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The Steroid–Receptor Complex. Some Considerations Based on sp²-Hybridized Systems[1a,b]

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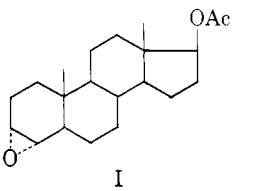
The preparation of a number of steroidal ring A olefins, epoxides, methano steroids, and spirooxiranyl steroids has been undertaken to verify the hypothesis that sp²-hybridization in the A-ring is required for highly biologically active androgens. The requisite intermediate olefins were prepared in the androstane series by application of the Bamford–Stevens reaction to 3-oxo steroids, and mixtures of 2,3- and 3,4-olefins were obtained in some cases. Epoxidation with peracetic acid gave the α-epoxides. In the estrane series the olefins were produced by solvolysis of the 3β-*p*-toluenesulfonate esters. Methano steroids were secured by treatment of the olefins with diiodomethane and zinc–copper couple. The interaction of 3-oxo steroids with dimethylsulfoxonium methylide in dimethyl sulfoxide gave the corresponding spiro-3β-oxiranyl steroids. Biological evaluation of a number of these compounds by means of the myotrophic–androgenic assay indicates that the methano steroids are the most active compounds in the series and that androgens are bound to their receptors through a π-bond to the β-face of the A-ring. The β-face of rings A, B, and C and the α-face of the D-ring apparently are involved in the steroid–receptor complex. It is concluded that the steroid may function as a *conformational cam* or *wedge* in initiating the biological response.

In a preceding paper,[2] evidence was presented for the hypothesis that androgens, as well as progesterone, function by β-face adsorption on the receptor site. We now describe studies intended to provide further information on the mode of combination of steroids with receptor sites.

This work was initiated in the hope of confirming our hypothesis[3] that a requirement for androgenic activity is the presence of high electron density at C-2 and/or C-3, such as is provided by sp² hybridization. Normally, sp²-hybridized bonds at C-2 or C-3 will produce electron clouds which are symmetrical with respect to a plane passing through C-2, C-4, and C-10, or C-1, C-3, and C-5. Under these conditions, information cannot be obtained relative to the steric requirements on the α- and β-faces of the sp²-hybridized systems. Ethylene oxide and cyclopropane, however, are special cases in this connection. Both the Walsh[4] and the Coulson and Moffitt[5] models (Fig. 1) show the bonds in these substances to be sp² hybridized. Whereas these groups have the electronic characteristics of ethylene, they are, of course, quite different sterically.[6] Thus, it is possible to evaluate steric as well as electronic factors by the fusion of these rings to the steroid nucleus. The biological evaluation of steroids having cyclopropane or ethylene oxide rings fused to C-2 and C-3 should thus be informative with reference to the validity of the sp² hypothesis, the steric requirements around C-2 and C-3, and, finally, the question of the mechanism by which the C-2 and/or C-3 substituent is involved in androgenic action.

Chemical Results.—For the synthesis of the epoxides, the preparation of 2,3-olefins was required. Although



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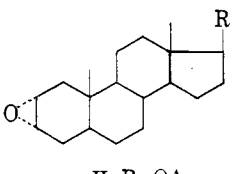
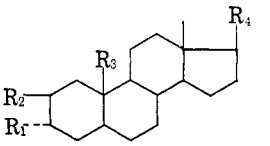


II, R=OAc
III, R=CH(OH)



IV,	R_1 = Cl;	R_2 = OH;	R_3 = CH_3;	R_4 = OAc
V,	R_1 = Cl;	R_2 = OH;	R_3 = CHNOH;	R_4 = OAc
VI,	R_1 = OH;	R_2 = OH;	R_3 = CN;	R_4 = OAc
VII,	R_1 = OAc;	R_2 = OAc;	R_3 = CN;	R_4 = OAc
XXII,	R_1 = H;	R_2 = OH;	R_3 = CH_3;	R_4 = OH
XXIII,	R_1 = H;	R_2 = O;	R_3 = CH_3;	R_4 = O

(1) (a) A preliminary account of portions of this work was presented at the Sixth Pan American Congress of Pharmacy and Biochemistry, Mexico City, Mexico, Dec. 11, 1963. (b) This investigation was supported by a PHS research grant (AM 05016) from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service. The n.m.r. spectrometer used in this study was provided by a grant (NSF-G 21268) from the National Science Foundation. (c) Portions of this work are taken from the Ph.D. Thesis of W. Ho, University of California, San Francisco, Calif., 1965.

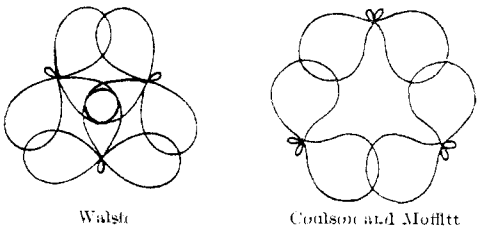
(2) M. E. Wolff and T. Jen, *J. Med. Chem.*, **6**, 726 (1963).

(3) R. Kwok, Ph.D. Thesis, University of California, San Francisco, Calif., Jan., 1963, p. 73–74. A similar hypothesis was independently proposed by A. Bowers, A. Cross, J. Edwards, H. Carpio, M. Calzada, and E. Denot, *ibid.*, **6**, 156 (1963).

(4) A. D. Walsh, *Trans. Faraday Soc.*, **45**, 179 (1949).

(5) C. A. Coulson and W. E. Moffitt, *Phil. Mag.*, **40**, 1 (1949).

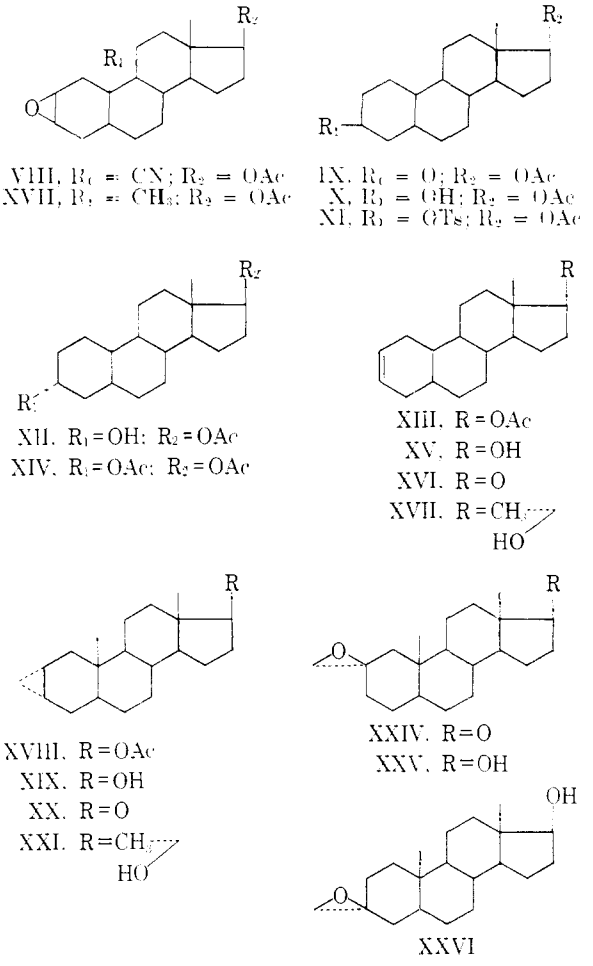
(6) For a review of the structure of cyclopropane, consult M. Y Lukina, *Russ. Chem. Rev.* (English Transl.), 419 (1962).



Walsh Coulson and Moffitt

Fig. 1.—Models of cyclopropane.

Marker[7] had prepared 17β-hydroxyandrost-2-ene acetate in unstated yield by treatment of 3-chloroandrostan-17-one with quinoline and subsequent reduction, in the present case the use of the Bamford–Stevens reaction was investigated.[8] Treatment of 17β-hydroxy-5α-androstan-3-one with *p*-toluenesulfonylhydrazine gave the corresponding hydrazone, which was subjected to the Bamford–Stevens reaction[9] in order to obtain the Δ²-olefin. Although this reaction had been applied to 7-oxo[10] and 2α-methyl-3-oxo steroids[11] with the production of mixtures of products in some cases, the identity of the side products had not been established. In the present case the reaction gave olefinic material which melted 10° below the literature value. Chromatography failed to raise the melting point, but on acetylation and subsequent chromatography, the melting point rose to the value reported for androst-2-en-17β-ol acetate. That the chromatographed acetylated product was in fact still a mixture of the Δ²- and Δ³-isomers was shown when epoxidation of this material gave two readily separated oxides, m.p. 108–110° and m.p. 184–186°, in the ratio 8:1, respectively. The higher melting oxide was found to be 3α,4α-epoxy-5α-androstan-17β-ol acetate (I) by comparison with an authentic sample,[12] whereas the major product was the expected 2α,3α-isomer (II). The formation of the two isomers indicates that the Bamford–Stevens reaction may lead to confusing mixtures of olefins, as in this particular case, where the sharply melting mixture could not be separated by our normal chromatographic procedures. It is noteworthy that collidine treatment of 3β-tosyloxy-5α-cholestane and 3β-tosyloxy-5α-androstan-17-one affords similar sharply melting isomer mixtures,[12,13] and in these cases even the oxide mixtures behave as homogeneous compounds.



To obtain 19-substituted steroidal epoxides and olefins, 2β,3β-epoxy-5α-androstan-17β-ol acetate[12] was cleaved with hydrogen chloride to afford 3α-chloro-5α-androstane-2β,17β-diol 17-acetate (IV), which on application of the Barton reaction[14] formed the corresponding 19-oxime (V). Treatment of V with zinc in acetic acid, in order to form the corresponding 2,3-olefin by analogy to our previous work,[3,15] gave instead a mixture of saturated products. Separation by chromatography afforded two major products. The first major compound to be eluted was found to be the epoxide VIII, apparently formed by internal displacement of the halogen by the hydroxyl group. In later fractions the diol monoacetate VI was obtained. The structure of VI was established by the elemental analysis and infrared spectrum, and by its conversion to the triacetate VII.[16]

In the 19-nor series, acetylation of 17β-hydroxy-5α-estran-3-one gave the corresponding acetate IX which, on reduction with lithium tri-*t*-butoxyaluminohydride, gave the corresponding 3β-hydroxy derivative. That reduction of 19-nor steroidal 3-ketones with metal hydrides affords the 3β-alcohols has been demonstrated previously.[17] Conversion to the tosylate XI, followed by treatment with sodium acetate, gave the corresponding Δ²-derivative XIII, together with some of the 3α-acetate inversion product XIV. The structure of XIV was confirmed by treatment of the 3β-tosylate with alumina to afford the 3α-hydroxy derivative XII. Acetylation gave a diacetate identical with XIV. For the 17α-methyl derivative, XIII was saponified to form XV which was oxidized to the ketone XVI. On treatment with methylmagnesium bromide, the 17α-methyl derivative XVII was obtained.

For the synthesis of methano steroids, the Simmons–

(7) R. Marker, O. Kamm, D. Jones, and L. Mixon, *J. Am. Chem. Soc.*, **59**, 1363 (1937).

(8) Another alternate preparation of the Δ²- and Δ³-androstenols was reported subsequent to the completion of this phase of the work in ref. 3.

(9) W. Bamford and T. Stevens, *J. Chem. Soc.*, 4735 (1952).

(10) D. Evans and G. Summers, *ibid.*, 4821 (1956).

(11) C. Djerassi, N. Finch, R. Cookson, and C. Bird, *J. Am. Chem. Soc.*, **82**, 5488 (1960).

(12) J. Fajkoš and F. Šorm, *Collection Czech. Chem. Commun.*, **24**, 3115 (1959).

(13) I. Malunowicz, J. Fajkoš, and F. Šorm, *ibid.*, **25**, 1359 (1960).

(14) D. H. R. Barton, J. M. Beaton, L. E. Geller, and M. M. Pechet, *J. Am. Chem. Soc.*, **82**, 2640 (1960); **83**, 4076 (1961).

(15) T. Jen and M. E. Wolff, *J. Med. Pharm. Chem.*, **5**, 876 (1962).

(16) R. Kwok and M. E. Wolff, *J. Org. Chem.*, **28**, 423 (1963).

(17) A. Bowers, H. J. Ringold, and E. Denot, *J. Am. Chem. Soc.*, **80**, 6115 (1958).

Smith[18] method was used. The preparation of several 2,3-dihalomethano and methano steroids using carbene syntheses has been disclosed recently,[19,20] but in some cases[19] only a probable stereochemical assignment could be made owing to the absence of stereochemical homogeneity in the addition of dihalocarbenes to steroidal olefins. The Simmons–Smith reagent, however, has been shown to be highly subject to steric effects. Thus, the Simmons–Smith reaction on Δ^7-cycloheptenyl acetate gives *only* the *trans* adduct[21] whereas the same reaction on the corresponding alcohol gives *only* the *cis* adduct, as a result of anchimeric stereochemical control resulting from complexation of the organozinc reagent with the basic oxygen function.[22] It has been reported that the Simmons–Smith reagent fails to add to steroidal 2,3-olefins.[20] In our hands, when iodomethylzinc iodide, prepared from zinc–copper couple,[23] was allowed to react with 5α-androst-2-en-17β-ol, only one product was isolated. This is quite certainly the 2α,3α-adduct XVIII, arising from the familiar sterically controlled α-face addition to the *trans* A/B ring system of steroids. Saponification of the adduct gave the corresponding alcohol XIX, which on chromic acid oxidation gave the ketone XX. Treatment of this compound with methylmagnesium bromide gave the corresponding 17α-methyl derivative (XXI).

For the synthesis of spirooxiranyl[24] steroids the method of Corey–Chaykovsky[25] was employed. Reduction of 2β,3β-epoxy-5α-androstan-17β-ol[12] with lithium aluminum hydride gave the diol XXII, which on oxidation with chromic acid in acetone gave the dione XXIII. Treatment of XXIII with dimethylsulfoxonium methylide in dimethyl sulfoxide solution gave only spiro-2β-oxiranyl-5α-androstan-17-one (XXIV). The 17-ketone group was unaffected under these conditions, as shown by the infrared spectrum. The stereochemistry of the product is assigned on the basis of backside attack by the bulky ylide. Reduction of the ketone gave the alcohol XXV. The action of the ylide on dihydrotestosterone gave the spiro compound XXVI.

Pharmacological Methods. Androgenic–Myotrophic Assay.[26]—The test materials in carboxymethylcellulose (CMC) suspension were given by subcutaneous injection, once daily for 7 days, to groups of five castrate male rats 21 days of age at the start of the test. Autopsy was performed on the day following the last day of administration.[27]

Statistical Analysis.—The significance of the data was established by the "t" test; the 95% confidence level was used as the limit of significance.

Discussion

The data from the pharmacological testing are displayed in Table I. Perhaps the most interesting finding is the remarkable activity shown by the methano steroid XIX, which is as active as testosterone propionate in the myotropic test and about one-third as active in the androgenic tests. Moreover, the epoxides II and III exhibit about one-fifth the myotrophic activity of testosterone propionate. The C-2 and C-3 atoms in all three compounds are in the sp^2-hybridized state. Compound I, a 3α,4α-epoxide, likewise has about one-fifth the myotrophic activity of testosterone propionate.

These results are of special interest in connection with the hypothesis that the reversible oxidation and reduction of a C-3 oxygen function is the basis for androgenic action.[28,29] It has been known for some time that steroids lacking an A-ring substituent still exhibit some androgenic activity,[30] and more recently it has been shown[31] that the hydrocarbon 5α-androstane, although inactive in the levator ani and seminal vesicle test when given subcutaneously, has some oral activity and weak local chick comb activity. The activity of all of these compounds may result from conversion to a C-3 oxygenated metabolite, and the possibility that such compounds are proandrogens has been suggested as an explanation for the fact that 5α-androstane has greater oral than subcutaneous activity. Even the 2,3-olefins described by Bowers[3] and the 2,3-epoxides obtained in the present work might owe their activity to conversion to C-3 ketonic metabolites. For example, enzymatic hydroxylation of double bonds is a well-known reaction. However, in the case of the methano steroid XII, this possibility is unlikely. Although comparatively little is known of the metabolism of the cyclopropane ring, cyclopropane and its methyl and ethyl ethers are metabolically inert and excreted unchanged. *trans*-α-Phenylcyclopropylamine (tranylcypromine) is extensively metabolized,[32] but this can be explained readily on the basis of an initial attack on the amino group. Thus, the marked biological action[33] of the ring-A olefins, epoxides, and cyclopropanes is most easily rationalized in terms of the activity of *the unaltered sp^2 system.* An oxidation–reduction mechanism, therefore, cannot be invoked to explain the androgenic response in this context, and we propose that the ring-A substituent functions by the *formation of a π-complex with the receptor site.* In this connection, it is noteworthy that the preparation of stable

(18) For leading references, consult H. E. Simmons, E. P. Blanchard, and R. D. Smith, *J. Am. Chem. Soc.*, **86**, 1347 (1964).

(19) L. H. Knox, E. Velarde, S. Berger, D. Cuadriello, P. Landis, and A. Cross, *ibid.*, **85**, 1851 (1963).

(20) R. C. Cookson, D. P. G. Hamon, and J. Hudec, *J. Chem. Soc.*, 5782 (1963).

(21) A. C. Cope and P. E. Peterson, *J. Am. Chem. Soc.*, **81**, 1943 (1959).

(22) W. G. Dauben and G. H. Berezin, *ibid.*, **85**, 468 (1963).

(23) R. S. Shank and H. Schechter, *J. Org. Chem.*, **24**, 1825 (1959).

(24) Although compounds of this type are named 1-oxaspiro[2.5]octane when dried from cyclohexanone, in the case of analogous compounds involving one of the steroid rings the stereochemistry of the new ring must be indicated. It is proposed that these compounds be named as spirooxiranyl steroids, using conventional symbols to indicate the position and configuration of the oxygen atom, *e.g.*, spiro-2β-oxiranyl-5α-androstan-17-one.

(25) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **84**, 868 (1962).

(26) L. G. Hershberger, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953).

(27) Pharmacological tests were performed at The Endocrine Laboratories, Madison, Wis.

(28) P. Talalay, B. Hurlock and H. G. Williams-Ashman, *Proc. Natl. Acad Sci. U. S.*, **44**, 862 (1958).

(29) H. J. Ringold in "Mechanism of Action of Steroid Hormones," C. A. Villee and L. L. Engel, Ed., Permagon Press, New York, N. Y., 1961, p. 214–215.

(30) C. D. Kochakian, *Proc. Soc. Exptl. Biol. Med.*, **80**, 386 (1952); C. H. Huggins and E. V. Jensen, *J. Exptl. Med.*, **100**, 241 (1954).

(31) A. Segaloff and R. B. Gabbard, *Endocrinology*, **71**, 949 (1962).

(32) J. J. Alleva, *J. Med. Chem.*, **6**, 621 (1963).

(33) For the purposes of this discussion, no sharp distinction is drawn between androgenic and myotrophic action except when specified, since the two effects both involve protein synthesis and may reflect changes in drug distribution or tissue sensitivity rather than more fundamental factors. For a discussion, see J. A. Szirmai in "Protein Metabolism," F. Gross, Ed., Springer Verlag, Berlin, 1962, pp. 45–74.

TABLE I
ANDROGENIC–MYOTROPHIC ASSAY

Compd. (total dose, mg.)		Body wt. gain, g./rat	Ventral prostate wt., mg.[a]	Seminal vesicle wt., mg.[a]	Levator ani wt., mg.[a]	Activity (vs. testosterone propionate) Androgenic	Myotrophic
Series A							
Castrate control		40	14.6 ± 1.4	12.0 ± 1.2	27.0 ± 1.8		
Testosterone propionate	(0.3)	45	52.4 ± 4.1	43.7 ± 7.5	47.4 ± 5.9	1.0	1.0
XXVII	(1.5)	40	17.4 ± 2.8	13.2 ± 2.7	30.4 ± 2.5	0	<0.1
VIII	(1.5)	34	12.8 ± 1.7	10.0 ± 0.9	23.7 ± 5.4	0	0
II	(1.5)	40	14.7 ± 3.6	11.5 ± 1.5	36.8 ± 4.2	0	<0.2
III	(1.5)	43	39.0 ± 9.1	21.4 ± 5.3	61.0 ± 4.5	<0.2	>0.2
I	(1.5)	40	26.2 ± 4.6	20.2 ± 4.4	48.7 ± 6.5	<0.2	0.2
Series B							
Castrate control		34	14.5 ± 4.1	11.8 ± 2.1	29.7 ± 1.6		
Testosterone propionate	(0.3)	36	32.2 ± 12.3	17.5 ± 2.1	34.2 ± 2.0	1.0	1.0
XXVII	(3.0)	34	22.1 ± 5.6	14.4 ± 1.1	34.3 ± 2.3	<0.1	0.1
Series C							
Castrate control		40	20.6 ± 1.2	13.4 ± 1.1	28.9 ± 5.0		
Testosterone propionate	(0.3)	45	67.8 ± 8.8	37.1 ± 2.9	47.0 ± 1.7	1.0	1.0
VIII	(3.0)	38	21.0 ± 4.6	12.1 ± 1.6	30.2 ± 4.5	0	0
II	(3.0)	43	45.0 ± 13.4	14.4 ± 1.4	52.7 ± 6.3	<0.1	>0.1
Series D							
Castrate control		34	13.5 ± 2.1	10.8 ± 1.7	21.5 ± 4.3		
Testosterone propionate	(0.3)	42	47.8 ± 9.8	32.2 ± 4.2	42.0 ± 8.8	1.0	1.0
XIX	(0.6)	39	25.3 ± 1.7	25.1 ± 1.6	54.7 ± 3.7	0.3	1.0
XIX	(0.9)	32	39.6 ± 11.0	29.8 ± 2.5	55.0 ± 4.4	0.3	1.0
XIX	(3.0)	41	46.2 ± 7.1	63.4 ± 9.8	70.9 ± 7.4	0.3	>1.0
XXI	(3.0)	39	35.3 ± 8.8	24.4 ± 2.9	49.8 ± 4.5	<0.1	>0.1
XXV	(3.0)	39	12.8 ± 2.7	8.5 ± 0.9	23.9 ± 2.2	0	0
XXVI	(3.0)	34	28.5 ± 11.5	16.3 ± 1.8	27.2 ± 2.6	<0.1	<0.1
XV	(3.0)	40	30.5 ± 9.3	31.4 ± 3.9	55.4 ± 6.8	<0.1	<0.1
XVII	(3.0)	40	32.8 ± 4.7	19.7 ± 1.3	49.1 ± 3.0	<0.1	0.1

[a] Mean ± standard deviation.

π-complexes of estrone and acetylergosterol with chromium tricarbonyl has been described recently.[34]

The manner in which the formation of such a complex can lead to androgenic action will be considered later in this section. First, however, it should be pointed out that compounds XXV, XXVI, and XXVII[12] are weakly active or inactive. The lack of potency of XXV should be compared with the response to 2-methylene-5α-androstan-17β-ol[35] which has one-tenth the androgenic and one-fifth the myotrophic activity of testosterone. Again, 17α-methyl-3-methylene-5α-androstan-17β-ol is reported to have appreciable myotrophic and androgenic activity.[35] Each of the present epoxides has sp^2 hybridization at C-2 or C-3, but the bulky oxide ring is on the β-face of the steroid. Thus, the tendency is for the asymmetrical sp^2 system to afford a more active compound when hindrance is greater on the α-face, than when hindrance is greater on the β-face. This result is compatible with our β-face adsorption hypothesis[2] for the A-ring of androgens. It is pertinent that 19-methyltestosterone is inactive[36] in the myotrophic–androgenic assay and the less bulky 19-methylenetestosterone is intermediate in activity between this compound and testosterone itself. Compounds XV and XVIII, the 2,3-olefins derived from 19-nortestosterone and its 17α-methyl derivative, respectively, are considerably less effective than 5α-androst-2-en-17β-ol[36] which is in harmony with an interaction between the 19-angular methyl group and the receptor site. The nitrile VIII is also inactive, as would be expected on the basis of all these considerations.

It is worthwhile to examine the structural requirements of other areas in the androgen molecule in connection with this new information about the A-ring. There is a low steric requirement on the α-face of the B-ring, since the 7α-methyl steroids have high activity.[37] When ring D is considered, however, a number of points are found where the steric requirement on the α-face is greater than on the β-face, indicating α-face adsorption in these areas. Thus, 17α-methyltestosterone has high activity, but larger 17α-substituents such as ethyl, vinyl, and ethinyl markedly diminish the response. Again, the 17α-methylmethano steroid XXI is less active than XIX. In the 19-nor series 17α-alkyl groups larger than ethyl abolish activity.[38] Conversely, the presence of bulky ester groups in the 17β-position does not decrease androgenic action. Although *a priori* it would be expected that the C-17 esters first hydrolyze and the free 17β-alcohols are the active species, the available data do not support

(34) A. Nakanura and M. Tsutsui, *J. Med. Chem.*, **6**, 796 (1963); *Z. Naturforsch.*, **18b**, 666 (1963).

(35) (a) A. D. Cross, J. A. Edwards, J. C. Orr, B. Berköz, L. Cervantes, M. C. Calzada, and A. Bowers, *J. Med. Chem.*, **6**, 162 (1963); (b) D. D. Evans, D. P. Evans, G. S. Lewis, and P. J. Palmer, *J. Chem. Soc.*, 4312 (1963); (c) K. Irmscher, H. G. Kraft, and K. Brückner, *J. Med. Chem.*, **7**, 345 (1964).

(36) R. I. Dorfman and F. A. Kincl, *Endocrinology*, **72**, 259 (1963); *Steroids*, **3**, 109 (1964).

(37) J. A. Campbell, S. C. Lyster, G. W. Duncan, and J. C. Babcock, *ibid.*, **1**, 317 (1963).

(38) F. J. Saunders and V. A. Drill, *Endocrinology*, **58**, 567 (1956).

this expectation and indicate instead that the intact esters are active as such.[39] A 17β-functional group is required for appreciable activity, however, since 5α-androstane is markedly less active than 5α-androstan-17β-ol.[31]

Bulky groups at C-13 do not decrease activity, as is apparent from the interesting work of Smith and his co-workers[40,41] in which active 19-nortestosterone derivatives having C-13 ethyl, propyl, and butyl groups were prepared. On the other hand, the presence of an 11β-hydroxyl function decreases activity.[36]

All of these data can be rationalized in terms of a steroid–receptor complex of the type shown in Fig. 2. It is suggested that the steroid is in contact with the receptor surface in two discrete areas: the β-face of rings A, B, and C and the α-face of ring D. The 13β- and 17β-substituents are in a relatively unhindered environment. It is proposed that the two principal binding sites are the A-ring, where a π-bond is formed, and the 17β-function, which can be attached by any of several types of nonbonded interactions (hydrogen bond, hydrophobic bond, etc.). The remaining areas in contact with the receptor would form ordinary hydrophobic bonds or van der Waals bonds. It is suggested that no chemical reaction (*e.g.*, oxidation–reduction) as such takes place, but that the effect of the steroid is to induce a conformational change in the receptor. The receptor surface on the β-face, for example, might be deformed to accommodate the C-19 angular methyl group.

Since the nature of the receptor is unknown, the diverse effects of steroids have resulted in a number of theories of action[42] including the previously mentioned oxidation–reduction theory, the action of steroids on various enzymes,[42] metal-ion chelation mechanisms,[43] and effects on cell membranes.[44,45] An important view of the mechanism of steroid hormone action is that steroids exert their effect at the level of DNA control of RNA synthesis, a process which has been called "gene activation."[46] The messenger RNA molecules whose synthesis is thus controlled are the templates for *de novo* enzyme synthesis, and it is these enzymes which regulate the processes resulting in the observed physiological effects.[47] Regulatory effects on RNA synthesis or *de novo* enzyme or protein synthesis have been observed for estradiol,[48–50] testosterone,[51,52] cortisol,[53] and aldosterone.[54,55]

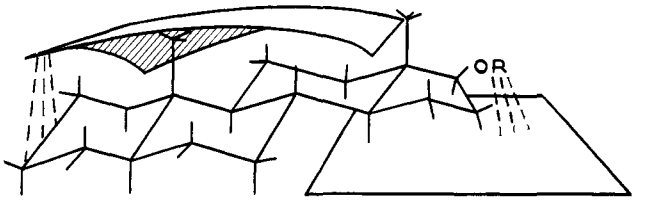


Fig. 2.—Simultaneous interaction of the androgen molecule with two spatially separate surfaces at the receptor site.

There is ample evidence for the occurrence of steroid–protein or steroid–DNA interactions. Munck, Scott, and Engel[56–58] have shown that testosterone, among other steroids, complexes with adenine and certain of its derivatives. More recently, it has been shown[59] that testosterone binds strongly to DNA and to a lesser extent to histone. The interaction between steroids and proteins is well established.[60] Dannenberg[61] has suggested that steroids may displace base pairs in the DNA double helix, and in fact it has been demonstrated that the coil form of DNA has a higher affinity for steroids than the helical form.[59]

How then can the peculiar steric and electronic relationships shown schematically in Fig. 2 be rationalized in terms of these concepts? The compact, rigid steroid molecule can be viewed as a *conformational cam*. Depending on the size and disposition of the nuclear substituents in space, specific conformational changes could be induced in the surface to which the fused ring system is bound, much as a complex cam can depress a number of levers. The steric requirements in Fig. 2 may result from the *wedging of the steroid molecule between adjacent surfaces in a helical structure*, each surface in the figure representing one turn of the helix. In this way, steroids could initiate RNA synthesis by serving as markers for the nucleotide sequence, by displacing a repressor from the DNA surface, or by combining with repressor substances.

Alternatively, steroids could become enzyme activators by inducing in the enzyme specific conformational changes necessary for catalytic action.[62] Either proteins or nucleic acids, both of which have helical structure, could be involved as receptors.

Experimental[63]

3α,4α-Epoxy-5α-androstan-17β-ol Acetate (I).—A solution of 20.0 g. (0.064 mole) of 17β-hydroxy-5α-androstan-3-one, 11.9 g. (0.064 mole) of *p*-toluenesulfonylhydrazine, and 20 ml. of concentrated HCl in 500 ml. of ethanol was heated under reflux for

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(63) Melting points were determined with a Thomas–Hoover apparatus and are corrected. Infrared spectra were obtained with a Beckman IR-5 instrument. Ultraviolet spectra were obtained with a Cary Model 11 instrument. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Optical rotations were obtained in a 0.5-dm. tube with a Rudolph photoelectric polarimeter.

1 hr. The mixture was poured into water and extracted with ether. The ether solution was washed with 2 *N* HCl, 10% $NaHCO_3$ solution, and water, dried, and evaporated. The gummy residue was recrystallized once from methanol–benzene to give 21.0 g. (66%) of product, m.p. 117–120°. A solution of 4.2 g. (0.009 mole) of the crude hydrazone and 4.2 g. of potassium hydroxide in 120 ml. of diethylene glycol monoethyl ether was heated under reflux for 5 hr. and diluted with water. The resulting precipitate was filtered and recrystallized once from hexane to give 1.2 g. (50%) of product, m.p. 153–155° (lit.[7] m.p. 165° for 17β-hydroxy-5α-androst-2-ene). The melting point could not be raised by chromatography.

A solution of 1.2 g. (0.0044 mole) of this olefin in 10 ml. of pyridine was treated with 2 ml. of acetic anhydride at 27° for 16 hr. The solution was poured onto ice, acidified with 5% HCl, and extracted with ether. The ether solution was washed with 10% $NaHCO_3$ solution and water, dried, and evaporated. The residue was chromatographed on 40 g. of neutral alumina. A crystalline product (0.90 g.) was obtained from the benzene and benzene–ether fractions, m.p. 92–94° (lit.[7] m.p. 96° for 17β-hydroxy-5α-androst-2-ene acetate). Recrystallization from methanol gave large crystals, m.p. 95–99°.

A stirred solution of 3.8 g. (0.012 mole) of the foregoing acetate in 30 ml. of chloroform was treated with 2.5 ml. of 40% peracetic acid (0.013 mole) containing 0.25 g. of sodium acetate trihydrate at 27° for 48 hr. The mixture was diluted with water and extracted with chloroform. The chloroform solution was washed with 10% $NaHCO_3$ solution and water, dried, and evaporated. There was obtained 4.0 g. of solid residue, which was recrystallized from methanol. The first crop was recrystallized further from acetone to give 0.3 g. of product, m.p. 184–184°.

The analytical sample, obtained from acetone, had m.p. 184–186°; $[\alpha]^{25}D$ +31° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 5.80, 8.05, and 12.1 μ [lit.[12] for I, m.p. 179–180°, $[\alpha]^{20}D$ +29.7 ± 1° (*c* 2.87), when prepared by another method].

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.44; H, 9.59.

A solution of 0.20 g. (0.00063 mole) of 17β-hydroxy-5α-androst-3-ene acetate[64] in 15 ml. of chloroform was stirred with 0.12 ml. of 40% peracetic acid and 0.05 g. of sodium acetate trihydrate at 27° for 48 hr. The mixture was washed with 5% $NaHCO_3$ solution and water, dried, and evaporated to give a crystalline residue. Two recrystallizations from acetone gave small needles, m.p. 184–186°, identical with the epoxide described above, as shown by mixture melting point and comparison of the infrared spectra.

2α,3α-Epoxy-5α-androstan-17β-ol Acetate (II).—The mother liquor from the first recrystallization of I was concentrated and the resulting residue was recrystallized from aqueous methanol to afford 2.5 g. of II, m.p. 100–103°. The analytical sample, obtained from aqueous methanol had m.p. 108–110°; $[\alpha]^{25}D$ +16° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 5.76, 8.00, 12.3, and 12.5 μ [lit.[12] m.p. 109–110°, $[\alpha]^{20}D$ +19.5° (*c* 2.56), when prepared by another method].

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.65; H, 9.53.

3α-Chloro-5α-androstane-2β,17β-diol 17-Acetate (IV).—A solution of 10.00 g. (0.030 mole) of 2β,3β-epoxy-5α-androstan-17β-ol acetate[12] in 500 ml. of anhydrous ether was treated with a stream of HCl gas for 5 min. The solution was kept at 27° for 16 hr., and the solvent was removed under reduced pressure. The crystalline residue was recrystallized from aqueous methanol to afford 8.00 g. (72%) of chlorohydrin. Further recrystallization from aqueous methanol gave the analytical sample, m.p. 161–162°, $[\alpha]^{25}D$ +40° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{21}H_{33}ClO_3$: C, 68.40; H, 9.02. Found: C, 68.62; H, 8.92.

3α-Chloro-*syn*-19-oximino-5α-androstane-2β,17β-diol 17-Acetate (V).—A solution of 8.00 g. (0.023 mole) of IV in 40 ml. of pyridine was treated with excess nitrosyl chloride at 0°. The mixture was poured into ice–water and the resulting precipitate was filtered, washed with water and aqueous methanol, and air-dried. It was dissolved in 200 ml. of toluene, the toluene solution was dried (Na_2SO_4), and the dried solution was irradiated under nitrogen for 1 hr. with a Hanovia 450-w. high-pressure mercury arc under ice bath cooling. The solvent was removed under reduced pressure and a crystalline residue was obtained. Recrystallization from acetonitrile afforded 3.0 g. (35%) of small crystals, m.p. 218–219.5°. The analytical sample had m.p. 220–221°; $[\alpha]^{25}D$ +12° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 3.14, 3.22, 5.74, and 8.00 μ.

Anal. Calcd. for $C_{21}H_{32}ClNO_3$: C, 63.40; H, 8.12. Found: C, 63.24; H, 7.98.

Treatment of V with Zinc–Acetic Acid.—A solution of 2.2 g. (0.0058 mole) of V in 70 ml. of acetic acid was treated with 4.4 g. of zinc dust at 93–95° for 16 hr. The mixture was cooled, filtered, and the filter cake was washed with acetic acid. The combined filtrate was diluted slowly with water and the resulting precipitate was collected, washed with water, and dried. It was chromatographed on 80 g. of neutral alumina: the following eluents were used: 80 ml. of benzene, 80 ml. of benzene–ether 1:1, 80 ml. of ether, four 40-ml. portions of 1% methanol in ether, four 40-ml. portions of 2% methanol in ether, four 40-ml. portions of 4% methanol in ether, four 40-ml. portions of 8% methanol in ether, four 40-ml. portions of 16% methanol in ether. From the ether fractions, a trace of what was apparently the expected olefin was isolated, m.p. 168–170°; λ_{max}^{KBr} 4.50, 5.80, 8.10, 9.59, and 14.65 μ; but there was insufficient material for analysis.

From the 2% methanol fractions there was obtained crystalline VIII. Recrystallization from benzene–hexane gave colorless needles, m.p. 214–216°; $[\alpha]^{25}D$ −25° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 4.49, 5.79, 8.05, 9.60, and 12.49 μ.

Anal. Calcd. for $C_{21}H_{29}NO_3$: C, 73.43; H, 8.51. Found: C, 73.32; H, 8.34.

The 16% methanol fraction gave VI, which on recrystallization from acetonitrile gave colorless microscopic needles, m.p. 271–273°; $[\alpha]^{25}D$ +2° (*c* 0.5, ethanol); λ_{max}^{KBr} 2.98, 4.48, 5.70, 8.00, and 9.64 μ.

Anal. Calcd. for $C_{21}H_{31}NO_3$: C, 69.77; H, 8.65. Found: C, 69.43; H, 8.47.

Acetylation of this compound gave VII,[58] as shown by mixture melting point and comparison of the infrared spectra.

2α,3α-Epoxy-17α-methyl-5α-androstan-17β-ol (III).—A solution of 1.7 g. (0.0059 mole) of 17β-hydroxy-17α-methyl-5α-androst-2-ene[8] in 15 ml. of chloroform was stirred with 1.12 ml. of 40% peracetic acid containing 0.10 g. of sodium acetate trihydrate at 27° for 48 hr. It was diluted with water and extracted with chloroform. The chloroform solution was washed with water, 10% sodium bicarbonate solution, and water, dried with sodium sulfate, and evaporated to give 1.8 g. of solid residue. Recrystallization from methanol gave a crystalline product, m.p. 195–199°. Further recrystallization from the same solvent gave an analytical sample, m.p. 200–203°; $[\alpha]^{25}D$ −5° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 2.95, 12.3, and 12.46 μ.

Anal. Calcd. for $C_{20}H_{32}O_2$: C, 78.78; H, 10.59. Found: C, 78.66; H, 10.44.

17β-Hydroxy-5α-estran-3-one Acetate (IX).—A solution of 0.50 g. of 17β-hydroxy-5α-estran-3-one[65] was acetylated with acetic anhydride in pyridine at 21° for 18 hr. It was diluted with water and the precipitate was collected and washed with water. Recrystallization from aqueous methanol gave 0.40 g. of product, m.p. 98–100°. Further recrystallization from hexane gave the analytical sample, m.p. 100–102°, $[\alpha]^{28}D$ +46° (*c* 0.5, $CHCl_3$).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.28; H, 9.42.

5α-Estrane-3β,17β-diol 17-Acetate (X).—A chilled solution of 1.9 g. of IX in 10 ml. of tetrahydrofuran was added to a solution of 4.0 g. of lithium tri-*t*-butoxyaluminohydride in 20 ml. of tetrahydrofuran chilled in an ice bath. After 0.5 hr. it was poured into water, acidified with acetic acid, and extracted with ether. The ether solution was washed with 5% $NaHCO_3$ and water, dried, and evaporated. The residue was recrystallized once from acetone–hexane to afford 1.5 g. (79%) of fine needles. Further recrystallization gave the analytical sample, m.p. 108–110, $[\alpha]^{28}D$ +13° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{20}H_{32}O_2$: C, 74.96; H, 10.06. Found: C, 75.05; H, 10.00.

5α-Estrane-3β,17β-diol 17-Acetate 3-*p*-Toluenesulfonate (XI).—A solution of 3.70 g. of X and 3.70 g. of *p*-toluenesulfonyl chloride in 20 ml. of pyridine was kept at 21° for 70 hr. It was poured into water and extracted with ether. The ether solution was washed with 5% HCl, 5% $NaHCO_3$ solution, and water, dried, and evaporated. One recrystallization from acetone–hexane gave 3.7 g. (67%) of flakes, m.p. 169–170°. Further recrystallization from the same solvents gave the analytical

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sample, m.p. 176–178; $[\alpha]^{28}D$ −4° (*c* 1, $CHCl_3$); λ_{max}^{KBr} 5.78, 7.91, 8.50, 9.50, 10.61, 11.71, and 14.92 μ.

Anal. Calcd. for $C_{27}H_{38}O_5S$: C, 68.33; H, 8.07. Found C, 68.33; H, 8.16.

Chromatographic purification of XI on neutral alumina gave the 3α-hydroxy compound XII, m.p. 155–156°, $[\alpha]^{28}D$ +18° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{20}H_{32}O_3$: C, 74.96; H, 10.00. Found: C, 74.71; H, 9.99.

17β-Hydroxy-5α-estr-2-ene 17-Acetate (XIII).—A mixture of 1.3 g. of XI, 1.3 g. of sodium acetate, 2 ml. of acetic anhydride, and 20 ml. of acetic acid was heated under reflux for 3 hr. It was diluted with water and extracted with ether. The ether solution was washed with 5% $NaHCO_3$ solution and water, dried, and evaporated. The residue was chromatographed on 40 g. of neutral alumina packed in hexane. Elution with hexane and benzene–hexane 1:1 afforded 500 mg. (60%) of crystals, m.p. 96–99°. The analytical sample, obtained by vacuum sublimation, had m.p. 96–98°, $[\alpha]^{28}D$ +68° (*c* 0.8, $CHCl_3$).

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.58; H, 10.24.

From the ether fractions, 200 mg. of material was recovered, m.p. 125°. Recrystallization from methanol gave the diacetate XIV, m.p. 137–139°, $[\alpha]^{28}D$ +24° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 72.91; H, 9.69.

Acetylation of XII gave the same product.

17β-Hydroxy-5α-estr-2-ene (XV).—A solution of 0.6 g. of XIII in 50 ml. of 5% KOH in methanol containing 10 ml. of water was heated under reflux for 1 hr. The solution was concentrated, diluted with water, and the precipitate was collected. Recrystallization from aqueous methanol gave 0.45 g. (87%) of long needles, m.p. 110–112°. The analytical sample, from aqueous methanol, had m.p. 111–113°, $[\alpha]^{28}D$ +100° (*c* 0.8, $CHCl_3$).

Anal. Calcd. for $C_{18}H_{28}O$: C, 83.02; H, 10.84. Found: C, 83.07; H, 10.56.

5α-Estr-2-en-17-one (XVI).—A solution of 0.50 g. of XV in 20 ml. of acetone was treated with 8 *N* chromic acid reagent under ice cooling. After a slight excess of oxidant had been added, the mixture was kept in an ice bath for 3 min., treated with isopropyl alcohol, and diluted with water. The resulting crystalline precipitate was filtered and washed with water. There was obtained 0.45 mg. (90%) of product, m.p. 121–123°. Recrystallization from methanol gave the analytical sample, m.p. 122–124°, $[\alpha]^{28}D$ +190° (*c* 0.4, $CHCl_3$).

Anal. Calcd. for $C_{18}H_{26}O$: C, 83.66; H, 10.14. Found: C, 83.27; H, 9.89.

17α-Methyl-5α-estr-2-en-17β-ol (XVII).—A solution of 0.4 g. of XVI and 8 ml. of 3 *N* methylmagnesium bromide in 50 ml. of anhydrous ether was heated under reflux for 16 hr. It was poured onto ice, acidified with HCl, and extracted with ether. The ether solution was washed with water, dried, and evaporated, and the solid residue was chromatographed on 25 g. of neutral alumina. The product, 0.32 g. (77%), m.p. 133–135°, was recovered from the 1% methanol in ether fractions. It formed a gel in all attempts at recrystallization. Vacuum sublimation gave the analytical sample, m.p. 135–137°, $[\alpha]^{28}D$ +56° (*c* 0.8, $CHCl_3$).

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 83.48; H, 11.22.

2α,3α-Methano-5α-androstan-17β-ol Acetate (XVIII).—A stirred mixture of 16.3 g. (0.25 mole) of Zn–Cu couple,[23] 54 g. of methylene iodide (0.2 mole), and 0.015 g. of iodine in 300 ml. of anhydrous ether was heated under reflux for 1 hr., and 6.3 g. (0.02 mole) of 5α-androst-2-en-17β-ol acetate dissolved in 30 ml. of anhydrous ether was added. The mixture was heated under reflux for 90 hr. and then filtered through alumina. The ether was washed with 5% HCl (to wash out any dissolved ZnI_2) and water and dried (Na_2SO_4). Evaporation and recrystallization of the residue from methanol afforded 3.5 g. (50%) of product, m.p. 98–104°. Further recrystallization gave the analytical sample, m.p. 105–106°, $[\alpha]^{28}D$ +105° (*c* 0.87, $CHCl_3$).

Anal. Calcd. for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 79.71; H, 10.17.

2α,3α-Methano-5α-androstan-17β-ol (XIX).—A solution of 3.0 g. (0.009 mole) of XVIII, 4.0 g. of KOH, and 5 ml. of water in 80 ml. of methanol was heated under reflux for 1 hr. It was diluted with water and the resulting precipitate was filtered and recrystallized from methanol to afford 2.5 g. (95%) of colorless crystals, m.p. 127–130°. Further recrystallization gave the analytical sample, m.p. 130–132°, $[\alpha]^{28}D$ +38° (*c* 1, $CHCl_3$); lit.,[19] for a product tentatively assigned this structure, m.p. 127–128°, $[\alpha]D$ +26°.

Anal. Calcd. for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Found: C, 83.52; H, 10.93.

2α,3α-Methano-5α-androstan-17-one (XX).—A solution of 0.5 g. (0.0017 mole) of XIX in 30 ml. of acetone was cooled in ice and treated with excess 8 *N* chromic acid solution. The excess chromic acid was decomposed with 2-propanol and the solvent was removed under reduced pressure. The residue was washed with water and recrystallized from aqueous methanol to give 0.5 g. (80%) of colorless crystals, m.p. 97–100°. The analytical sample had m.p. 100–102°, $[\alpha]^{28}D$ +113° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{20}H_{30}O$: C, 83.86; H, 10.56. Found: C, 83.84; H, 10.53.

2α,3α-Methano-17α-methyl-5α-androstan-17β-ol (XXI).—A solution of 0.5 g. (0.0017 mole) of XX and 10 ml. of methylmagnesium bromide in 60 ml. of anhydrous ether was heated under reflux for 18 hr. It was poured onto ice, acidified with 20% HCl solution, and extracted with ether. The ether solution was washed with water and dried (Na_2SO_4). Evaporation gave a crystalline residue, which on recrystallization from methanol afforded 0.41 g. (78%) of product, m.p. 175–178°. Further recrystallization from methanol gave the analytical sample, m.p. 177–179°, $[\alpha]^{28}D$ +22° (*c* 0.6, $CHCl_3$).

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.10; H, 11.28.

Androstane-2,17-dione (XXIII).—A solution of 0.50 g. of XXII[65] in 50 ml. of acetone was treated with excess 8 *N* chromic acid reagent. The excess oxidant was decomposed with isopropyl alcohol, the mixture was diluted with water, and the precipitate was filtered and washed with water. It was recrystallized once from acetonitrile to give 0.40 g. (80%) of the dione. Further recrystallization from the same solvent gave the analytical sample, m.p. 154–155°, $[\alpha]^{28}D$ +114° (*c* 0.4, $CHCl_3$); λ_{max}^{KBr} 5.75 and 5.90 μ (lit.[66] m.p. 152.5–154.5, $[\alpha]^{20}D$ +119.5°, when prepared by another method).

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 78.79; H, 9.48.

Spiro-2β-oxiranyl-5α-androstan-17-one (XXIV).—A stirred solution of 3.2 g. of trimethylsulfoxonium iodide[67] in 50 ml. of dimethyl sulfoxide was treated with 0.35 g. of sodium hydride under nitrogen. After a clear solution had been obtained, 1.0 g. of XXIII was added, and stirring was continued at room temperature for 16 hr., followed by 4 hr. at 50°. It was diluted with water and the resulting precipitate was collected and washed with water. Recrystallization from acetonitrile gave 0.60 g. of long needles, m.p. 162–165°. The analytical sample obtained from the same solvent had m.p. 165–167°; $[\alpha]^{28}D$ +52° (*c* 0.4, $CHCl_3$); λ_{max}^{KBr} 5.79, 9.93, and 10.97 μ.

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.40; H, 9.97.

Spiro-2β-oxiranyl-5α-androstan-17β-ol (XXV).—A solution of 0.45 g. of XXIV in 20 ml. of tetrahydrofuran was treated with 1.0 g. of lithium tri-*t*-butoxyaluminohydride in 30 ml. of tetrahydrofuran for 30 min. under ice cooling. It was diluted with water, acidified with acetic acid, extracted with ether, and the ether solution was washed with 5% sodium bicarbonate solution and water and dried. Evaporation gave a crystalline residue. Recrystallization from acetonitrile gave 0.30 g. (67%) of flakes, m.p. 192–194°. Further recrystallization from acetonitrile gave the analytical sample, m.p. 200–202°; $[\alpha]^{28}D$ +10° (*c* 0.8, $CHCl_3$); λ_{max}^{KBr} 2.98, 9.49, 11.10, and 12.57 μ.

Anal. Calcd. for $C_{20}H_{32}O_2$: C, 78.89; H, 10.59. Found: C, 79.05; H, 10.48.

Spiro-3β-oxiranyl-5α-androstan-17-one (XXVI).—A solution of 4.8 g. (0.038 mole) of trimethylsulfoxonium iodide[67] in 100 ml. of dimethyl sulfoxide was stirred with 0.52 g. of powdered sodium hydride under nitrogen at 21°. After a clear solution had been obtained and hydrogen evolution had ceased, 3.1 g. (0.019 mole) of 17β-hydroxy-5α-androstan-3-one was added. The stirred solution was maintained at 21° for 16 hr., and then at 50° for 2 hr. It was poured into water, and the precipitate was filtered, washed with water, and dried. It was recrystallized from acetonitrile to give 2.0 g. (63%) of product, m.p. 170–173°. Further recrystallization from acetonitrile gave the analytical sample,

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m.p. 173–175°; $[\alpha]^{28}D$ +3° (*c* 0.8, $CHCl_3$); λ_{max}^{KBr} 2.90, 10.89, and 11.09 μ.

Anal. Calcd. for $C_{30}H_{42}O_2$: C, 78.89; H, 10.59. Found: C, 78.71; H, 10.59.

Heterocyclic Steroids in the Antiinflammatory Series[1]

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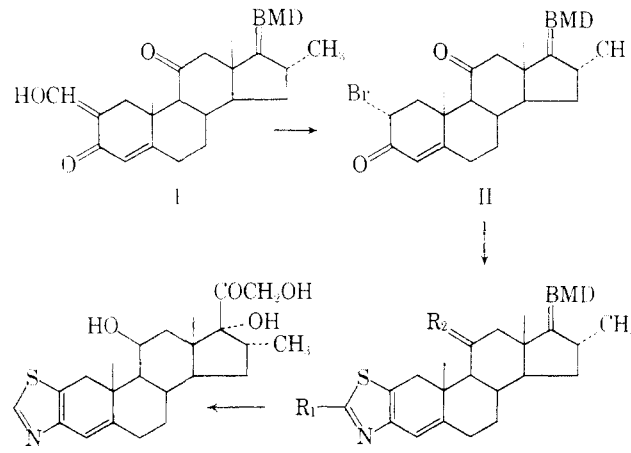
A number of heterocyclic-fused steroids have been prepared as an extension of the lead provided by the steroidal [3,2-*c*]pyrazoles as antiinflammatory agents. The syntheses of steroidal [3,2-*d*]thiazoles, [2,3-*d*]imidazoles, [3,2-*d*]triazoles, and [3,2-*d*]pyrimidines related to cortisone are described. The 3′-phenyl[3,2-*d*]-3′*H*-1′,2′,3′-triazole function has been found to be a powerful activity-enhancing group.

Pharmacologically active steroids with a pyrazole function fused to positions C-2 and -3 were first reported in the androgen series.[2] More recent reports from these laboratories[3] demonstrated that the [3,2-*c*]-pyrazoles of antiinflammatory steroids were also consistent with activity. Particularly interesting was the finding that the 2′-phenyl[3,2-*c*]pyrazole group[3a] was the most potent activity-enhancing function in the antiinflammatory series yet discovered.

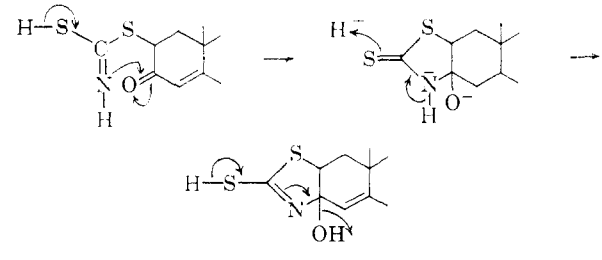
It was a matter of interest to determine if the antiinflammatory activity of the steroidal pyrazoles was unique, or could be maintained by other heterocyclic fusions. To that end a number of representative compounds were synthesized.

Preparation of a thiazolo steroid was undertaken because this structure is known to retain biological activity in the androgen series[4,5] (see Chart I). A convenient starting material was 17α,20;20,21-bismethylenedioxy-2-formyl-16α-methyl-4-pregnene-3,11-dione (I), which was obtained from 17α,20;20,21-bismethylenedioxy-16α-methyl-4-pregnene-3,11-dione[6] by condensation with ethyl formate. Bromination and subsequent deformylation afforded the 2α-bromo ketone II.[7] Reaction of II with a thioamide was expected to yield a thiazole, since 4,5-allodihydro-2-bromo-3-keto steroids readily undergo this reaction.[4,5,8] In the present case it appears that the first step, replacement of the C-2 bromine by the sulfur of thiourea, proceeded normally. However, the subsequent ring closure with the Δ^4-analogs could not be accomplished even by reflux in dimethylformamide. In contrast, ammonium dithiocarbamate[10] reacted smoothly at room temperature to yield the 2-mercaptothiazole III. The facile ring closure is most probably assisted by the 2′-mercapto group as indicated.

CHART I



BMD = bismethylenedioxy



Removal of the 2′-mercapto group could be carried out by nitric acid oxidation, or better with alkaline hydrogen peroxide and subsequent acidification of the sulfinic acid sodium salt.[10] Reduction of the C-11 ketone and removal of the BMD protecting group[11]

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