

## Steroidal Heterocycles. VII.<sup>1</sup> Androstano[2,3-d]isoxazoles and Related Compounds

A. J. MANSON, F. W. STONNER, H. C. NEUMANN, R. G. CHRISTIANSEN, ROBERT L. CLARKE, J. H. ACKERMAN, D. F. PAGE, J. W. DEAN, D. K. PHILLIPS, G. O. POTTS, A. ARNOLD, A. L. BEYLER, AND R. O. CLINTON

*Sterling-Winthrop Research Institute, Rensselaer, N. Y.*

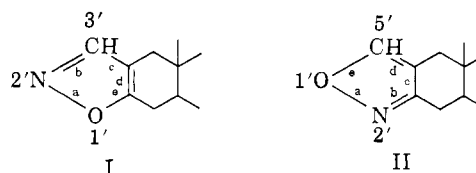
*Received June 11, 1962*

The preparation of steroidal heterocycles containing an isoxazole ring fused to the 2,3-positions of the steroid nucleus is described. These were prepared by the reaction of hydroxylamine with either a 2-hydroxymethylene-3-ketosteroid or a 2-acyl-3-ketosteroid. The factors which influence the ratio of androstano[2,3-d]isoxazole to androstano[3,2-c]isoxazole afforded by this reaction are discussed. Several of these novel steroidal heterocycles possess interesting, and often unpredictable, endocrinological activity. The cleavage of steroidal[2,3-d]-isoxazoles by means of base leads to the corresponding 2-cyano-3-ketosteroids.

In a previous publication<sup>2</sup> we outlined attempts to alter the nucleophilic environment near position 3 of the steroid nucleus<sup>3</sup> by the attachment of a pyrazole ring at the 2,3-positions. This approach was based upon the rationalization that the cellular receptor sites would vary sufficiently to make the question of fit one of paramount importance.

The success of our initial efforts in synthesizing steroidal[3,2-c]pyrazoles with very high anabolic/androgenic ratios<sup>2</sup> has led us to investigate the very closely related steroidal isoxazoles fused at the 2,3-positions.<sup>4,5</sup>

The reaction of hydroxylamine with a 2-hydroxymethylene-3-ketosteroid can conceivably afford both of the two isomeric derivatives,<sup>6</sup> the steroidal[2,3-d]-isoxazole (I) and the steroidal[3,2-c]isoxazole (II). I can be distinguished readily from II by its facile base-catalyzed conversion to the corresponding 2 $\alpha$ -cyano-3-ketosteroid. Under the same conditions II is recovered unchanged.<sup>7</sup>



The literature contains inferences<sup>8</sup> that the ratio between the two possible isoxazole isomers is dependent upon pH, solvent and temperature. Very early in our work it became desirable to obtain more specific information regarding the preferential formation of the steroidal[2,3-d]isoxazoles,<sup>9</sup> since it soon became apparent that they possessed more noteworthy endocrinological activities than did their [3,2-c] analogs.

When the 2-hydroxymethylene-3-ketosteroids were made to react with hydroxylamine under mildly basic conditions (*e.g.*, in alcoholic solution containing an excess of sodium acetate, or in pyridine solution<sup>10</sup>) mixtures of isomeric isoxazoles usually resulted. Purification of these mixtures was not possible by direct methods, such as recrystallization or chromatography. However, the steroidal [3,2-c]isoxazoles could be easily isolated from the mixtures since the isomeric [2,3-d]isoxazoles were readily converted by sodium methoxide to the alkali-soluble  $\alpha$ -cyanoketones.

Optimum yields of the androstano[2,3-d]isoxazoles were obtained by the direct reaction between a 2-hydroxymethylene-3-ketosteroid and hydroxylamine hydrochloride in either alcoholic or glacial acetic acid

(1) Paper VI: R. O. Clinton, R. L. Clarke, F. W. Stonner, A. J. Manson, K. F. Jennings and D. K. Phillips, *J. Org. Chem.*, **27**, 2800 (1962).

(2) R. O. Clinton, A. J. Mason, F. W. Stonner, H. C. Newmann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, W. B. Dickinson and C. Carabateas, *J. Am. Chem. Soc.*, **83**, 1478 (1961).

(3) A similar concept has been used recently by J. C. Orr, O. Halpern and A. Bowers, *J. Med. Pharm. Chem.*, **5**, 409 (1962).

(4) Preliminary communication: R. O. Clinton, A. J. Manson, F. W. Stonner, R. G. Christiansen, A. L. Beyler, G. O. Potts and A. Arnold, *J. Org. Chem.*, **26**, 279 (1961).

(5) Since the completion of our work there have appeared two other reports dealing with androstanoisoxazoles: (a) J. A. Zderic, O. Halpern, H. Carpio, A. Ruiz, D. C. Limon, L. Magaña, H. Jiménez, A. Bowers and H. J. Ringold, *Chem. and Ind.*, 1625 (1960), (b) E. Marchetti and P. Donini, *Gazz. chim. ital.*, **86**, 1133 (1961). Concerning the question of priority raised in the latter publication, *cf.* our Belgium Patent 580,902 (January 22, 1960).

(6) *Cf.* the reaction of hydroxylamine with the hydroxymethylene derivative of cyclohexanone, K. V. Auwers, Th. Bahr and E. Frese, *Ann.*, **441**, 54 (1925).

(7) L. Claisen, *Ber.*, **36**, 3664 (1903).

(8) W. S. Johnson and W. E. Shelberg, *J. Am. Chem. Soc.*, **67**, 1745 (1945).

(9) F. Winternitz, C. Menow and E. Arnal, *Bull. soc. chim. France*, 505 (1960), have reported the preparation of 2 $\alpha$ -cyanocholestan-3-one via the intermediate cholestan-2,3-d] isoxazole; the latter compound was not isolated.

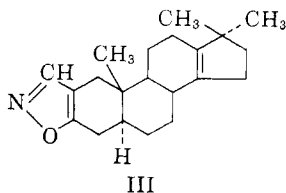
(10) *Cf.* references 5a and 5b.

solution.<sup>11</sup> However, these conditions were not suitable when a tertiary alcohol was present in the steroid, since extensive dehydration resulted (see below). In order to prevent dehydration when the 2-hydroxymethylene-3-ketosteroid contained a tertiary hydroxyl group, it was found necessary to employ the addition of sodium acetate so that the condensation was carried out under mildly acidic conditions (see Experimental).

For the preparation of 4,4-dimethylandrostan- and 4,4-dimethylandrostan-5-eno[2,3-d]isoxazoles possessing a tertiary hydroxyl group at the 17-position, a slight excess of sodium acetate over the hydroxylamine hydrochloride completely prevented any dehydration of the 17 $\beta$ -hydroxyl group while affording excellent yields of the steroidal [2,3-d]isoxazole as the sole product. Apparently the 4,4-dimethyl group sterically hinders the nucleophilic attack of hydroxylamine at the 3-position to such an extent that under these experimental conditions the steroidal [2,3-d]isoxazole is formed exclusively.

The preparative methods described above were successfully applied to the preparation of the unsaturated androst-4-eno[2,3-d]isoxazoles and androst-4,6-dieno[2,3-d]isoxazoles. In both of these cases the steroidal [2,3-d]isoxazoles were the sole products of these reactions. Their structures were demonstrated by their ready conversion to 2 $\alpha$ -cyano-3-ketosteroids.<sup>12</sup>

A specific example of the acid-catalyzed dehydration of tertiary alcohols mentioned above was provided by the isolation of 17,17-dimethyl-18-norandrost-13-eno[2,3-d]isoxazole (III) in high yield from the reaction of equimolar amounts of 2-hydroxymethylene-17 $\alpha$ -methyl-



androstan-17 $\beta$ -ol-3-one<sup>13</sup> and hydroxylamine hydrochloride in glacial acetic acid. The structure of the product was demonstrated by elemental analysis and by ultraviolet, infrared, and nuclear magnetic resonance (n.m.r.) spectra. The infrared spectrum contained two prominent bands at 7.22  $\mu$  and 7.36  $\mu$  which are indicative of the presence of a *gem*-dimethyl group.<sup>14</sup>

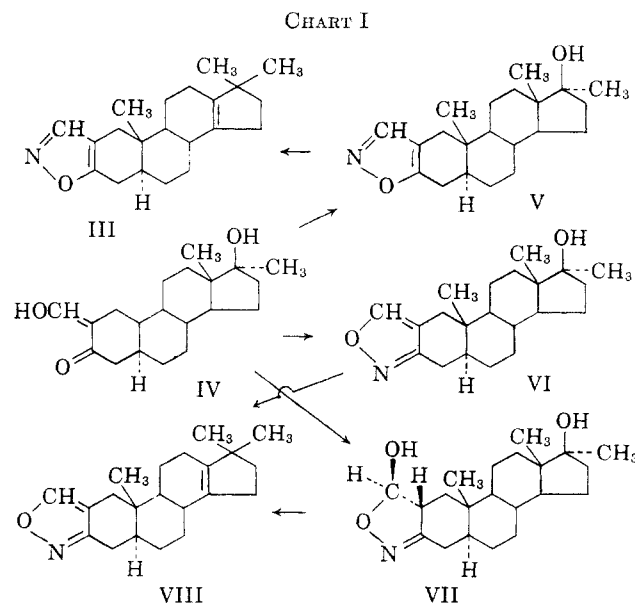
The n.m.r. spectrum<sup>15</sup> showed a signal at 8.55 p.p.m. which was assigned to the hydrogen on the heterocyclic ring. The absence of any other signal in the vinyl region eliminated the alternative structure containing a double bond at the 12-position.<sup>16</sup> The as-

sumption that migration of the C<sub>18</sub> methyl group had taken place was supported by the signal at 1.48 p.p.m., which was assigned to the *gem*-dimethyl group at the 17-position. The signal at 1.23 p.p.m. was assigned to the C<sub>19</sub> methyl group. These assignments of the signals were made on the basis of their position, intensity and line-shape.

In order to determine what effect substitution on the isoxazole ring would have on the structure-activity relationship, 17 $\beta$ -hydroxyandrostan[2,3-d]-3'-methylisoxazole and 17 $\beta$ -benzoxyandrostan[2,3-d]-3'-propylisoxazole were prepared *via* the appropriate 2-acyl-3-ketoandrostanes. These intermediates were prepared by the boron trifluoride-catalyzed reaction between androstan-17 $\beta$ -ol-3-one and the appropriate anhydride in methylene dichloride solution. Base catalysis (*e.g.*, sodium hydride) of the reaction between ethyl acetate and a 3-ketoandrostan failed to produce the desired product. The expectation that the 2-acyl-3-ketoandrostanes, like the 2-hydroxymethylene-3-ketosteroids, would exist predominantly in the chelated form was confirmed by the ultraviolet and infrared spectra.<sup>17</sup>

Subsequent condensations of the 2-acyl-3-ketosteroids with hydroxylamine hydrochloride provided excellent yields of the pure isoxazoles. The structures of these isoxazoles are tentatively assigned on the basis that they were prepared under conditions which favor the nucleophilic attack of hydroxylamine on the hydroxymethylene-3-ketosteroids. The substitution at position 3' of these isoxazoles prevents any ready demonstration of their structures by conversion to  $\alpha$ -cyano-ketones.

During the preparation of 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan[2,3-d]isoxazole (V), an unusual by-product was isolated in addition to the isomeric [3,2-c]isoxazole VI. This readily-crystalline, colorless by-product was shown to be the 5-hydroxy- $\Delta^2$ -isoxazoline (VII) by means of the reactions illustrated in Chart I and other evidence:



(17) The preparation of 2-acetylandrostan-17 $\beta$ -ol-3-one acetate under similar conditions was presented without experimental detail by R. Ledeen and G. I. Fujimota before the Division of Medicinal Chemistry, 138th Meeting, American Chemical Society, St. Louis, Mo., March, 1961.

(11) The latter solvent usually acetylated secondary alcohols (*e.g.*, at C<sub>17</sub>) under these conditions.

(12) Several 2 $\alpha$ -cyano- $\Delta^4$ -3-ketosteroids have been prepared by H. M. Kissman, A. S. Hoffman and M. J. Weiss, *J. Org. Chem.*, **26**, 2610 (1961), by the treatment of 2-hydroxymethylene- $\Delta^4$ -3-ketosteroids with O,N-bis-(trifluoroacetyl)hydroxylamine. *Cf.* also ref. 5a.

(13) H. J. Ringold, E. Batres, O. Halpern and E. Necoechea, *J. Am. Chem. Soc.*, **81**, 427 (1959).

(14) A. R. H. Cole, D. W. Thornton and D. E. White, *Chem. and Ind.*, 795 (1956).

(15) All n.m.r. spectra were recorded on a Varian A-60 spectrometer using 15% solutions of the compounds in deuteriochloroform. Tetramethylsilane was used as an external standard. The positions of the signals were measured on precalibrated charts and expressed as p.p.m. toward low field from tetramethylsilane.

(16) *Cf.* H. L. Herzog, C. C. Joyner, M. G. Gentles, M. T. Hughes, E. P. Oliveto, E. B. Hershberg and D. H. R. Barton, *J. Org. Chem.*, **22**, 1413 (1957).

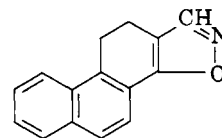
(1) The analysis of VII was in agreement with that expected for the isoxazole plus a molecule of water. (2) The ultraviolet spectrum showed no selective absorption above 220  $m\mu$ . Its infrared spectrum revealed no ketone absorption but exhibited bands at 3.10  $\mu$  ( $-\text{OH}$ ) and 6.14  $\mu$  ( $=\text{C}=\text{N}-$ ). (3) On treatment with a catalytic amount of concentrated hydrochloric acid in boiling acetic acid solution, the by-product VII lost two molecules of water and was converted quantitatively to 17,17 - dimethyl - 18 - norandrost - 13 - eno[3,2 - c]-isoxazole (VIII), which was also obtained from VI under the same conditions. The structure of this [3,2-c]isoxazole was firmly established by elemental analysis, its non-identity with 17,17-dimethyl-18-norandrost-13-eno[2,3-d]isoxazole (III), and by ultraviolet, infrared and n.m.r. spectra. The n.m.r. spectrum<sup>15</sup> of VIII exhibited a signal at 8.63 p.p.m. which was assigned to the hydrogen on the heterocyclic ring.<sup>18</sup> As was observed for the n.m.r. spectrum of the [2,3-d]isoxazole III (see above) no signal appeared in the vinyl region. Signals at 1.52 and 1.27 p.p.m. were assigned to the 17,17-*gem*-dimethyl group and the  $\text{C}_{19}$  methyl group, respectively. The positions, intensities and line-shapes were consistent with the assignments. (4) Under conditions which do not ordinarily acetylate a tertiary hydroxyl group, *i.e.*, pyridine and acetic anhydride, a monoacetyl derivative of VII was prepared readily. The infrared spectrum of this acetyl derivative showed carbonyl absorption at 5.69  $\mu$ , which further supports the assumption that acetylation had occurred at a position other than 17. Finally, the n.m.r. spectrum of this relatively soluble acetyl derivative provided the additional evidence necessary to allow the unambiguous assignment of structure VII to the by-product (due to the highly insoluble nature of VI, its n.m.r. spectrum could not be obtained). The n.m.r. spectrum<sup>15</sup> of this acetate showed a narrow doublet at 6.88 p.p.m. ( $J = 2$  cps.) which was assigned to the hydrogen at position 5'. That this signal is a doublet is consistent with the assigned structure; however, the corresponding splitting of the signal of the  $\text{C}_2$  hydrogen could not be demonstrated since it was not recognizable. The signals observed at 2.62, 1.75, 1.53 and 1.40 p.p.m. were assigned to the methyl group of the acetate, the methyl group at the 17 position, the  $\text{C}_{19}$  methyl and the  $\text{C}_{18}$  methyl, respectively, on the basis of comparison with known steroids. The assignments were consistent with the observed positions, intensities and line shapes of the signals.

Although several 5-acetoxy- $\Delta^2$ -isoxazolines are known,<sup>19</sup> an example of a 5-hydroxy- $\Delta^2$ -isoxazoline was not reported until recently.<sup>20</sup>

The formation and isolation of 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostano[3,2-c]-5'-hydroxy- $\Delta^2$ -isoxazoline may be explained in the following way. A part of the hydroxylamine reacts with 2-hydroxymethylene-17 $\alpha$ -methylandrostano-17 $\beta$ -ol-3-one (IV) to yield its 3-

oximino-derivative which can cyclize to either the hydroxyisoxazoline VII or its  $\text{C}_5'$  epimer. Subsequent dehydration of these two hydroxyisoxazolines to the isoxazole VI can proceed by either a unimolecular elimination (E1) reaction *via* the carbonium ion at  $\text{C}_5'$  or by a bimolecular elimination (E2) reaction which is subject to steric requirements. The isolation of a stable hydroxyisoxazoline suggests that only an E2 mechanism is operative and that the compound possesses a configuration unfavorable for an E2 dehydration (*i.e.*, the 5'-hydroxy group is *cis* relative to the 2 $\beta$ -hydrogen). This isolable hydroxyisoxazoline then must be represented by the structure VII. The  $\text{C}_5'$  epimeric isoxazoline would be expected to dehydrate too readily to the isoxazole VI to permit its isolation. It should be noted that the isolation of VII requires that its reconversion to the 3-oximino-derivative of IV must be relatively slow. Confirmation of this fact was provided by quantitative recovery of the hydroxyisoxazoline after it had been heated for 4 hours in an ethanolic solution containing the same ratio of hydroxylamine hydrochloride to sodium acetate as had been used in the reaction from which it was isolated.

Several of the derived steroidal [2,3-d]isoxazoles were converted to their corresponding 2 $\alpha$ -cyano-3-ketosteroids by treatment with an excess of one equivalent of sodium methoxide, utilizing either methanol or tetrahydrofuran as solvent. The use of tetrahydrofuran as the solvent was found to enhance greatly the rate of conversion to the  $\alpha$ -cyanoketone, relative to the use of methanol.<sup>21</sup> In methanol solution the reaction usually



required about 48 hours at room temperature for completion. In tetrahydrofuran solution under the same conditions the reaction was completed in less than 30 minutes (see Experimental).

The  $\Delta^{4,6}$ -steroidal isoxazoles were found to be very labile to base and although they apparently were transformed to 2 $\alpha$ -cyano derivatives ( $\lambda_{\text{max}}$  288  $m\mu$  ( $\sim 18,200$ ) and 341  $m\mu$  ( $\sim 3700$ ) on crude product), the instability of the reaction product prevented further identification.

The ultraviolet absorption maxima of the androstano- and androst-4-eno[2,3-d]isoxazoles were unaffected by the presence of excess hydrochloric acid. In the case of the androstano[3,2-c]isoxazoles a slight hypsochromic shift (2  $m\mu$ ) of the maxima was observed on the addition of excess hydrochloric acid; however, subsequent neutralization with potassium hydroxide restored the original absorption maximum. In the presence of excess hydrochloric acid the absorption maxima at 319  $m\mu$  of the androsta-4,6-dieno[2,3-d]isoxazoles underwent a hypsochromic shift to 296  $m\mu$  ( $\epsilon \sim 15,000$ ) over a period of 96 hours at room temperature.

It is interesting to note that the androstano[2,3-d]-isoxazoles were found to absorb at a longer wave length in the ultraviolet than their corresponding isomeric

(18) In comparing the signals observed for the hydrogen on the heterocyclic rings of the isomeric isoxazoles III and VIII, it is interesting to note that the relative positions of these signals, 8.55 and 8.63 p.p.m., respectively, are in agreement with the expected trends based on chemical shift considerations; see L. H. Meyer, A. Saika and H. S. Gutowsky, *J. Am. Chem. Soc.*, **75**, 4567 (1953).

(19) (a) G. S. D'Alcontres and P. Grünanger, *Gazz. chim. ital.*, **80**, 741 (1950), and references cited therein. (b) T. Makaiyama and T. Hoshino, *J. Am. Chem. Soc.*, **82**, 5339 (1960).

(20) K. Brückner, K. Irmscher, F. v. Werder, K. Bork and H. Metz, *Ber.*, **94**, 2879 (1961).

(21) W. S. Johnson, J. W. Petersen, and C. D. Gutsche, *J. Am. Chem. Soc.*, **69**, 2942 (1947), reported rapid transformation of 10,11-dihydro-7-methoxyphenanthro-[2,1-d]isoxazole to its corresponding  $\alpha$ -cyano ketone utilizing potassium *tert*-butoxide in *tert*-butyl alcohol.

[3,2-c]fused analogs (see Experimental). Almost without exception the ultraviolet absorption maxima for the androstano[2,3-d]isoxazoles and their [3,2-c]-analogs were  $227 \pm 2 \text{ m}\mu$  and  $222 \pm 1 \text{ m}\mu$ , respectively; notable exceptions to this were 17,17-dimethyl-18-norandrost-13-eno[2,3-d]isoxazole III,  $\lambda_{\text{max}} 222 \text{ m}\mu$  ( $\epsilon = 5800$ ) and its [3,2-c]analogue VIII which exhibited no absorption above  $218 \text{ m}\mu$ . Hence the introduction of  $\Delta^{13}$  unsaturation into a 2,3-fused androstanoisoxazole results in a hypsochromic shift of the absorption maximum, which in the case of the [3,2-c]isoxazole VIII is obscured by end absorption. This rather dramatic shift can be interpreted as a manifestation of "long range conformational transmission" effects.<sup>22</sup>

A convenient method for the evaluation of the purity of a steroidal [2,3-d]isoxazole, with respect to contamination by its [3,2-c]isomer, utilizes the fact that the [2,3-d]isomer is converted quantitatively to an  $\alpha$ -cyanoketone by base. A specific example of this method is cited: A sample of 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostano[2,3-d]isoxazole,  $\lambda_{\text{max}} 227 \text{ m}\mu$  ( $\epsilon = 5000$ ), was dissolved in alcohol containing an excess of potassium hydroxide. By recording the ultraviolet absorption maximum of this solution at appropriate time intervals, it was found that the solution acquired an essentially constant absorption value after 48 hr., i.e.,  $\lambda_{\text{max}} 265 \text{ m}\mu$  ( $\epsilon = 11,800$ ). A solution of the pure potassium salt of 2 $\alpha$ -cyano-17 $\alpha$ -methylandrostan-17 $\beta$ -ol-3-one exhibited  $\lambda_{\text{max}} 265 \text{ m}\mu$  ( $\epsilon 11,900$ ) and was stable under analogous conditions. Thus the purity of the steroidal [2,3-d]isoxazole can be readily ascertained.

**Structure-Activity Relationships.**—In regard to anabolic/androgenic properties, the androstano-isoxazoles exhibited a pattern of activity similar to that reported for their corresponding androstano[3,2-c]-pyrazoles.<sup>2,23</sup> The most interesting member of the isoxazole series in terms of the separation of anabolic and androgenic activities was 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostano[2,3-d]isoxazole (V). It was 0.25 as anabolic, 0.43 as myotrophic and 0.11 as androgenic as testosterone propionate when compared parenterally (to be published). Furthermore, this steroid was 9.7 times as anabolic, 2 times as myotropic and 0.24 times as androgenic as methyltestosterone when compared orally.<sup>24,25</sup>

17 $\beta$ -Hydroxyandrostano[2,3-d]isoxazole was comparable in activities to the preceding isoxazole V when administered parenterally but inactive when given orally. Extension of the isoxazole series to the higher homologs (e.g., 17 $\beta$ -hydroxy-17 $\alpha$ -ethylandrostano[2,3-

d]isoxazole) resulted in a decline of activity indicating a structure-activity relationship with regard to substituents of the 17-position.

17 $\beta$  - Hydroxy - 17 $\alpha$  - methylandrost - 4 - eno[2,3-d]isoxazole was 0.10–0.33 as anabolic, 0.50 to equally as myotrophic and 0.10–0.20 as androgenic as testosterone propionate when compared parenterally. In addition this steroid was approximately 1.2 times as anabolic, equally as myotrophic and 0.10 as androgenic as methyltestosterone when given orally. The 17 $\beta$ -hydroxyandrost-4-eno[2,3-d]isoxazole was comparable in activities to the corresponding 17 $\alpha$ -methyl analog when administered parenterally, but was inactive when given orally. When the 17 $\alpha$ -alkyl was larger than methyl (e.g., 17 $\beta$ -hydroxy-17 $\alpha$ -ethylandrost-4-eno[2,3-d]isoxazole) a decrease in these activities was noted.

The 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrost-4,6-dieno[2,3-d]isoxazole exhibited approximately 0.1 the anabolic and androgenic activities of 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostano[2,3-d]isoxazole on parenteral administration. The extension of the 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrost-4,6-dieno[2,3-d]isoxazole series to the higher homologs (e.g., 17 $\beta$ -hydroxy-17 $\alpha$ -ethylandrosta-4,6-dieno[2,3-d]isoxazole) resulted in a decline of activity.

No estrogenicity was observed for 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrost-4-eno[2,3-d]isoxazole or 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrost-4,6-dieno[2,3-d]isoxazole which is in contrast to their corresponding [3,2-c]pyrazoles.<sup>2,23,26</sup>

17 $\alpha$  - Ethynyl - 17 $\beta$  - hydroxyandrostano[2,3 - d]-isoxazole and 17 $\alpha$ -ethynyl-17 $\beta$ -hydroxyandrost-4-eno[2,3-d]isoxazole were at most only weakly myotrophic and androgenic. The former compound exhibited a low degree of estrogenicity (1/40,000 estradiol when compared parenterally) which was absent in the endocrinological profile of the latter unsaturated analog. This finding of estrogenicity is in direct contrast to the results obtained in the steroidal [3,2-c]pyrazoles, where estrogenicity appeared to be a manifestation of the introduction of unsaturation (i.e., a double bond at the 4-position) into ring A of the steroid.<sup>2,23,26</sup>

The effect of substituents on the androstanoisoxazole molecule other than at the 17-position was examined by preparing various derivatives containing 4,4-dimethyl,<sup>27</sup> 6 $\alpha$ -methyl<sup>28</sup> and 3'-alkyl substituents. In all derivatives thus prepared myotrophic activity was minimal (e.g., 6 $\alpha$ ,17 $\alpha$ -dimethyl-17 $\beta$ -hydroxyandrostano[2,3-d]isoxazole and 6 $\alpha$ ,17 $\alpha$ -dimethyl-17 $\beta$ -hydroxyandrost-4-eno[2,3-d]isoxazole). This effect is in marked contrast to the enhancement of myotrophic activity conferred by the 6 $\alpha$ -methyl group on testosterone and dihydrotestosterone.<sup>23</sup>

In the 19-nor series, 19-nor-17 $\beta$ -hydroxy-17 $\alpha$ -methylandrost-4-eno[2,3-d]isoxazole proved to be the most interesting. It was found to be progestational, equal in activity to progesterone intramuscularly and at least as active as ethisterone when given orally. This compound has the same order of myotrophic and androgenic activities as 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrost-4-eno[2,3-d]isoxazole when compared parenter-

(22) D. H. R. Barton and G. A. Morrison, *Progress in the Chemistry of Organic Natural Products*, Springer-Verlag, Berlin, 1961, p. 166, and refs. 34, 41, 42 and 49 cited therein.

(23) A. L. Beyler, G. O. Potts and A. Arnold, *Endocrinology*, **68**, 987 (1961).

(24) (a) A. Arnold, G. O. Potts, A. L. Beyler and R. O. Clinton, *Fed. Proc.*, **20**, 198 (1961); (b) A. Arnold, G. O. Potts and A. L. Beyler, *Fed. Proc.*, **21**, 212 (1962); (c) G. O. Potts, A. Arnold and A. L. Beyler, *Excerpta Medica, International Congress Series*, No. 51, 211 (1962); (d) A. Arnold, G. O. Potts and A. L. Beyler, in press.

(25) The anabolic activity was determined by nitrogen retention; myotrophic activity by the growth response of the levator ani muscle; androgenicity by the gain in weight of the ventral prostate; and estrogenicity by vaginal cornification; all in castrated rats. Progestational activity was evaluated by the Clauberg test in rabbits. Myotrophic and estrogenic data are based on results obtained by subcutaneous injection of the compounds. All compounds were administered by intramuscular injection for progestational evaluation.

(26) G. O. Potts, A. Arnold and A. L. Beyler, *Endocrinology*, **67**, 849 (1960).

(27) For the intermediate 3-keto steroids see H. J. Ringold and G. Rosenkranz, *J. Org. Chem.*, **22**, 602 (1957); cf. W. J. Adams, D. K. Patel, V. Petrow, I. A. Stuart-Webb and B. Sturgeon, *J. Chem. Soc.*, 4490 (1956).

(28) H. J. Ringold, E. Batres and G. Rosenkranz, *J. Org. Chem.*, **22**, 99 (1957).

ally. In addition, the 19-nor compound had a low degree of estrogenic activity. The estrogenic response was atypical, since considerable mucification and leukocytic infiltration accompanied the cornifying effects on the vaginal epithelium. 19-Nor-17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan-2,3-d]isoxazole was approximately 0.25 as myotrophic and androgenic as 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan-2,3-d]isoxazole when it was compared parenterally. The 19-nor saturated isoxazole was not estrogenic. It should be noted also that the 19-nor-unsaturated isoxazole was somewhat more myotrophic and androgenic than the 19-nor-saturated isoxazole.

In search for a long-acting anabolic agent, several esters of 17 $\beta$ -hydroxyandrostan-2,3-d]isoxazole and 17 $\beta$ -hydroxyandrostan-4-eno-2,3-d]isoxazole were prepared. All showed some degree of myotrophic activity except 17 $\beta$ -(trimethylacetoxymethyl)androstan-2,3-d]isoxazole. The lack of myotrophic activity in this latter ester can be attributed to its resistance to hydrolysis. The compound of most interest in this group was 17 $\beta$ -(3-cyclohexylpropionyloxy)androstan-2,3-d]isoxazole which proved to be a potent anabolic agent with a long duration of action and minimal androgenicity. The administration of this agent to castrated immature male rats as a single subcutaneous injection at 10 mg. produced a marked increase in the weight of the levator ani muscle which reached its peak response 3 weeks after administration and which was maintained for an additional 5 weeks. The marked myotrophic response was accompanied by only a moderate androgenic response as measured by increase in the weight of the ventral prostate over the same time period.

In several cases where the steroidal [2,3-d]isoxazole had been found to possess outstanding myotrophic activity, *e.g.*, 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan-2,3-d]isoxazole and 17 $\beta$ -(3-cyclopentylpropionyloxy)androstan-2,3-d]isoxazole, the corresponding [3,2-c]isoxazole was investigated. In each case the [3,2-c] analog (*e.g.*, 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan-3,2-c]isoxazole) was found to exhibit a lower order of activity.

To summarize, the relationship of myotrophic and androgenic activities to structure has been demonstrated to be dependent upon rather critical requirements. Apparently only minor structural features can be altered on the 17 $\beta$ -hydroxyandrostan-2,3-d]isoxazole molecule if a high degree of activity is to be maintained or enhanced. The presence or absence of the  $\Delta^4$  or  $\Delta^{4,6}$  unsaturated positions appeared critical in that for all cases, except for the 19-nor members, the fully saturated analogs were the more active. In the 19-nor series where anabolic activity was demonstrated, the  $\Delta^4$  unsaturated member was clearly more active than its saturated congener. Only limited variations could be made without greatly diminishing the degree of activity demonstrated for the 17 $\beta$ -hydroxy- or 17 $\beta$ -hydroxy-17 $\alpha$ -methyl members. This information is presented with the idea that the accumulation of such structure-activity relationships can lead to a better understanding of cellular requirements for androgenic and anabolic activity.

**Acknowledgment.**—The authors are indebted to Dr. F. C. Nachod, Miss Catherine Martini and Mr. M. Priznar for spectral determinations and to Mr. K. D. Fleischer and staff for analytical services. They also

thank Dr. R. K. Kullnig for his helpful discussions regarding the interpretation of the n.m.r. spectral data.

## Experimental<sup>29</sup>

**2-Hydroxymethylene-3-ketosteroids.**—Except for the compounds listed in Table I, the 2-hydroxymethylene-3-ketosteroid intermediates used in the present work have been reported in reference 2.<sup>30</sup>

TABLE I

## 2-HYDROXYMETHYLENE-3-KETOSTEROID INTERMEDIATES

2-Hydroxymethylene derivative of	Method <sup>a</sup>	Time, days	Yield, % <sup>b</sup>
17 $\alpha$ -Propinylandrost-4-en-17 $\beta$ -ol-3-one <sup>c</sup>	C	4	88
4,4-Dimethyl-17 $\alpha$ -ethinylandrost-5-en-17 $\beta$ -ol-3-one 17-(2-tetrahydropyran ether) <sup>d</sup>	E	2	65 <sup>e</sup>
4,17 $\alpha$ -Dimethylandrostan-4-en-17 $\beta$ -ol-3-one <sup>f</sup>	C	1	80 <sup>g</sup>
4-Ethyl-17 $\alpha$ -methylandrostan-4-en-17 $\beta$ -ol-3-one <sup>d</sup>	E	3	95
4,4-Diethyl-17 $\alpha$ -methylandrostan-5-en-17 $\beta$ -ol-3-one <sup>d</sup>	E	1	100 <sup>h</sup>

<sup>a</sup> The capitalized letters listed under method refer to the methods of preparation for 2-hydroxymethylene-3-ketosteroids listed in reference 2, (page 1484). <sup>b</sup> Yields are based on the total amount of base-soluble product. <sup>c</sup> For the intermediate 3-ketosteroid see S. P. Barton, D. Burn, G. Cooley, B. Ellis and V. Petrow, *J. Chem. Soc.*, 1957 (1959). In our hands this 3-ketosteroid had a m.p. 181–182°, whereas these authors report a m.p. 151–152°. (The infrared spectra of these two substances in CHCl<sub>3</sub> solution were identical. We thank Dr. V. Petrow of the British Drug Houses Ltd., London, for this information). <sup>d</sup> For intermediate 3-ketosteroid see Experimental. <sup>e</sup> Resinous,  $\lambda_{\max}$  277 m $\mu$  (4930) and 228 m $\mu$  (2110). <sup>f</sup> For the intermediate 3-ketosteroid see N. W. Atwater, *J. Am. Chem. Soc.*, **82**, 2847 (1960). <sup>g</sup> A sample of the crude product which was recrystallized from ether afforded pale yellow crystals, m.p. 197–204°,  $[\alpha]_D -0.4^\circ$ ,  $\lambda_{\max}$  262 m $\mu$  (10,500) and 308 m $\mu$  (6650). *Anal.* Calcd. for C<sub>25</sub>H<sub>32</sub>O<sub>3</sub>: C, 76.70; H, 9.36. Found: C, 76.73; H, 9.35. <sup>h</sup> A sample of the crude product which was recrystallized from ethyl acetate afforded needles, m.p. 177–178°,  $[\alpha]_D -40.3^\circ$ ,  $\lambda_{\max}$  281 m $\mu$  (7810). *Anal.* Calcd. for C<sub>25</sub>H<sub>32</sub>O<sub>3</sub>: C, 77.67; H, 9.91. Found: C, 77.64; H, 9.76.

**4,4-Dimethyl-17 $\alpha$ -ethinylandrost-5-en-17 $\beta$ -ol-3-one 17-(2-Tetrahydropyran ether).**—A partial suspension of 20.0 g. of 17 $\alpha$ -ethinylandrost-4-en-17 $\beta$ -ol-3-one (0.064 mole), 75 ml. of dihydropyran (distilled from potassium hydroxide) and 10 drops of phosphorus oxychloride in 225 ml. of tetrahydrofuran (distilled from calcium hydride) was stirred for 5.5 hr. and then allowed to stand at room temperature for 16 hr. Care was taken to exclude moisture during the reaction period. Excess solid sodium methoxide was added and the mixture was stirred for 2 hr. Solid material was removed by filtration and the filtrate was concentrated *in vacuo* on a steam bath. The residue was dissolved in a minimum amount of ether, and allowed to stand for several hr. The precipitate of unreacted 17 $\alpha$ -ethinyltestosterone (1.4 g., 0.0045 mole) was removed by filtration, and the ethereal filtrate

(29) Melting points were taken in a Hershberg-type apparatus and are corrected. Except as noted, rotations were taken in chloroform solution at 25°, *C* ~ 1%; ultraviolet spectra in 95% ethanol (Cary) and infrared spectra in a potassium bromide disc (Perkin-Elmer 21). In the chromatographic purifications the silica gel used was Davison Type 923, 100–200 mesh.

(30) The following three compounds, which were reported in crude form in ref. 2, subsequently have been obtained in pure form: 2-Hydroxymethylene-17 $\alpha$ -ethinylandrost-4-en-17 $\beta$ -ol-3-one, which was recrystallized from acetone, was obtained as fine needles, m.p. 195–199°,  $[\alpha]_D -67.4^\circ$ ,  $\lambda_{\max}^{EtOH}$  250 m $\mu$  (12,000) and 306 m $\mu$  (5200). *Anal.* Calcd. for C<sub>25</sub>H<sub>32</sub>O<sub>3</sub>: C, 77.61; H, 8.29. Found: C, 77.33; H, 7.99. 2-Hydroxymethylene-17 $\alpha$ -ethinylandrost-17 $\beta$ -ol-3-one, which was recrystallized from benzene, had m.p. 148–150°,  $[\alpha]_D +34.7^\circ$ ,  $\lambda_{\max}$  281 m $\mu$  (9300). *Anal.* Calcd. for C<sub>22</sub>H<sub>30</sub>O<sub>3</sub>: C, 76.26; H, 9.89. Found: C, 76.07; H, 9.93. 2-Hydroxymethylene-17 $\alpha$ -ethinylandrost-17 $\beta$ -ol-3-one, which was recrystallized from acetone, had m.p. 172–177°,  $[\alpha]_D -18.5^\circ$ ,  $\lambda_{\max}^{EtOH}$  283 m $\mu$  (15,300). *Anal.* Calcd. for C<sub>22</sub>H<sub>30</sub>O<sub>3</sub>: C, 77.15; H, 8.83. Found: C, 77.07; H, 8.53.

TABLE II  
STEREODAL[2,3-d]ISOXAZOLES

	[2,3-d]isoxazole	Method <sup>1a</sup>	M.p., °C.	[α] <sub>D</sub> <sup>b</sup> CHCl <sub>3</sub>	λ <sub>max</sub> <sup>b</sup> (ε)	Formula	Carbon		Hydrogen		Nitrogen		Oxygen	
							Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
17β-Hydroxyandrostano- <sup>b</sup>		A	180-182	61.6	228 (4900)	C <sub>26</sub> H <sub>32</sub> NO <sub>2</sub>	76.15	75.93	9.27	9.38			10.14	9.90
17β-Acetoxyandrostano- <sup>c</sup>		B	164-166	39.6	226 (4300)	C <sub>22</sub> H <sub>31</sub> NO <sub>3</sub>	73.91	73.66	8.74	8.64			13.43	13.15
17β-(Trimethylacetoxyl)androstano- <sup>c</sup>		d	188-189	42.0	227 (5100)	C <sub>25</sub> H <sub>37</sub> NO <sub>3</sub>	75.17	75.46	9.33	9.20			12.01	12.25
17β-(3-Cyclopentylpropionyloxy)androstano- <sup>c</sup>		d	142-143	41.2	227 (4900)	C <sub>28</sub> H <sub>41</sub> NO <sub>3</sub>	76.49	76.53	9.40	9.37			10.92	10.60
17β-(3-Cyclohexylpropionyloxy)androstano- <sup>c</sup>		f	140-142	40.6	227 (4900)	C <sub>29</sub> H <sub>43</sub> NO <sub>3</sub>	76.78	76.54	9.55	9.63	3.09	3.12		
17β-(4-Chlorophenoxyacetoxyl)androstano- <sup>g</sup>		d	152-153	38.2	226 (16100)	C <sub>28</sub> H <sub>34</sub> ClNO <sub>4</sub>	69.48	69.16	7.08	7.11	<sup>h</sup>	<sup>h</sup>		
17β-Hydroxy-17α-methylandrostano- <sup>i</sup>		D <sup>1,k</sup>	171-173	36.2	228 (5100)	C <sub>21</sub> H <sub>31</sub> NO <sub>2</sub>	76.55	76.70	9.48	9.55	4.08	4.16		
17α-Ethyl-17β-hydroxyandrostano- <sup>i</sup>		D	159-160	38.4	228 (4900)	C <sub>22</sub> H <sub>33</sub> NO <sub>2</sub>	77.84	77.55	8.61	8.39			9.71	10.00
17α-Ethyl-17β-hydroxyandrostano- <sup>i</sup>		D	197-198	-8.0	228 (5150)	C <sub>22</sub> H <sub>33</sub> NO <sub>2</sub>	77.84	77.55	8.61	8.39			9.43	9.70
17β-Hydroxy-17α-propylandrostano- <sup>e</sup>		D	125-135	-18.5	227 (5300)	C <sub>23</sub> H <sub>35</sub> NO <sub>2</sub>	78.14	77.85	8.84	9.13			9.05	9.15
6α,17α-Dimethyl-17β-hydroxyandrostano- <sup>g</sup>		D <sup>l</sup>	161-166	47.8	228 (4800)	C <sub>22</sub> H <sub>33</sub> NO <sub>2</sub>	76.92	77.04	9.68	9.75	4.08	3.99		
4,4-Dimethyl-17β-hydroxyandrostano- <sup>m</sup>		E	222-228	32.6	228 (5200)	C <sub>22</sub> H <sub>33</sub> NO <sub>2</sub>	76.92	76.64	9.68	9.70	4.08	4.02		
17β-Hydroxy-4,4,17α-trimethylandrostano- <sup>g</sup>		D <sup>k</sup>	207-210	7.1	228 (5500)	C <sub>23</sub> H <sub>35</sub> NO <sub>2</sub>	77.26	77.58	9.87	9.98			8.95	9.03
17β-Hydroxy-17α-methyl-19-norandrostano- <sup>c</sup>		E	198-200	—	227 (4700)	C <sub>20</sub> H <sub>29</sub> NO <sub>2</sub>	76.39	76.02	8.98	9.15			10.18	10.40
17β-Hydroxyandrost-4-eno- <sup>i</sup>		A <sup>o</sup>	183-184	137.9	285 (10700)	C <sub>20</sub> H <sub>27</sub> NO <sub>2</sub>	76.64	76.95	8.68	8.63	4.47	4.56		
17β-Propionylandrost-4-eno- <sup>i</sup>		B	130-134	103.1	285 (11300)	C <sub>22</sub> H <sub>31</sub> NO <sub>3</sub>	74.76	74.80	8.46	8.26			12.99	13.00
17β-(3-Cyclohexylpropionyloxy)androst-4-eno- <sup>e</sup>		f	86-87	93.3	285 (10700)	C <sub>23</sub> H <sub>35</sub> NO <sub>3</sub>	77.12	77.36	9.15	8.97	3.10	3.34	10.63	10.90
17β-(4-Chlorophenoxyacetoxyl)androst-4-eno- <sup>i</sup>		d	173-174	92.9	226 (13600) <sup>p</sup>	C <sub>28</sub> H <sub>32</sub> ClNO <sub>4</sub>	69.77	69.72	6.69	6.48			9.77	9.80
17β-Hydroxy-17α-methylandrost-4-eno- <sup>i</sup>		D	175-179	107.5	285 (11900)	C <sub>21</sub> H <sub>29</sub> NO <sub>2</sub>	77.02	76.90	8.93	8.77			9.37	9.33
17α-Ethyl-17β-hydroxyandrost-4-eno- <sup>i</sup>		D	145-148	94.8	286 (10800)	C <sub>22</sub> H <sub>31</sub> NO <sub>2</sub>	77.37	77.33	9.15	9.27			9.00	9.15
17β-Hydroxy-17α-propylandrost-4-eno- <sup>c</sup>		C <sup>n</sup>	123-128	79.7	286 (10200)	C <sub>23</sub> H <sub>33</sub> NO <sub>2</sub>	77.84	77.82	8.61	8.90	3.94	3.82	9.43	9.15
17β-Hydroxy-17α-vinylandrost-4-eno- <sup>i</sup>		C	139-145	82.1	285 (10600)	C <sub>22</sub> H <sub>31</sub> NO <sub>2</sub>	78.30	78.00	8.07	8.06	4.15	4.06	9.48	9.10
17α-Ethyl-17β-hydroxyandrost-4-eno- <sup>c</sup>		E	224-227	21.9	286 (11300)	C <sub>22</sub> H <sub>31</sub> NO <sub>2</sub>	76.90	76.88	8.62	8.75	3.74	3.83	<sup>t</sup>	<sup>t</sup>
17β-Hydroxy-17α-propylandrost-4-eno- <sup>c</sup>		D	139-144	-8.3	284 (11100)	C <sub>24</sub> H <sub>32</sub> NO <sub>2</sub> <sup>s</sup>	78.59	78.27	8.32	8.06	3.99	3.97		
17β-Hydroxy-17α-propargylandrost-4-eno- <sup>c</sup>		C <sup>n</sup>	182-188	72.6	285 (11200)	C <sub>23</sub> H <sub>31</sub> NO <sub>2</sub>	77.37	77.14	9.15	8.91	4.10	4.39		
4,4-Dimethyl-17β-hydroxyandrost-5-eno- <sup>g</sup>		E	193-195	-39.6	229 (5800)	C <sub>22</sub> H <sub>31</sub> NO <sub>2</sub>	77.70	77.80	9.36	9.37			9.00	9.20
4,4-Dimethyl-17β-methoxyandrost-5-eno- <sup>m</sup>		E	139-141	-49.8	229 (5900)	C <sub>23</sub> H <sub>33</sub> NO <sub>2</sub>	77.70	77.74	9.36	9.11			9.00	8.90
17β-Hydroxy-4,4,17α-trimethylandrost-4-eno- <sup>i</sup>		E	176-178	-60.5	228 (5800)	C <sub>23</sub> H <sub>33</sub> NO <sub>2</sub>	77.70	77.74	9.36	9.13	4.10	3.93		
6α,17α-Dimethyl-17β-hydroxyandrost-4-eno- <sup>g</sup>		C	177-182	90.2	285 (10700)	C <sub>22</sub> H <sub>31</sub> NO <sub>2</sub>	77.37	77.58	9.15	8.97	3.91	3.89		
4-Ethyl-17β-hydroxy-17α-methylandrost-4-eno- <sup>i</sup>		D	107-111 <sup>n</sup>	73.9	288 (11400)	C <sub>23</sub> H <sub>33</sub> NO <sub>2</sub>	77.70	77.64	9.36	9.45	3.65	3.54		
4,4-Diethyl-17β-hydroxy-17α-methylandrost-5-eno- <sup>c</sup>		D	130-135 <sup>c</sup>	-54.1	230 (6100)	C <sub>26</sub> H <sub>37</sub> NO <sub>2</sub>	78.28	78.44	9.72	9.45	4.10	4.05		
4,17α-Dimethyl-17β-hydroxyandrost-4-eno- <sup>i</sup>		E	193-194	83.8	288 (12100)	C <sub>22</sub> H <sub>31</sub> NO <sub>2</sub>	77.37	77.63	9.15	9.41			9.83	10.05
4,4-Dimethyl-17α-ethyl-17β-hydroxyandrost-5-eno- <sup>c</sup>		E <sup>w</sup>	211-219	-100.5	229 (5800)	C <sub>24</sub> H <sub>34</sub> NO <sub>2</sub>	78.86	79.08	8.55	8.63	3.24	3.74	9.43	9.60
17β-Hydroxy-17α-methyl-19-norandrost-4-eno- <sup>i</sup>		D	160-161	-43.3	287 (10400)	C <sub>20</sub> H <sub>27</sub> NO <sub>2</sub>	76.63	76.78	8.69	8.65	4.47	4.51		
17β-Acetoxyandrost-4,6-dieno- <sup>i</sup>		B <sup>x</sup>	157-161	-182.6	319 (18200) <sup>c</sup>	C <sub>22</sub> H <sub>31</sub> NO <sub>3</sub>	77.13	76.89	8.09	7.85	3.96	3.92	13.58	13.25
17β-Hydroxyandrost-4,6-dieno <sup>r</sup>		B <sup>x</sup>	216-220	-134.7	319 (17800) <sup>p</sup>	C <sub>20</sub> H <sub>25</sub> NO <sub>2</sub>	77.50	77.19	8.36	8.29	4.50	4.49		
17β-Hydroxy-17α-methylandrost-4,6-dieno- <sup>c</sup>		F	193-199	-187.8	319 (19800) <sup>aa</sup>	C <sub>21</sub> H <sub>27</sub> NO <sub>2</sub>	77.50	77.19	8.36	8.29			9.83	10.05
17α-Ethyl-17β-hydroxyandrost-4,6-dieno- <sup>i</sup>		D	177-185	-209.4	319 (17800) <sup>bb</sup>	C <sub>22</sub> H <sub>29</sub> NO <sub>2</sub>	77.84	77.74	8.61	8.34			9.43	9.60

was evaporated to a clear syrup. This material was suitable for the subsequent alkylation step. A small portion of this syrup was crystallized from an ether-pentane mixture to afford crystals of crude 17 $\alpha$ -ethinylandrosta-4-en-17 $\beta$ -ol-3-one 17-(2-tetrahydropyranyl ether), m.p. 158–164°,  $\lambda_{\text{max}}$  240 m $\mu$  (17,500),  $\lambda_{\text{max}}^{\text{KBr}}$  (no OH band), 3.07, 4.75, 5.99, 6.21  $\mu$ .

Under anhydrous conditions a solution of the above tetrahydropyranyl ether (0.058 M) in 50 ml. of *tert*-butyl alcohol was added to a freshly prepared solution of 7.5 g. of potassium in 400 ml. of *tert*-butyl alcohol at 20°. The resulting wine-red solution was treated dropwise with 24 ml. of methyl iodide, while the temperature of the reaction was maintained at 20°. This addition required 1 hr. The yellow reaction mixture was stirred at room temperature for 16 hr. The cream colored suspension then was treated with just enough water to effect a clear solution. The *tert*-butyl alcohol was removed by distillation *in vacuo* and the residual aqueous suspension was extracted with ether. The ether extract was washed with saturated salt solution, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and evaporated to dryness. The residue was purified by chromatography on 800 g. of Merck alumina. Elution with a mixture of 9:1 pentane-ether through 3:1 pentane-ether gave 16.3 g. of product (mixture of stereoisomeric ethers) as a semicrystalline mass, which did not absorb above 220 m $\mu$  in its ultraviolet spectrum. A small amount of this product was subjected to hydrolysis in boiling aqueous acetic acid (20 min.). The resulting crude product was recrystallized from acetone to afford pure 4,4-dimethyl-17 $\alpha$ -ethinylandrosta-5-en-17 $\beta$ -ol-3-one as colorless crystals, m.p. 212–214.5°,  $[\alpha]_D -68.7^\circ$ ,  $\lambda_{\text{max}}$  2.90, 3.10, 4.77, 5.88, 6.08  $\mu$ .

Anal. Calcd. for C<sub>28</sub>H<sub>32</sub>O<sub>2</sub>: C, 81.13; H, 9.47. Found: C, 80.82; H, 9.40.

**4,4-Diethyl-17 $\alpha$ -methylandrosta-5-en-17 $\beta$ -ol-3-one and 4-Ethyl-17 $\alpha$ -methylandrosta-4-en-17 $\beta$ -ol-3-one.**—17 $\alpha$ -Methylandrosta-4-en-17 $\beta$ -ol-3-one (20.1 g.) was subjected to the conditions described above for the alkylation of 17 $\alpha$ -ethinylandrosta-4-en-17 $\beta$ -ol-3-one 17-(2-tetrahydropyranyl ether), except that an equivalent amount of ethyl bromide was substituted for the methyl iodide. Workup of the reaction mixture in the previously described manner afforded a crude solid which after two recrystallizations from ethanol gave 7.86 g. of a crystalline compound, m.p. 185–192°,  $\lambda_{\text{shd}}$  240 m $\mu$  (215). The mother liquors were concentrated to dryness and purified by chromatography on a column containing 200 g. of Florisil,<sup>31</sup> on top of which was 1500 g. of silica gel. Elution with 2.5 and 5% ethyl acetate in benzene yielded an additional 6.52 g. of the same compound as obtained above; thus this product was obtained in a 60% yield. Recrystallization of a sample from methanol gave colorless cubes, m.p. 186–190°,  $[\alpha]_D -30.0^\circ$ ,  $\lambda_{\text{max}}$  280–290 m $\mu$  (50);  $\lambda_{\text{max}}$  2.84, 5.91, 6.08  $\mu$ . These properties are consistent with the assigned structure of 4,4-diethyl-17 $\alpha$ -methylandrosta-5-en-17 $\beta$ -ol-3-one.

Anal. Calcd. for C<sub>24</sub>H<sub>32</sub>O<sub>2</sub>: C, 80.39; H, 10.68. Found: C, 80.41; H, 10.44.

Elution of the above chromatograms with 7.5 and 10% ethyl acetate in benzene afforded 4.24 g. of a solid. A sample of this compound was recrystallized from ethyl acetate. The pure compound was identified as 4-ethyl-17 $\alpha$ -methylandrosta-4-en-17 $\beta$ -ol-3-one by ultraviolet and infrared spectra and had m.p. 117–118°,  $[\alpha]_D +88.3^\circ$ ,  $\lambda_{\text{max}}$  251 m $\mu$  (15,400),  $\lambda_{\text{max}}$  2.94, 6.09, 6.27  $\mu$ .

(31) The Floridin Company, Tallahassee, Florida.

Anal. Calcd. for C<sub>22</sub>H<sub>34</sub>O<sub>2</sub>: C, 79.95; H, 10.37. Found: C, 80.07; H, 10.35.

**Preparation of Steroidal [2,3-d]isoxazoles.**—The steroidal [2,3-d]isoxazoles are listed in Table II. The various methods of preparation of these compounds are described in subsequent methods A–E. With few exceptions the yield of these isoxazoles was greater than 60%.

**Method A.**—To a solution of 0.01 mole of 2-hydroxymethylene-3-ketosteroid in 100 ml. of ethanol was added a solution containing 0.01–0.011 mole of hydroxylamine hydrochloride in 2 ml. of water. The resulting solution was boiled 0.5–2 hr.<sup>32</sup> Frequently the product crystallized on cooling the reaction mixture. In other cases the reaction mixture was diluted with water. The product was then isolated by filtration or extraction in the usual manner.

**Method B.**—The above procedure was modified by the use of 25 ml. of glacial acetic acid instead of the ethanol and solid hydroxylamine hydrochloride was added. The reaction mixture was stirred and maintained at 70° for 8 hr.

**Method C.**—Method A was modified by the initial addition of 0.0095–0.0098 mole of sodium acetate to the reaction mixture.

**Method D.**—Method A was modified as follows: The ethanol was replaced by *ca.* 25 ml. of glacial acetic acid and 0.0095–0.01 mole of sodium acetate was dissolved in the aqueous hydroxylamine hydrochloride solution. During the reaction period the reaction mixture was maintained at its boiling point from 5 to 10 min. In a few cases this procedure was altered by maintaining the mixture at 70° from 4 to 6 hr.; no apparent difference in yield was noted.

**Method E.**—Method D was modified by increasing the amount of sodium acetate to 0.012 mole.

**17 $\beta$ -Hydroxy-17 $\alpha$ -methylandrosta[3,2-c]-5'-hydroxy- $\Delta^2$ -isoxazoline (VII) and 17 $\alpha$ -Hydroxy-17 $\alpha$ -methylandrosta[2,3-d]isoxazole (V).**<sup>33</sup>—To a solution of 10.0 g. of 2-hydroxymethylene-17 $\alpha$ -methylandrosta-17 $\beta$ -ol-3-one, m.p. 162–177°, in 300 ml. of ethanol was added a solution of 2.09 g. of hydroxylamine hydrochloride and 3.88 g. of sodium acetate trihydrate in 10 ml. of water. The resultant solution was boiled under reflux for 4 hr. and then concentrated under reduced pressure to 50 ml. Water and ethyl acetate were added to the residue, the layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic solutions were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to a volume of 100 ml. The cooled solution afforded 2.58 g. of fine colorless crystals, m.p. 218–238°. Several recrystallizations from ethyl acetate gave 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrosta[3,2-c]-5'-hydroxy- $\Delta^2$ -isoxazoline as fine colorless needles (1.52 g.), m.p. 233–237° dec.,  $[\alpha]_D -138.5^\circ$ . Only end absorption was observed in the ultraviolet spectrum,  $\lambda_{\text{max}}$  3.10, 3.44, 6.14, 6.19  $\mu$ .

Anal. Calcd. for C<sub>21</sub>H<sub>30</sub>N<sub>2</sub>O<sub>3</sub>: C, 72.58; H, 9.57; N, 4.03. Found: C, 72.80; H, 9.32; N, 3.98.

The residue from the mother liquors of the preceding crystalline crop, m.p. 218–238°, was chromatographed on 280 g. of silica gel pre-wet with benzene. The center fractions that were eluted with

(32) The completion of the reaction could be determined by a negative ferric chloride test.

(33) The preferred method for the preparation of this isoxazole is given in Table II (Method D). The preparation of the isoxazole by Method C is given in detail since it yields a separable mixture of products. These products are significant in that they provide an insight into the actual mode of formation of the isoxazole.

<sup>a</sup> See Experimental. <sup>b</sup> Recrystallized from ethyl acetate; solvate obtained on recrystallization from methanol or acetone. <sup>c</sup> Recrystallized from acetone. <sup>d</sup> Prepared by treatment of the isoxazole with the acid chloride-pyridine, 24 hours at room temperature. <sup>e</sup> Recrystallized from methanol. <sup>f</sup> Prepared by treatment of the isoxazole with an anhydride in pyridine, 24 hr. at room temperature. <sup>g</sup> Recrystallized from ether. <sup>h</sup> Calcd.: Cl, 7.33. Found: Cl, 7.70. <sup>i</sup> Recrystallized from ethyl acetate. <sup>j</sup> Method D afforded a 85% yield of the isoxazole plus a by-product identified as 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrosta[3,2-c]-5'-hydroxy- $\Delta^2$ -isoxazoline (see Experimental). <sup>k</sup> Preparation of the isoxazole by method C is reported in detail (see Experimental). <sup>l</sup> Crude product subjected to chromatography on silica gel pre-wet with pentane; isoxazole eluted with 7:3 pentane-ether. <sup>m</sup> Recrystallized from isopropyl alcohol. <sup>n</sup> Crude product subjected to chromatography on silica gel, pre-wet with pentane; isoxazole eluted with 19:1 benzene-ether. <sup>o</sup> Crude product subjected to chromatography on silica gel prewet with pentane; isoxazole eluted with 9:1 benzene-ether. <sup>p</sup> Also maxima at 282 and 287 m $\mu$ ,  $\epsilon = 12,500$  and 12,500. <sup>q</sup> Calcd.: Cl, 7.36. Found: Cl, 7.48. <sup>r</sup> Recrystallized from ethanol. <sup>s</sup> Hemietanolate. <sup>t</sup> Calcd.: C<sub>2</sub>H<sub>3</sub>O, 5.79. Found: C<sub>2</sub>H<sub>3</sub>O, 5.97. <sup>u</sup> Resolidified and remelted at 148–154°. <sup>v</sup> Resolidified and remelted at 161–163°. <sup>w</sup> 17-(2-Tetrahydropyranyl ether) derivative used as starting material. <sup>x</sup> Application of Method B afforded both the 17 $\beta$ -hydroxyandrosta-4,6-dieno[2,3-d]isoxazole and its corresponding 17-acetate in a 1:1 ratio. The two isoxazoles were separated readily by chromatography on silica gel, pre-wet with pentane; the acetate was eluted with 19:1 pentane-ether and the 17-hydroxyl derivative was eluted with 9:1 pentane-ether. <sup>y</sup> Also maxima at 235 and 254 m $\mu$ ,  $\epsilon = 2900$  and 2500, respectively. <sup>z</sup> Also maxima at 244 and 252 m $\mu$ ,  $\epsilon = 3200$  and 2700, respectively. <sup>aa</sup> Also maxima at 236, 245, 253 m $\mu$ ,  $\epsilon = 3400$ , 3000 and 2500, respectively. <sup>bb</sup> Also maxima at 243 and 252 m $\mu$ ,  $\epsilon = 2900$  and 2500, respectively.



9:1 benzene-ether amounted to 7.52 g. of solid material which on recrystallization from ethyl acetate afforded 4.29 g. of crystals, m.p. 168–172°.

This compound was demonstrated to be identical with 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan[2,3-d]isoxazole (reported in Table II) by mixture melting point and infrared spectrum ( $\lambda_{\max}$  2.94, 3.44, 3.52, 6.09, 6.20, 6.78, 6.91  $\mu$ ).

In a similar experiment the ethyl acetate extract was concentrated to dryness. The total crude product was treated with excess sodium methoxide in methanol for 3 days at room temperature. After separation of the alkali-soluble  $\alpha$ -cyanoketone by the method of Johnson and Shelberg<sup>8</sup> the neutral portion from the reaction mixture was subjected to chromatography on 110 g. of silica gel, pre-wet with pentane. Elution with 19:1 benzene-ether gave a series of crystalline fractions. These were combined and recrystallized from acetone. The pure 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan[3,2-c]isoxazole (0.73 g.) formed triangular plates of m.p. 171–172°,  $[\alpha]_D +30.6^\circ$ ,  $\lambda_{\max}$  223 m $\mu$  (3700).

Anal. Calcd. for  $C_{21}H_{31}NO_2$ : C, 76.55; H, 9.84; O, 9.71. Found: C, 76.52; H, 9.79; O, 9.35.

The physical data reported here are in agreement with those reported for 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan[3,2-c]isoxazole by Marchetti and Doneni.<sup>5b</sup> On the other hand, the physical data (i.e.,  $[\alpha] +19$ ,  $\lambda_{\max}$  229 m $\mu$ ) for this isoxazole which are reported by Zderic, *et al.*,<sup>5a</sup> suggest that these workers had a mixture.

**17,17-Dimethyl-18-norandrost-13-eno[2,3-d]isoxazole (III).**—A solution of 2-hydroxymethylene-17 $\alpha$ -methylandrostan-17 $\beta$ -ol-3-one (3.33 g.) in 25 ml. of glacial acetic acid was mixed with a solution of hydroxylamine hydrochloride (0.70 g.) in 5 ml. of glacial acetic acid. The resultant mixture was boiled for 5 min., cooled to room temperature, and then diluted with water until a slight turbidity was apparent. The mixture was allowed to stand overnight at room temperature. There resulted a solid precipitate (2.56 g.) which was collected by filtration and subjected to chromatography on 100 g. of silica gel, prewet with pentane. Elution with benzene afforded a crystalline mass. Recrystallization from methanol gave 0.98 g. of colorless crystals, m.p. 134–139°,  $[\alpha]_D -7.8^\circ$ ,  $\lambda_{\max}$  222 m $\mu$  (5800),  $\lambda_{\max}^{CHCl_3}$  6.08, 7.22, 7.36  $\mu$ .

Anal. Calcd. for  $C_{21}H_{29}NO$ : C, 80.98; H, 9.39; N, 4.50. Found: C, 80.94; H, 9.39; O, 5.20.

**17,17-Dimethyl-18-norandrost-13-eno[3,2-c]isoxazole (VIII).**—(a) A solution of 0.50 g. of 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan[3,2-c]isoxazole and 1 ml. of 3 N ethereal hydrochloride solution in 15 ml. of glacial acetic acid was subjected to reflux for 7 min. Dilution with water gave a crystalline precipitate (0.41 g., m.p. 110–112°); recrystallization of the dried precipitate from methanol gave 0.15 g. of colorless prisms, m.p. 112–116°,  $[\alpha]_D -10.4$ ,  $\lambda_{\max}^{CHCl_3}$  6.21, 7.23, 7.37 ultraviolet spectrum revealed no absorption above 220 m $\mu$ .

Anal. Calcd. for  $C_{21}H_{29}NO$ : C, 80.98; H, 9.39; N, 4.50. Found: C, 81.12; H, 9.43; N, 4.36.

(b) A solution of 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan[3,2-c]-5'-hydroxy- $\Delta^2$ -isoxazoline (2.50 g.) and 3 N ethereal hydrochloride (5 ml.) in 60 ml. of acetic acid was boiled for 5 min. Work-up of the reaction mixture in the same manner as described above gave an excellent yield of the isoxazole, m.p. 115–117°, identical with that prepared by method (a).

**17 $\beta$ -Hydroxy-17 $\alpha$ -methylandrostan[3,2-c]-5'-acetoxy- $\Delta^2$ -isoxazoline (VII).**—A solution of 10.0 g. of 17 $\beta$ -hydroxy-17 $\alpha$ -methylandrostan[3,2-c]-5'-hydroxy- $\Delta^2$ -isoxazoline and 7.5 ml. of acetic anhydride in 100 ml. of pyridine was heated on the steam bath for 1.25 hr. The reaction mixture was diluted with 1 l. of water and extracted with ether. The ethereal extract was dried ( $Na_2SO_4$ ) and evaporated to dryness *in vacuo*. The residual oil (11.2 g.) was dissolved in 30 ml. of ether. On standing the solution deposited 2.21 g. of colorless needles, m.p. 166–172°. The residual material from the mother liquor was subjected to chromatography on 360 g. of Florisil. Elution with 7:3 pentane-ether gave several crystalline fractions. The center fractions were combined (3.12 g.) and recrystallized from ether. There was obtained 2.66 g. of colorless needles, m.p. 170–173°. The melting point of this compound was not depressed on admixture with the above crop (2.21 g.). Recrystallization from ether gave the pure acetate as needles, m.p. 169–170°,  $[\alpha]_D -269.2^\circ$ ,  $\lambda_{\max}$  2.81, 3.45, 5.69, 6.90, 8.24  $\mu$ .

Anal. Calcd. for  $C_{23}H_{35}NO_4$ : C, 70.92; H, 9.06; N, 3.60. Found: C, 71.07; H, 8.91; N, 3.45.

**17 $\beta$ -(3-Cyclopentylpropionyloxy)androstan[3,2-c]isoxazole.**—To a solution of 5.93 g. of 17 $\beta$ -hydroxyandrostan[3,2-c]isoxazole<sup>6b</sup> in 100 ml. of pyridine, 6.0 g. of 3-cyclopentylpropionyl chloride was added dropwise with stirring. After 18 hr. at room temperature, the reaction mixture was poured into 1 l. of water. A yellow gum separated. The mixture was extracted with 1:1 ether-benzene. The combined organic layers were washed with water and dried ( $Na_2SO_4$ ). The residue, obtained on evaporation of the solvent, was dissolved in benzene and filtered through 30 g. of silica gel. The column was washed with 19:1 benzene-ether. The combined filtrates were evaporated. The solid residue was recrystallized from methanol to yield 6.27 g. of pale yellow needles, m.p. 155–156°. Three recrystallizations from methanol afforded the pure ester as colorless needles, m.p. 154–156°,  $[\alpha]_D +35.8^\circ$ ,  $\lambda_{\max}$  223 m $\mu$  (3900),  $\lambda_{\max}$  3.44, 5.82, 6.22, 6.91, 8.47  $\mu$ .

Anal. Calcd. for  $C_{28}H_{41}NO_3$ : C, 76.49; H, 9.40; N, 3.19. Found: C, 76.19; H, 9.14; N, 3.28.

**17-Ketoandrostan[2,3-d]isoxazole.**—A solution of 7.21 g. of 17 $\beta$ -hydroxyandrostan[2,3-d]isoxazole in 250 ml. of glacial acetic acid was cooled in an ice bath. Water (ca. 18 ml.) was added until the solution developed a slight turbidity. There was added dropwise a solution of 2.3 g. of chromium trioxide in 2 ml. of water. The solution was mixed thoroughly and then allowed to warm up to room temperature over a period of 2 hr. Ethanol was added to decompose excess chromium trioxide. After dilution with water and partial concentration of the mixture under reduced pressure, the crude product was isolated by filtration and washed with water. The pale green solid was recrystallized from chloroform. There was obtained 6.47 g. of colorless rhomboids, m.p. 192–198°. Further recrystallization from ethyl acetate gave the analytical sample as colorless rods, m.p. 198–200°,  $[\alpha]_D +135.4$ ,  $\lambda_{\max}$  227 (4800).

Anal. Calcd. for  $C_{20}H_{27}NO_2$ : C, 76.64; H, 8.68; N, 4.47. Found: C, 76.95; H, 8.73; N, 4.35.

**17-Ketoandrost-4-eno[2,3-d]isoxazole.**—Treatment of 17 $\beta$ -hydroxyandrost-4-eno[2,3-d]isoxazole (6.53 g.) in the above described manner gave the title compound in a 33% yield as colorless, fine prisms, m.p. 242–246°,  $[\alpha]_D +200.2$ ,  $\lambda_{\max}$  285 m $\mu$  (9970),  $\lambda_{\max}$  3.26, 3.43, 3.51, 5.75, 6.13, 6.24, 6.78, 6.91  $\mu$ .

Anal. Calcd. for  $C_{20}H_{25}NO_2$ : C, 77.13; H, 8.09; N, 4.50. Found: C, 76.87; H, 7.84; N, 4.31.

**2 $\alpha$ -Cyano-4,4,17 $\alpha$ -Trimethylandrostan-5-en-17 $\beta$ -ol-3-one.**—Under anhydrous conditions, a stirred solution of 11.7 g. of 17 $\beta$ -hydroxy-4,4,17 $\alpha$ -trimethylandrostan-5-eno[2,3-d]isoxazole in 90 ml. of tetrahydrofuran (distilled from calcium hydride) was treated with 3.6 g. of powdered sodium methoxide. The resulting solution was stirred at room temperature for 1 hr. During this period the sodium salt of the  $\alpha$ -cyanoketone precipitated. Ether (200 ml.) and water (250 ml.) were added to the reaction mixture and after a thorough mixing which dissolved any solid material present, the aqueous layer was separated. The ether layer was washed again with 100 ml. of water. The combined aqueous layers were acidified with a slight excess of aqueous hydrochloric acid (1:1) at room temperature. The solid precipitate was collected by filtration and washed with water. The crude product (11.5 g.) had m.p. 225–230°. Recrystallization from tetrahydrofuran-ethyl acetate gave the pure cyanoketone, m.p. 227–230°,  $[\alpha]_D -23.4^\circ$ ,  $\lambda_{\max}$  237 m $\mu$  (7600),  $\lambda_{\max}$  2.98, 3.24, 3.40–3.45, 4.52, 5.77, 6.01  $\mu$ .

Anal. Calcd. for  $C_{25}H_{33}NO_2$ : C, 77.70; H, 9.36; O, 9.00. Found: C, 77.64; H, 9.22; O, 9.10.

These 2 $\alpha$ -cyano-3-keto steroids were prepared in the same manner as described for 2 $\alpha$ -cyano-4,4,17 $\alpha$ -trimethylandrostan-5-en-17 $\beta$ -ol-3-one (see above). In all cases the crude yield of the  $\alpha$ -cyanoketone was essentially quantitative.

**2 $\alpha$ -Cyanoandrostane-3,17-dione**, fine prisms (recrystallized from ethyl acetate), m.p. 206–219° dec.,  $[\alpha]_D +47.8^\circ$ ,  $\lambda_{\max}$  235 m $\mu$  (10,000).

Anal. Calcd. for  $C_{20}H_{27}NO_2$ : C, 76.64; H, 8.68; N, 4.47. Found: C, 76.46; H, 8.34; N, 4.41.

**2 $\alpha$ -Cyano-17 $\alpha$ -ethinylandrostan-17 $\beta$ -ol-3-one**, recrystallized from tetrahydrofuran, m.p. 264–270°,  $[\alpha]_D -5.9^\circ$  (1% in pyridine),  $\lambda_{\max}$  235 m $\mu$  (10,300).

Anal. Calcd. for  $C_{22}H_{29}NO_2$ : C, 77.84; H, 8.61; N, 4.13. Found: C, 77.50; H, 8.31; N, 4.10.

**2 $\alpha$ -Cyano-17 $\alpha$ -ethinylandrostan-4-en-17 $\beta$ -ol-3-one**, recrystallized from acetone, m.p. 184–189°,  $[\alpha]_D +34.8^\circ$ ,  $\lambda_{\max}$  242 m $\mu$  (15,400),  $\lambda_{\max}$  2.86, 3.07, 3.42, 4.46, 4.77, 5.99, 6.22, 6.90  $\mu$ .

Anal. Calcd. for  $C_{22}H_{33}NO_2$ : C, 76.92; H, 9.68; N, 4.08. Found: C, 76.75; H, 9.41; N, 3.99.



**2 $\alpha$ -Cyano-4,4-dimethylandrostan-5-en-17 $\beta$ -ol-3-one**, recrystallized from isopropyl alcohol, m.p. 215–218°,  $[\alpha]_D -23.5^\circ$ ,  $\lambda_{\max}$  238 m $\mu$  (7500).

*Anal.* Calcd. for  $C_{22}H_{31}NO_2$ : C, 77.37; H, 9.15; N, 4.10. Found: C, 77.00; H, 9.10; N, 4.02.

**2-Acetylandrostan-17 $\beta$ -ol-3-one 17-Acetate**.—In a flask with a mechanical, glass-blade stirrer, a gas-inlet tube; an addition funnel and a plug of cotton, were placed 9.6 g. (9.2 ml., 0.16 mole) of glacial acetic acid and 50 ml. of dry ethylene dichloride. The flask was immersed in an ice bath and a stream of anhydrous boron trifluoride was passed into the mixture until it was completely saturated as evidenced by the separation of the solid acetic acid-boron trifluoride complex and escape of boron trifluoride fumes above the cotton plug. To the mixture was added rapidly a solution of 11.60 g. (0.040 mole) of androstan-17 $\beta$ -ol-3-one and 12.2 g. (0.12 mole) of acetic anhydride in 100 ml. of ethylene dichloride. Stirring and addition of boron trifluoride were continued until resaturation was achieved. The gas entry tube was removed and the solution was stirred in the ice bath for 30 min., and then for 4 hr. at room temperature. The clear, dark orange colored solution was poured into a solution of 60 g. of sodium acetate trihydrate in 400 ml. of water. The ethylene dichloride was distilled and the residual heterogeneous mixture was refluxed for 45 min. After cooling in ice, the yellow-brown solid was filtered, washed thoroughly with water, and pressed dry. The wet solid was suspended in 200 ml. of methanol, 23 ml. of 35% aqueous sodium hydroxide solution was added, and the mixture was stirred until the solids had completely dissolved. After the mixture had stood for 1 hr. at room temperature it was diluted with water, acidified with glacial acetic acid, and the methanol was removed *in vacuo*. The resulting suspension of gum was extracted with methylene dichloride and the extracts were washed with water, dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*, yielding 14.76 g. of a brittle resin. The latter was dissolved in a mixture of 20 ml. of dry pyridine and 25 ml. of acetic anhydride and the solution was heated on the steam bath for one-half hr. The solution was quenched in cold, dilute sulfuric acid solution and the precipitated material was filtered, washed thoroughly with water and dried at 40° *in vacuo*. The crude acylated product was dissolved in hexane and chromatographed on 500 g. of silica gel prewet with pentane. Elution with 20% and 30% ether-pentane mixtures gave a total of 8.95 g. of the title compound. On recrystallization from acetone, an analytical sample was obtained which had m.p. 183.0–184.6°,  $[\alpha]_D +39.4^\circ$ ,  $\lambda_{\max}$  290 m $\mu$  (9100),  $\lambda_{\max}$  5.76, 6.15–6.25, 8.02  $\mu$ . The compound gave a deep purple-brown color with ferric chloride.

*Anal.* Calcd. for  $C_{23}H_{34}O_4$ : C, 73.76; H, 9.15. Found: C, 73.80; H, 9.55.

The pyrazole derivative, which was prepared in the usual manner,<sup>2</sup> showed m.p. 254–263°,  $[\alpha]_D +41.7^\circ$ ,  $\lambda_{\max}$  224 m $\mu$  (5300).

*Anal.* Calcd. for  $C_{23}H_{34}N_2O_2$ : C, 74.55; H, 9.25; O, 8.64. Found: C, 74.25; H, 9.06; O, 8.90.

Continued elution of the above chromatogram with a 40% ether-pentane mixture gave a total of 4.15 g. of a second product melting at 141–161°. The latter compound gave a negative ferric chloride test and crystallized from absolute ethanol in clusters of long, thin needles, m.p. 170–171°, resolidified and remelted at 177.0–178.0°,  $[\alpha]_D +59.2^\circ$ ,  $\lambda_{\max}$  240 m $\mu$  (8900),  $\lambda_{\max}$  5.69, 5.72, 6.01, 6.07, 7.96, 8.26  $\mu$ .

*Anal.* Calcd. for  $C_{25}H_{36}O_5$ : C, 72.08; H, 8.71. Found: C, 72.15; H, 8.63.

Since this compound exhibited properties expected of an enol acetate, it was assigned the structure **3-acetoxy-2-acetylandrostan-2-en-17 $\beta$ -ol-17 $\alpha$ -acetate**. Saponification of the 17-monoacetate or the enol diacetate by means of methanolic potassium hydroxide solution (1 hr., room temperature) gave **2-acetylandrostan-17 $\beta$ -ol-3-one** as rosettes of flattened needles (from an ethyl acetate-

heptane mixture), m.p. 149.6–154.0°,  $[\alpha]_D +62.3^\circ$ . The compound gave an intense purple color with ferric chloride.

*Anal.* Calcd. for  $C_{21}H_{32}O_3$ : C, 75.86; H, 9.70. Found: C, 75.92; H, 9.42.

**2 $\alpha$ -n-Butyrylandrostan-17 $\beta$ -ol-3-one**.—A mixture of 24.0 g. of androstan-17 $\beta$ -ol-3-one, 40 ml. of dry pyridine and 70 ml. of redistilled butyric anhydride was heated on the steam bath for 45 min. The yellow mixture was poured into a solution of 75 g. of sodium bicarbonate in 1000 ml. of water and the heterogeneous mixture was vigorously stirred for 2 hr. The insoluble solid was filtered, washed thoroughly with water and air-dried. Recrystallization from 150 ml. of hexane gave a total of 25.4 g. of androstan-17 $\beta$ -ol-3-one 17-butyrate, m.p. 98–100°.

The boron trifluoride-catalyzed acylation was carried out as described above for the 2-acetyl homolog, using 27.7 g. (0.0768 mole) of androstan-17 $\beta$ -ol-3-one 17-butyrate, 24.3 g. (0.154 mole), of butyric anhydride, 27.1 g. (0.307 mole), of butyric acid, and proportionate increases in the other reagents. The product from the sodium hydroxide-methanol saponification was an orange-brown crystalline solid (27.5 g.), m.p. 105–130°. The crude product was chromatographed on 500 g. of silica gel prewet with pentane. After preliminary elution with 5–20% ether pentane mixtures, elution with 25% ether-pentane gave a total of 25.1 g. of crystalline product. Recrystallization from ethyl acetate gave material melting at 130.8–132.8°, with but little loss. The m.p. was unchanged on further recrystallization from methanol. The compound formed white prisms with  $[\alpha]_D +55.8^\circ$ ,  $\lambda_{\max}$  290 m $\mu$  (9500),  $\lambda_{\max}$  2.80, 6.21  $\mu$ . The ferric chloride test gave an intense purple color.

*Anal.* Calcd. for  $C_{23}H_{36}O_3$ : C, 76.62; H, 10.07. Found: C, 76.70; H, 10.10.

The pyrazole derivative, which was prepared in the usual manner,<sup>2</sup> showed m.p. 169–172°,  $\lambda_{\max}$  224 m $\mu$  (5800).

*Anal.* Calcd. for  $C_{23}H_{36}N_2O$ : C, 77.48; H, 10.18; N, 7.86. Found: C, 77.38; H, 10.20; N, 7.84.

**17 $\beta$ -Acetoxyandrostan-2,3-d]-3'-methylisoxazole and 17 $\beta$ -Hydroxyandrostan-2,3-d]-3'-methylisoxazole**.—A mixture of 3.32 g. of 2-acetylandrostan-17 $\beta$ -ol-3-one and 9.73 g. of hydroxylamine hydrochloride in 25 ml. of glacial acetic acid was boiled for 10 min., cooled and diluted with water. The solid precipitate was collected by filtration and subjected to chromatography on 200 g. of silica gel, prewet with pentane. Elution with 1:4 ether-pentane yielded 1.21 g. of solid material, m.p. 180–190°. Recrystallization from methanol afforded 17 $\beta$ -acetoxyandrostan-2,3-d]-3'-methylisoxazole as slender needles, m.p. 189–195°,  $[\alpha]_D +35.2^\circ$ .

*Anal.* Calcd. for  $C_{23}H_{38}NO_3$ : C, 74.36; H, 8.75; N, 3.77. Found: C, 74.32; H, 9.34; N, 3.73.

Continued elution of the above chromatogram with 2:3 ether-pentane yielded 2.07 g. of crystals, m.p. 176–204°. Recrystallization from ethyl acetate afforded the 17 $\beta$ -hydroxy derivative as large flat needles, m.p. 209–220°,  $[\alpha]_D +56.4^\circ$ ,  $\lambda_{\max}$  227 m $\mu$  (5500).

*Anal.* Calcd. for  $C_{21}H_{34}NO_3$ : C, 76.55; H, 9.48; N, 4.25. Found: C, 76.33; H, 9.41; N, 4.14.

**17 $\beta$ -Benzyloxyandrostan-2,3-d]-3'-propylisoxazole**.—A mixture of 3.60 g. of 2 $\alpha$ -butyrylandrostan-17 $\beta$ -ol-3-one, 0.73 g. of hydroxylamine hydrochloride and 1.29 g. of sodium acetate trihydrate in 50 ml. of ethyl alcohol was boiled for 2 hr. and then poured into water. A gummy solid precipitate resulted. The crude product was chromatographed on 150 g. of silica gel, prewet with 1:19 ether: pentane. Elution with 3:7 ether-pentane gave 3.10 g. of a resin. Treatment of this resin with 3.0 g. of benzoyl chloride in pyridine for 16 hr. at room temperature and subsequent work-up in the usual manner afforded a crude crystalline product. Recrystallization from methanol afforded 2.47 g. of colorless needles, m.p. 199–203°,  $[\alpha]_D +82.4^\circ$ ,  $\lambda_{\max}$  229 m $\mu$  (20500), 268 m $\mu$  (820), 272 m $\mu$  (930), 281 m $\mu$  (730).

*Anal.* Calcd. for  $C_{30}H_{40}NO_3$ : C, 78.05; H, 8.52; N, 3.03. Found: C, 78.31; H, 8.77; N, 3.03.