compounds, **10** being the major one. Chromatography on alumina, using benzene as eluent, followed by three recrystallizations from ethanol yielded pyrazole **10**: 3.0 g; mp 206.5–209.5°; $[\alpha]_D$ −53°; λ_{max} 221 mμ (ε 8000); λ_{max} 2.73 (w), 2.90 (w, broad, 3β-OH, free and hydrogen bonded), 6.20 (w), and 6.56 μ (m, pyrazole ring stretching); $R_{cholesterol}$, 0.42; relative retention time, 0.84.

Anal. Calcd for $C_{23}H_{34}N_2O$: C, 77.92; H, 9.67; N, 7.90. Found: C, 77.63; H, 9.39; N, 8.40.

Of the two compounds present in the 210–232° mixture, **10** had the higher R_f value on tlc. The substance with the lower R_f was found to be **11** (see below).

17β-(1-Methyl-3-pyrazolyl)-5-androsten-3β-ol Acetate (12).—A solution of **10** (1.0 g) in a 1:1 mixture (22 ml) of pyridine and acetic anhydride was left at room temperature for 15 hr. After evaporation, *in vacuo*, the residue was chromatographed on alumina, using benzene as solvent. Recrystallization from acetonitrile yielded **12**: 650 mg; mp 171–173°; $[\alpha]_D$ −55°; λ_{max} 221 mμ (ε 6700); λ_{max} 5.76 (s), 7.95 (s, 3β-acetate), 6.22 (w), and 6.56 μ (m, pyrazole ring); $R_{cholesterol}$, 0.94; relative retention time, 1.20.

Anal. Calcd for $C_{26}H_{36}N_2O_2$: C, 75.72; H, 9.15; N, 7.06. Found: C, 75.49; H, 8.97; N, 7.16.

17β-(1-Methyl-5-pyrazolyl)-5-androsten-3β-ol Acetate (13).—A mixture of **2** (10.0 g), acetic acid (150 ml), and methylhydrazine (8.0 g) was refluxed for 1 hr. The solvent was evaporated, *in vacuo*, and chloroform and water were added. The organic layer was separated, washed with water, dried, and evaporated. The infrared spectrum of the residue showed a hydroxy group. Tlc and vpc indicated that pyrazoles **10** and **11** were present.

The residue was dissolved in acetic acid (100 ml) and concentrated hydrochloric acid (20 ml) was added, to give the corresponding acetates **12** and **13**. Evaporation *in vacuo* and five recrystallizations from methanol yielded **13**: 53 mg; mp 196–199°; $[\alpha]_D$ −57°; λ_{max} 214 mμ (ε 6800); λ_{max} 5.78 (s), 7.98 (s, 3β-acetate), 6.25 (w), and 6.55 (w, pyrazole ring); $R_{cholesterol}$, 0.89; relative retention time, 1.64.

Anal. Calcd for $C_{26}H_{36}N_2O_2$: C, 75.67; H, 9.05; N, 6.98. Found: C, 75.72; H, 9.15; N, 7.06.

The isomeric N-methylpyrazolyl derivatives **12** and **13** were also separated by preparative tlc. Chromatoplates were prepared from silica gel G (100.0 g) and distilled water (170 ml). After vigorous shaking for 50 sec the suspension was poured into the applicator where it was left for 30 sec. The slurry was then slowly distributed on three 20 × 20 cm plates, using a thickness slit of 1 mm. The chromatoplates were air dried and not activated.

The mixture of **12** and **13** (100 mg) dissolved in chloroform (0.25 ml) was applied on a line at about 3 cm from one of the plate's edges, using a syringe. The eluent was benzene–ethyl acetate (1:1). After drying, the plate was covered, except for a narrow band in the direction of elution. This band was sprayed with a 1% solution of iodine in methanol, which permitted locating the partially overlapping compounds. The upper and lower parts of the overlapping bands were individually scraped and washed repeatedly with chloroform. Evaporation and recrystallization from acetonitrile gave **12** (20 mg, mp 171–173°) and **13** (12

mg, mp 196–199°). The purity of these compounds was confirmed by mixture melting point, tlc, and vpc.

3β-Hydroxy-21-formylpregn-5-en-20-one 22-(2-Methylsemicarbazone) (14).—To a solution of **2** (7.0 g) in acetic acid (70 ml) was added another solution of 2-methylsemicarbazide[21] (20 g) in water (10 ml). This mixture was refluxed for 15 min and after cooling, water (50 ml) was added; then it was kept in the refrigerator for 2 days. The product which separated was filtered to give **14**: 3.5 g; mp 202–210°; λ_{max} 234 mμ (ε 9500) and 286 mμ (ε 2700); λ_{max}^{KBr} 2.90 (m), 5.80 (s, C_{20} carbonyl), 5.90 (s, shoulder, C_3 carbonyl), and 6.10 μ (m, C=N double bond).

The intermediate **14** gave an intense green color with ethanolic ferric chloride.

17β-(1-Methyl-5-pyrazolyl)-5-androsten-3β-ol (11).—The 22-(2-methylsemicarbazone) derivative **14** (3.0 g) was melted and kept in a wax bath at 245–250° for 2 hr. The pyrolyzed product was dissolved in a 1:1 mixture of benzene–chloroform and chromatographed on an alumina column, using benzene as the eluent. Three recrystallizations from ethyl acetate yielded **11**: 500 mg, mp 252–254°; $[\alpha]_D$ −65°; λ_{max} 214 mμ (ε 4500); λ_{max} 2.75 (w), 2.95 (w, broad, 3β-OH free and hydrogen bonded), 6.25 (w), and 6.55 μ (m, pyrazole ring); $R_{cholesterol}$, 0.32; relative retention time, 1.09.

Anal. Calcd for $C_{23}H_{34}N_2O$: C, 77.92; H, 9.67; N, 7.90. Found: C, 78.20; H, 10.02; N, 7.71.

17β-(1-Phenyl-5-pyrazolyl)-5-androsten-3β-ol (15).—Phenylhydrazine (1.5 g) was added to a solution of the β-ketoaldehyde **3** (2.0 g) in acetic acid (100 ml). After 48 hr at room temperature, the mixture was poured into ice-cold water; a precipitate formed. After filtration and drying, the product was chromatographed on alumina, using benzene–ethyl acetate (10:1) as the eluent. Two recrystallizations from benzene afforded a compound, 1.0 g, mp 220–224°. The nmr spectrum showed that the compound melting at 220–224° was a solvate made of 2 moles of pyrazole **15** and 1 mole of benzene: C_3 (δ 7.61, d, J_{3-4} = 2 cps), NC_6H_5 (δ 7.40, s, five protons), benzene (δ 7.35, s, three protons), C_4 (δ 6.28, d, J_{3-4} = 2 cps), C_{19} (δ 0.93), C_{18} (δ 0.67). When this solvate was heated at 100° *in vacuo* for 24 hr or when the temperature was raised very slowly, the melting point rose to 222–224°. Pyrazole **15** had $[\alpha]_D$ −111°; λ_{max} 230 mμ (ε 9100); λ_{max} 2.75 (w), 2.94 (m, broad, 3β-HO, free and hydrogen bonded), 6.23 (m), 6.64 μ (m, pyrazole ring); $R_{cholesterol}$, 0.83; relative retention time, 3.53.

Anal. Calcd for $C_{28}H_{36}N_2O$: C, 80.72; H, 8.71; N, 6.73. Found: C, 80.55; H, 8.43; N, 6.89.

Pyrazole **15** also formed solvates with methanol, mp 190–192°, acetonitrile, mp 182–185°, and acetone, mp 177–179°. These melting points were obtained when the temperature was raised rapidly; when it was raised very slowly the melting point was always 222–224°.

The reaction of **2** or **3** with phenylhydrazine hydrochloride in ethanol or acetic acid afforded **15** in good yield, as the sole product.

3β-Hydroxy-21-formylpregn-5-en-20-one 22-(2-Phenylsemicarbazone) (16).—A mixture of a solution of the β-ketoaldehyde **3** (7.0 g) in acetic acid (150 ml), a 15% sodium acetate aqueous solution (25 ml), and an aqueous solution of 2-phenylsemicarbazide hydrochloride[26] were allowed to stay at room temperature for 2 hr and then poured into ice-cold water. This mixture was extracted with chloroform, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to give a solid glass: mp 110–120°; λ_{max} 234 mμ (ε 7800) and 290 mμ (ε 6300); λ_{max} 2.78 (w), 2.90 (w), 5.83 (s, C_{20} carbonyl), 5.88 (s, shoulder, C_3 carbonyl), and 6.12 μ (w, C=N double bond). This compound gave an intense green color with ethanolic ferric chloride.

Pyrolysis was achieved by heating **16** (6.0 g) in a wax bath at 245–250° for 2 hr. The dark residue was dissolved in a chloroform–benzene mixture and chromatographed on alumina, using benzene as eluent. Two recrystallizations from benzene yielded a compound (2.0 g) which proved to be identical with **15**. The identity was confirmed by undepressed mixture melting point, comparison of spectra, and tlc and vpc.

17β-(1-Phenyl-5-pyrazolyl)-5-androsten-3β-ol Acetate (23).—The solution of **15** (6.0 g) in a 1:1 mixture (180 ml) of pyridine and acetic anhydride was obtained by heating on the steam bath, and then it was left for 15 hr at room temperature. The reaction mixture was worked up in the same way as **12**, to give compound **23** (5.5 g). Recrystallization from acetonitrile yielded the analytical sample: mp 205–206°; $[\alpha]_D$ −110°; λ_{max} 230 mμ (ε 9100); λ_{max} 5.76 (s), 7.98 (s, 3β-acetate), 6.23 (w), and 6.64 μ (m, pyrazole ring); $R_{cholesterol}$, 1.35; relative retention time, 5.05.

Anal. Calcd for $C_{30}H_{38}N_2O_2$: C, 78.56; H, 8.35; N, 6.11. Found: C, 78.37; H, 8.66; N, 6.19.

17β-(1-Phenyl-5-pyrazolyl)-4-androsten-3-one (17).—A mixture of **15** (6.0 g), aluminum isopropoxide (9.0 g), toluene (200 ml), and cyclohexane (30 ml) was refluxed for 3 hr. After cooling, a saturated solution of potassium bitartrate (250 ml) was added. The organic layer was separated, washed with water, dried, and evaporated *in vacuo*. The oily residue was triturated with petroleum ether (bp 30–60°) and crystals separated. Chromatography on alumina, using benzene as eluent, and one recrystallization from methanol gave analytical **17**: 4.4 g.; mp 196–197°; $[\alpha]_D$ +10°; λ_{max} 237 mμ (ε 24,000); λ_{max} 5.99 (s), C=O), 6.18 (w, C=C), 6.22 (w), and 6.62 μ (m, pyrazole ring); $R_{cholesterol}$, 1.09; relative retention time, 5.18.

Anal. Calcd for $C_{28}H_{34}N_2O$: C, 81.11; H, 8.27; N, 6.76. Found: C, 80.76; H, 8.12; N, 6.61.

17β-[3(5)-Pyrazolyl]-5-androsten-3β-ol (18).—The reaction of β-ketoaldehyde **3** (2.0 g) with hydrazine hydrate (2.0 g) was carried out in acetic acid (100 ml), at room temperature. After 48 hr the solution was poured in ice-cold water and the solid which separated was filtered, dried, and chromatographed on alumina, using benzene–ethyl acetate (1:1) as the eluent. Three recrystallizations from methanol gave analytical **18**: 1.1 g; mp 233–235.5°; $[\alpha]_D$ −52°; $\lambda_{max}^{EtOH, HCl}$ 219 mμ (ε 8900); λ_{max} 2.75 (w, 3β-OH),[31] 2.86 (m, ring NH), 6.22 (w), and 6.40 μ (w, pyrazole ring); $R_{cholesterol}$, 0.25; relative retention time, 1.23.

Anal. Calcd for $C_{22}H_{32}N_2O$: C, 77.60; H, 9.47; N, 8.23. Found: C, 77.22; H, 9.68; N, 8.34.

17β-[3(5)-Pyrazolyl]-5-androsten-3β-ol Acetate (24).—The reaction of pyrazole **18** (500 mg) with acetic anhydride (10 ml) in pyridine (10 ml), was carried at room temperature for 15 hr. The residue obtained upon evaporation *in vacuo* was recrystallized twice from ethanol to give 350 mg of white needles, mp 135°.[33] This product was chromatographed on alumina, using benzene as eluent. Two recrystallizations from ethyl acetate gave **24**: 150 mg; mp 208.5–211°; $[\alpha]_D$ −40°; $\lambda_{max}^{EtOH, HCl}$ 219 mμ (ε 9700); λ_{max} 2.87 (m, ring NH), 5.80 (s), 7.98 (s, 3β-acetate), and 6.40 μ (w, pyrazole ring); $R_{cholesterol}$, 0.64; relative retention time, 1.77.

Anal. Calcd for $C_{24}H_{34}N_2O_2$: C, 75.35; H, 8.96; N, 7.32. Found: C, 74.98; H, 8.90; N, 7.51.

3β-Hydroxy-21-formylpregna-5,16-dien-20-one (20).—A solution of 3β-acetoxypregna-5,16-dien-20-one (106.0 g) in benzene (500 ml) was added to a suspension of sodium methoxide (21.6 g) in benzene (50 ml), while stirring and cooling with ice-water. After 10 min, a mixture of ethyl formate (29.6 g) and benzene (100 ml) was added dropwise. Stirring was continued for 2 hr and then the reaction mixture was left overnight at room temperature. The mixture was acidified with acetic acid (24.0 g), while keeping the temperature below 10°, and then washed twice with water (15 ml). The benzene solution was dried over anhydrous sodium sulfate and was employed to carry out the reactions of β-ketoaldehyde **20** described below. Samples of compound **20** for infrared and ultraviolet spectra were prepared by evaporation of the solvent *in vacuo*. The impure β-ketoaldehyde **20** showed λ_{max}^{EtOH} 240 and 278 mμ; $\lambda_{max}^{EtOH, NaOH}$ 238 and 287 mμ; λ_{max} 2.75 (w), 2.85 (w, broad, 3β-OH), and 6.13–6.30 μ (m, broad, enolic β-ketoaldehyde). The compound gave an intense red color with an alcoholic solution of ferric chloride.

17-(1-Phenyl-5-pyrazolyl)-5,16-androstadien-3β-ol (21).—The benzene solution of the β-ketoaldehyde **20** (100 ml), a solution of phenylhydrazine hydrochloride (12.4 g) in water (150 ml), and sodium acetate (13.0 g) were mixed and shaked for 2 hr at room temperature. The benzene layer was separated, washed twice with water (20 ml), and dried over anhydrous sodium sulfate. Evaporation *in vacuo* gave a residue which was dissolved by addition of water and chloroform. The chloroform layer was washed with 1 *N* hydrochloric acid (20 ml), water, a 5% solution of sodium bicarbonate, and finally three times with water. After drying and evaporation *in vacuo*, a gum was obtained, which was chromatographed on neutral alumina (activity grade I) using benzene as eluent. The first eluted fractions yielded a gum, which, after dissolving in acetone and letting stand for 2 weeks, crystallized. Five recrystallizations from acetone yielded analytical **21**: 430 mg; mp 208.5–210°; $[\alpha]_D$ −32°;

(33) This compound was not identified, but it may be the O,N-diacetyl derivative of **18** (see ref 10b, p 1483).

λ_{max} 246 mμ (ε 9900); λ_{max} 2.75 (w), 2.90 μ (w, broad, 3β-OH), 6.22 (m), and 6.63 μ (m, pyrazole ring); $R_{cholesterol}$, 0.93; relative retention time, 3.20.

Anal. Calcd for $C_{28}H_{34}N_2O$: C, 81.11; H, 8.27; N, 6.76. Found: C, 80.75; H, 8.06; N, 6.89.

17-[3(5)-Pyrazolyl]-5,16-androstadien-3β-ol (25).—The benzene solution of β-ketoaldehyde **20** (100 ml) was treated with 85% hydrazine (4.0 g); a solid separated which redissolved after adding acetic acid (30 ml). After 24 hr the solution was evaporated to dryness and the gummy residue was chromatographed on alumina, using ethyl acetate as the eluent. A gum was obtained which, after rechromatography and trituration with methanol yielded crystals. Four recrystallizations from methanol gave analytical **25**: 500 mg; mp 222–223.5°; $[\alpha]_D$ −40°; λ_{max} 247 mμ (ε 11,000); λ_{max}^{KBr} 3.02 (m, broad, 3β-OH, ring NH), 6.11 (w), and 6.43 μ (w, pyrazole ring); $R_{cholesterol}$, 0.43; relative retention time, 1.25.

Anal. Calcd for $C_{22}H_{30}N_2O$: C, 78.06; H, 8.93; N, 8.28. Found: C, 77.84; H, 8.97; N, 7.82.

17-(1-Methyl-5-pyrazolyl)-5,16-androstadien-3β-ol (26).—The benzene solution of the β-ketoaldehyde **20** (100 ml) was treated with methylhydrazine (4.0 g). A precipitate formed which was redissolved by the addition of acetic acid (30 ml). After 24 hr the benzene layer was washed twice with water (50 ml), dried over anhydrous sodium sulfate, and chromatographed on alumina, using benzene as eluent. The product was recrystallized from ethanol (four times) to give analytical **26**: 1.7 g; mp 242–244°; $[\alpha]_D$ −33°; λ_{max} 241 mμ (ε 10,300); λ_{max} 2.75 (w), 2.90 (w, broad, 3β-OH), and 6.62 μ (w, pyrazole ring); $R_{cholesterol}$, 0.44; relative retention time, 0.93.

Anal. Calcd for $C_{23}H_{32}N_2O$: C, 78.36; H, 9.15; N, 7.95. Found: C, 78.25; H, 9.28; N, 7.93.

17-(1-Methyl-5-pyrazolyl)-5,16-androstadien-3β-ol Acetate (22).—Pyrazole **26** (700 mg) was dissolved in a 1:1 mixture (80 ml) of pyridine and acetic anhydride, by warming. After keeping it at room temperature for 24 hr, evaporation *in vacuo* and chromatography on alumina, using benzene as eluent, gave pyrazole **22**. Recrystallization from acetonitrile yielded the analytical sample: 450 mg; mp 165–168°; $[\alpha]_D$ −28°, λ_{max} 241 mμ (ε 8600); λ_{max} 5.78 (s), 7.95 (s, 3β-acetate), and 6.63 μ (w, pyrazole ring); $R_{cholesterol}$, 0.88; relative retention time, 1.36.

Anal. Calcd for $C_{25}H_{34}N_2O_2$: C, 76.10; H, 8.69; N, 7.10. Found: C, 75.88; H, 8.51; N, 7.12.

Hydrogenation of Pyrazole 21.—A solution of pyrazole **21** (200 mg) in glacial acetic acid (20 ml) was hydrogenated at room temperature and 60 psi for 3 hr, using 10% palladium-on-charcoal (100 mg) catalyst. After filtration, the catalyst was washed with acetic acid (10 ml) and the combined filtrates were evaporated *in vacuo*. A gum was obtained, which crystallized from acetone. Two recrystallizations from this solvent gave a compound (mp 222–224°; melting point obtained by heating very slowly), which proved to be identical with pyrazole **15** by mixture melting point, tlc, vpc, and comparison of infrared and ultraviolet spectra.

Hydrogenation of Pyrazole 22.—A solution of compound **22** (200 mg) in glacial acetic acid (50 ml) was hydrogenated at room temperature and 60 psi for 17 hr, using 10% palladium-on-charcoal (400 mg) as the catalyst. After filtration and evaporation *in vacuo*, the residue was chromatographed on alumina, using benzene as eluent. Recrystallization from acetonitrile gave pyrazole **13**, mp 196–199°. The identity of this product was confirmed by mixture melting point and comparison of tlc, vpc, and infrared and ultraviolet spectra.

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Selenium Dioxide Oxidation of 5α-Androstane-3,17-dione. The Stereochemistry of Dehydrogenation

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Oxidation of 5α-androstane-3,17-dione with selenium dioxide produced 5α-androst-1-ene-3,17-dione, androst-4-ene-3,17-dione, and androsta-1,4-diene-3,17-dione. Oxidation of 1α-deuterio-5α-androstane-3,17-dione to 5α-androst-1-ene-3,17-dione proceeded with 93% loss of dueterium and apparently with a small isotope effect. The androst-4-ene-3,17-dione also isolated from the isotope reaction contained almost all of the original deuterium, indicating that it was not formed by isomerization of the Δ^1 compound.

Since Riley's introduction of selenium dioxide as an oxidant for organic compounds,[3] it has been the subject of many investigations.[4] Although it was soon applied to steroids,[5] a major contribution to its use in steroid chemistry came with the discovery that oxidation of a 12-keto steroid produced the $\Delta^{9,11}$-12 ketone, not the 11,12 diketone.[6] Subsequently, this finding was used in the synthesis of 11-dehydrocorticosterone.[7] In 1956 two groups reported that selenium dioxide in refluxing tertiary alcohols introduced a double bond at the 1,2 position in either 5α-3-keto steroids or Δ^4-3-keto steroids.[8,9] This fact coupled with the information that the introduction of a double bond at the 1,2 position in cortisone increased its antirheumatic and antiallergic activity[10] promoted interest in this reagent. However, since the mechanism and steric course of the reaction had not been completely elucidated, we undertook a study of the stereochemistry of the dehydrogenation reaction.

Results and Discussion

Oxidation of 5α-androstane-3,17-dione with 1 equiv of selenium dioxide in refluxing *t*-amyl alcohol gave a complex mixture of products, which were separated by preparative thin layer chromatography. In addition to 4.8% recovered starting material, 5α-androst-1-ene-3,17-dione in 13.3% yield, androst-4-ene-3,17-dione in 8.1% yield, and androsta-1,4-diene-3,17-dione

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