Heterocyclic Steroids. 2.[1a] Synthesis and Androgenic Activity of A-Ring Oxaandrostanes[1b]

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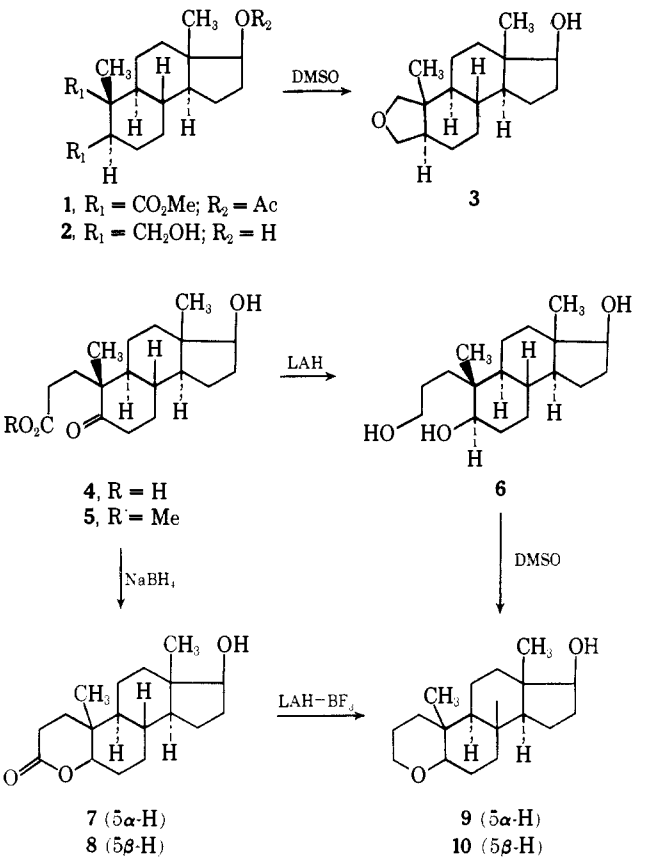
Received February 17, 1971

The preparation of 2-oxa-, 3-oxa-, and 4-oxa-5α-androstane derivatives is described. The synthesis of 2-oxa-A-nor-5α-androstan-17β-ol is also outlined. Biological evaluation of these products indicates that 2-oxa-5α-androstan-17β-ol is the most active material (50–100% of testosterone) and the 3-oxa, 4-oxa, and A-nor-2-oxa materials are weakly active or inactive. These results indicated that a group which flattens ring A in the vicinity of C-2 is required for androgenic activity.

In previous papers describing work in this laboratory, the androgenic–anabolic activity of thia-,[2] selena-, and tellurioandrostane[1a] derivatives was taken as evidence that it is the ring-flattening steric effects of such diverse groups as carbonyl, thia, and tellurio which are responsible for the activity-engendering effects of these substituents in the A ring of androstane derivatives. The localization of the requirement for such an atom was taken to be C-2 and/or C-3. Since it is possible to prepare various A-ring heterosteroids, *viz.* with the hetero atom replacing C-1, C-2, C-3, and C-4, in principle it would be possible to identify the exact area of the A ring where the flattening effect is required by the preparation of such derivatives. The studies decribed in this paper are, therefore, directed to the preparation of oxaandrostanes in which the oxygen atoms has been systematically moved around the A ring. Biological activities of the resulting compds are correlated with these changes. In addition, the effect of changing the ring size by contraction to 5 members has been investigated.

Treatment of dimethyl ester **1**[2] with LAH gave diol **2** which was cyclized to the *A*-noroxasteroid **3** in refluxing DMSO.[3] For the preparation of 4-oxaandrostane derivatives, lactone **7** was prepared by the procedure of Atwater and Ralls.[4] The melting point of this material corresponded to the literature value, but an examination of the nmr spectrum indicated the possibility that it was contaminated by 5β isomer **8**. Reduction with LAH–BF_3 in Et_2O gave a mixture of 4-oxa compounds **9** and **10**. Although the melting point of this mixture corresponded quite well with the literature value,[5] the nmr spectrum showed conclusively, through the presence of 4 separate Me peaks, that a mixture of the C-5 isomers **9** and **10** was at hand. Repeated recrystallization of the mixture from acetone–hexane gave a sample of pure **9**, and from the mother liquors there was isolated a sample of **10** by recrystallization from hexane. The identity of both of these compds was established through elemental analysis as well as high-resolution mass spectra and nmr spectra.

The configuration of C-5 of **9** and **10** was readily apparent from the spin-coupling patterns[6] of the C-5 H with the protons at C-6. In the 5α isomer **9** the 5α-H has an axial–axial coupling of 10 Hz to the 6β-H and an axial–equatorial coupling of 5 Hz to the 6α-H resulting in a quartet centered on δ 2.92. On the other hand, the 5β isomer **10** is clearly identified by the "triplet" at δ 3.15 resulting from the equatorial–equatorial coupling of the 5β-H to the 6α-H (5 Hz), and the axial–equatorial coupling of the 5β-H to the 6β-H (5 Hz). The formation of a mixture of **9** and **10** through the LAH–BF_3 reduction is apparently due to a process in the reduction reaction, but the possibility of an isomer mixture of **7** and **8** cannot be excluded. Compd **9** was also obtained by esterification of acid **4**[7] with CH_2N_2 to give ester **5** which was reduced with LAH to diol **6**. This compd was cyclized in refluxing DMSO[3] to give **9**



(1) (a) For the preceding paper in this series, see M. E. Wolff and G. Zanati, *Experientia*, **26**, 1115 (1970). (b) This research was supported in part by a Public Health Service Grant (AM-05016) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

(2) M. E. Wolff and G. Zanati, *J. Med. Chem.*, **12**, 629 (1969).

(3) B. T. Gillis and P. E. Beck, *J. Org. Chem.*, **28**, 1388 (1963).

(4) N. W. Atwater and J. W. Ralls, *J. Amer. Chem. Soc.*, **82**, 2011 (1960).

(5) (a) G. R. Pettit and T. R. Kasturi, *J. Org. Chem.*, **26**, 4557 (1961); (b) G. R. Pettit and T. R. Kasturi, *ibid.*, **26**, 986 (1961).

(6) N. S. Bhacca and D. H. Williams, "Applications of Nmr Spectroscopy in Organic Chemistry," Holden-Day Inc., San Francisco, Calif., 1964, p 51.

(7) H. J. Ringold and G. Rosenkrans, *J. Org. Chem.*, **22**, 602 (1957).

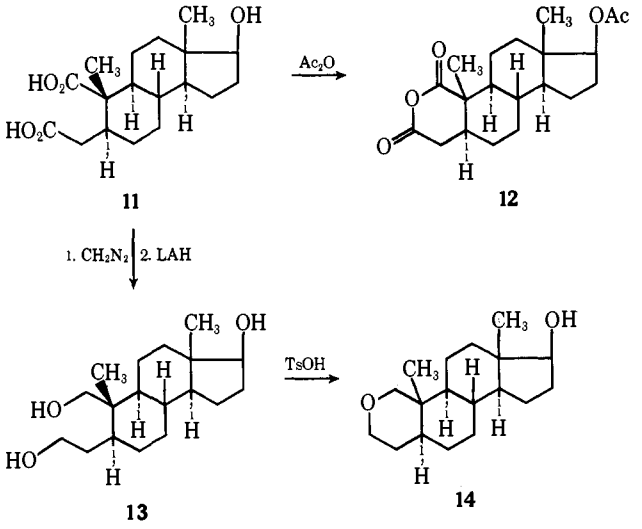
TABLE I
ANDROGENIC-MYOTROPHIC ASSAY

Compd (total dose, mg)	Wt, mg[a] (assay) Ventral prostate	Seminal vesicle	Levator ani	Body wt, g Initial	Final
Castrate control	15.8 ± 1.51	12.6 ± 1.21	25.2 ± 3.54	57	80
Testosterone propionate (0.6)	50.5 ± 3.38 (<0.001)	30.3 ± 2.27 (<0.001)	39.0 ± 1.35 (<0.01)	57	90
Testosterone (3.0)	143.2 ± 10.76 (<0.001)	89.0 ± 6.37 (<0.001)	71.0 ± 2.62 (<0.001))	57	95
3 (3.0)	23.3 ± 2.56 (<0.05)	13.0 ± 0.22 (N.S.)[b]	29.2 ± 2.36 (N.S.)	57	89
9 (3.0)	14.7 ± 0.86 (N.S.)	12.3 ± 0.64 (N.S.)	28.0 ± 2.09 (N.S.)	57	92
10 (3.0)	16.0 ± 1.25 (N.S.)	12.0 ± 0.07 (N.S.)	31.1 ± 0.80 (<0.10–0.05)	55	94
12 (3.0)	17.5 ± 1.85 (N.S.)	10.6 ± 0.36 (N.S.)	27.3 ± 1.91 (N.S.)	54	88
13 (3.0)	34.3 ± 3.08 (<0.01)	14.5 ± 0.36 (N.S.)	28.0 ± 0.63 (N.S.)	51	83
14 (3.0)	86.3 ± 7.11 (<0.001)	52.1 ± 3.00 (<0.001)	71.6 ± 3.99 (<0.001)	51	92
20 (2.4)	26.0 ± 0.31 (<0.01)	15.6 ± 1.44 (*ca.* 0.05)	37.9 ± 0.77 (<0.01)	58	95

[a] Mean ± standard error. [b] Not significant.

having the same melting point as the product of the other reaction sequence.

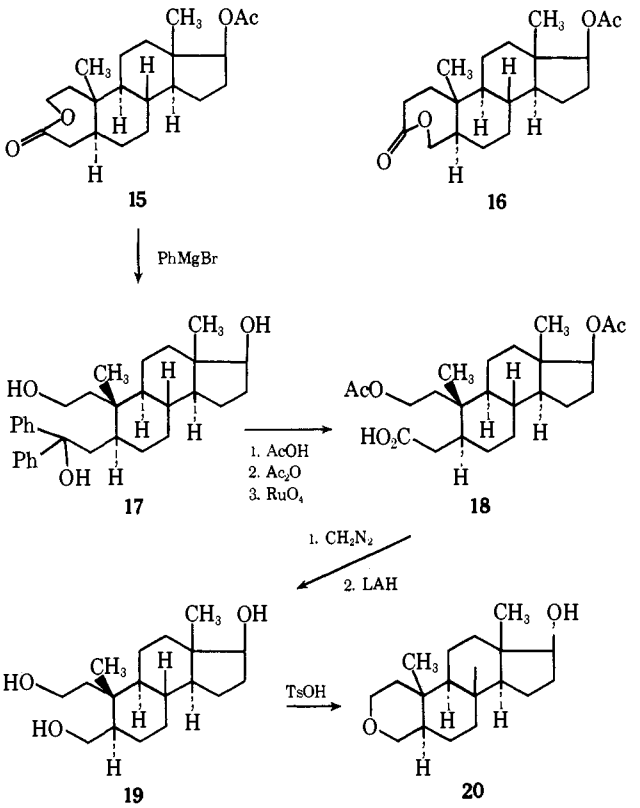
For the preparation of 2-oxasteroids, diacid **11**[8] was



11 **12** **13** **14**

refluxed in Ac_2O to give cyclic anhydride **12**. Esterification of **11** with CH_2N_2 followed by reduction with LAH gave diol **13** which on refluxing in PhH contg p-$MeC_6H_4SO_3H$ gave 2-oxasteroid **14**.

For the production of 3-oxaandrostane derivatives, dihydrotestosterone was oxidized with p-chloroperbenzoic acid to obtain a mixture of isomeric lactones **15** and **16**. This mixture was treated with C_6H_5MgBr to obtain a mixt of carbinols from which **17** was isolated by crystallization. The structure of **17** was established in the following way. Dehydration and acetylation of this material gave an olefin which was examined by nmr and exhibited a doublet (4-H, 5α-H, J = 10 Hz) centered on δ 6.06 arising from the C-4 H. This material was oxidized with RuO_4 to give acid **18**. The residue from the mother liquors of the isolation of **17** was dehydrated with AcOH followed by acetylation to give an olefin, which was purified by chromatography. The nmr spectrum of this product showed a triplet at δ 6.11 arising from the C-2 proton coupled to both protons at C-1. This olefin is therefore the product



15 **16** **17** **18** **19** **20**

derived from **16** in the mixture of **15** and **16**. Esterification of acid **18** with CH_2N_2 followed by reduction with LAH gave diol **19** which on cyclization with p-$MeC_6H_4SO_3H$ gave the desired **20**.

Discussion

The data from the biological testing are displayed in Table I.[9,10] An interesting pattern of activities is evident and useful conclusions can be drawn from it. Substitution of O at the 2 position of a 6-membered ring (**14**) results in an active androgen, whereas, a corresponding substitution in a 5-membered ring (**3**) results in an inactive compound. This is in sharp con-

(8) S. M. Pitiak, H. B. Bhat, and E. Caspi, *J. Org. Chem.*, **34**, 112 (1969).

(9) Pharmacological tests were performed at the Endocrine Laboratories, Madison, Wis.

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

trast to the highly active *A*-northiaandrostane derivatives[2] and reflects the fact that S is isosteric with CH=CH, whereas, O is isosteric with CH_2. Thus, a minimum ring size is required for activity in systems of this type, and that minimum is a 6-membered ring or its isosteric equivalent. This is further supported by the activity of the selena and tellurio steroids.[1a] As would be expected if the nature of the effect of activity-engendering groups in the A ring is steric, the position of the O atom in the A ring is of crucial importance to the biological activity. The substitution of O in place of C-2 (**14**) gives rise to the most active compd. Substitution in place of C-3 (**20**) results in a much less active compd, whereas, substitution at C-4 (**9**) results in a compd which is inactive. These results parallel those found by Bowers, *et al.*,[11] who "moved" a double bond around the A ring in a similar way. Since a double bond necessarily affects the geometry of 2 C atoms in the A ring, their data cannot be compared exactly with ours. Nevertheless, the most active olefin is the Δ^2, whereas, the least active is the Δ^4, so that the data, to the extent that it is possible to compare them, are in general agreement. Bowers, *et al.*,[7] attributed their results primarily to changes in the electron density in the A ring although they recognized that stereochemical differences might be important as well. Taken together, however, the data are clearly in strong support for the concept that the function of the structure of the activity-engendering group in ring A is wholly steric and that in principle isosteric groups of any type could be used to construct an androgenic molecule. Such a study is described in the following paper.

Experimental Section[12]

1,2-Seco-*A*-bisnor-5α-androstane-1,2,17β-triol (2).—A soln of 0.10 g of **1**[2] in 5 ml of Et_2O was added to 0.2 g of LAH in 50 ml of Et_2O and refluxed for 2 hr. It was cooled, Na-K tartrate was added, and the mixt was filtered. The filtrate was washed with dil HCl and H_2O and then evapd. The product was crystd from Me_2CO to give 0.05 g of product, mp 202–205°. *Anal.* ($C_{17}H_{30}O_3$) C, H.

2-Oxa-*A*-nor-5α-androstan-17β-ol (3).—A soln of 0.050 g of **2** in 5 ml of DMSO was heated at 156–166° for 13 hr. After cooling 50 ml of H_2O was added and the reaction mixt was extd with 2 × 50 ml of Et_2O. The combined exts were concd by distn and the residue was purified by preparative tlc on silica gel using hexane–Et_2O. There was obtained 0.025 g of pure product, mp 140–143° after recrystn from hexane, M^+ 264. *Anal.* ($C_{17}H_{28}O_2$) C, H.

Methyl 17β-Hydroxy-3,5-seco-5-oxo-*A*-norandrostan-3-oate Acetate (5).—A soln of 5 g of **4**[7] in 200 ml of Et_2O was cooled to 0° and esterified with CH_2N_2. After the usual work-up 4.5 g of **5** was obtained from MeOH as colorless needles. The anal. sample had mp 101–102°. *Anal.* ($C_{21}H_{32}O_5$) C, H.

3,5-Seco-*A*-norandrostane-3,5β,17β-triol (6).—To a soln of 1.0 g of LAH in 300 ml of Et_2O there was added dropwise a soln of 0.40 g of **5** in 50 ml of Et_2O. The mixt was stirred and refluxed for 3 hr, cooled, decompd carefully with a satd soln of Na-K tartrate and filtered. The ppt was washed with Et_2O, and the combined Et_2O soln was washed with dil HCl and H_2O and dried (Na_2SO_4). The residue from evapn of the dried soln gave 0.20 g of colorless crystals after several crystns from Me_2CO, mp 211–213°. *Anal.* ($C_{18}H_{32}O_3$) C, H.

4-Oxa-5α-androstan-17β-ol (9).—A mixt of the C-5 epimers **7** and **8** was reduced with LAH–BF_3 following the method of Pettit and Kasturi.[5] The product, mp 198–205°, lit.[5] 202–204°, was a mixt of **9** and **10** as shown by the nmr spectrum. Further recrystn from Me_2CO–hexane gave a pure sample: mp 186–187; nmr, δ 0.72 (18-H), 0.93 (19-H), 2.92 (q, 5α-H) ($J_{5\alpha,6\alpha}$ = 5 Hz, $J_{5\alpha,6\beta}$ = 10 Hz) ppm.

The same compd was obtd by heating 0.08 g of **6** in 5 ml of DMSO at 160° for 13 hr. To the cooled soln there was added 50 ml of H_2O and the mixt was extd with 2 × 50 ml of Et_2O. The combined ether ext was evapd and the residue was purified by tlc on silica gel using hexane–Et_2O. The resulting yellow solid gave **9**, mp 186–187° from Me_2CO, nmr as above, M^- 278.22418. *Anal.* ($C_{18}H_{30}O_2$) C, H.

4-Oxa-5β-androstan-17β-ol (10).—The mother liquor from the Me_2CO–hexane recrystn in the prepn of **9** was evapd, and the residue was crystd repeatedly from hexane giving **10**: mp 143–145°; M^+ 278.22480; nmr, δ 0.75, 0.85 (angular Me) 3.15 (t, 5β-H) ($J_{5\beta,6\alpha}$ = 5 Hz, $J_{5\beta,6\beta}$ = 5 Hz) ppm. *Anal.* ($C_{18}H_{30}O_2$) C, H.

17β-Hydroxy-2-oxa-5α-androstane-2,4-dione Acetate (12).—A soln of 0.12 g of **11**[8] in 10 ml of Ac_2O was refluxed for 6 hr. The mixt was cooled, poured into ice water, and extd with Et_2O. The Et_2O was evapd to give 0.10 g of residue which was crystd several times from hexane–Et_2O to give the anal. sample: mp 243–245; M^+ 348.19307. *Anal.* ($C_{20}H_{28}O_5$) C, H.

1,2-Seco-*A*-nor-5α-androstane-1,2,17β-triol (13).—A sample of **11**[8] was converted to the dimethyl ester with CH_2N_2. A soln of 1.0 g of this ester was reduced with 1.5 g of LAH in refluxing Et_2O and was worked up in a manner similar to that described for the prepn of **6**. The product (0.6 g) was crystd from Me_2CO–hexane to give the anal. sample: mp 179–182°; $M^+ - H_2O$ 278.22416. *Anal.* ($C_{18}H_{32}O_3$) C, H.

2-Oxa-5α-androstan-17β-ol (14).—A soln of 0.10 g of **13** and 0.05 g of *p*-$MeC_6H_4SO_3H$ in PhMe was refluxed for 2.5 hr and evapd under reduced pressure. The residue was dissolved in Et_2O, and the soln was washed with H_2O and dried (Na_2SO_4). The solid resulting from evapn of the Et_2O was crystd from Me_2CO–hexane to give **14**, mp 139–140°. *Anal.* ($C_{18}H_{30}O_2$) C, H.

2,3-Seco-3,3-diphenyl-5α-androstane-2,3,17β-triol (17).—A soln of 9.0 g of 17β-hydroxy-5α-androstan-3-one and 8 g of 85% *p*-chloroperbenzoic acid in 80 ml of $CHCl_3$ was stirred for 45 hr at room temp and then heated for 2 hr at reflux, under protection from light. To the soln was added 35 ml of H_2O and 15 ml of Et_2O, the layers were sepd, and the org layer was washed with 5% H_2SO_4, 5% Na_2CO_3 soln, and H_2O. The org layer was dried (Na_2SO_4), filtered, and evapd to give a solid mixt of **15** and **16** which was crystd from PhH. The product had mp 220–227°. *Anal.* ($C_{21}H_{32}O_4$) C, H.

The foregoing mixt (2 g) in 300 ml of dry Et_2O was added dropwise during 1 hr to 10 ml of stirred ice-cold 3 *M* PhMgBr in Et_2O. The mixt was stirred at 25° for 8 hr and kept for 10 hr. It was cooled in an ice bath and satd NH_4Cl soln was added slowly until the org layer became clear. This layer was filtered, and the pptd Mg salts were washed well with Et_2O. The combined org exts were steam distd in order to remove biphenyl. The distn flask contents were extd with Et_2O, dried ($MgSO_4$), and evapd. Crystn from Et_2O–hexane yielded 0.9 g of **17**: mp 213–215°; M^+ 462. *Anal.* ($C_{31}H_{42}O_3$) C, H.

3,17-Dihydroxy-2,3-seco-*A*-nor-5α-androstan-3-oic Acid Diacetate (18).—A soln of 0.8 g of **17** in 50 ml of glacial AcOH was boiled under reflux for 3 hr. Evapn of the solvent under reduced pressure gave a mixt which was acetylated in 10 ml of C_5H_5N and 7 ml of Ac_2O at 25°. After the usual work-up, the product was chromatogd on silica gel using Me_2CO–hexane to give 0.7 g of residue. A mixt of 0.8 g of $NaIO_4$, 0.1 g of RuO_2, and 10 ml of H_2O was stirred at 0° for 30 min. An addl 1 g of $NaIO_4$ was added, followed by dropwise addn of the foregoing residue dissolved in 40 ml of cold Me_2CO (distd from $KMnO_4$). A black ppt formed immediately. During the next 8 hr at room temp under stirring, a total of 1.5 g of $NaIO_4$ was added in small portions in order to remove the black ppt whenever it appeared. Excess RuO_4 was then destroyed by addn of 6 ml of *i*-PrOH. The mixt was added to aq NaCl contg 1 ml of 36% HCl and extd with Et_2O (4 × 50 ml). The combined Et_2O exts were washed with H_2O. The product was extd into satd $NaHCO_3$ soln which was then acidified with HCl and extd with Et_2O. Evapn of the dried (Na_2SO_4)

(11) A. Bowers, A. D. Cross, J. A. Edwards, H. Carpio, M. C. Calzada, and E. Denot, *J. Med. Chem.*, **6**, 156 (1963).

(12) Melting points were determined with a Thomas-Hoover apparatus equipped with a corrected thermometer. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Nmr spectra were obtained at 60 MHz on samples in $CDCl_3$ on a Varian A-60A instrument or at 100 MHz on a Jeolco JMH-100 instrument (TMS). Mass spectra were obtained by Mr. William Garland on a MS-902 high-resolution instrument. Where analyses are indicated only by symbols of the elements or functions, anal. results obtained for those elements or functions were within ±0.4% of the theor values.

Et_2O gave 0.33 g of **18** which was crystd from MeCN to give the anal. sample: mp 148–149°; M^+ − HOAc 334.21499. *Anal.* ($C_{22}H_{34}O_6$) C, H.

2,3-Seco-5α-*A*-norandrostane-2,3,17β-triol (19).—A soln of **18** in Et_2O was esterified with CH_2N_2. The ester (0.3 g) was dissolved in 50 ml of dry Et_2O and added to 0.7 g of LAH in 100 ml of dry Et_2O. It was refluxed and stirred for 3 hr after which no starting material remained as shown by tlc. A satd soln of Na-K tartrate was carefully added, and the mixt was filtered. The ppt was washed with Et_2O and the combined Et_2O soln was washed (dil HCl, H_2O) and evapd. The residue was crystd several times from Me_2CO giving colorless crystals: 0.05 g; mp 225–227°; M^+ 296.23624. *Anal.* ($C_{18}H_{32}O_3$) C, H.

3-Oxa-5α-androstan-17β-ol (20).—A soln of 0.040 g of **19** in PhMe contg 0.040 g of *p*-$MeC_6H_4SO_3H$ was refluxed for 3 hr and evapd under reduced pressure. The residue was dissolved in Et_2O, and the soln was washed with H_2O, dried (Na_2SO_4), and evapd to give 0.015 g of solid. Crystn from hexane gave the anal. sample, mp 121–123°, M^+ 278.22471. *Anal.* ($C_{18}H_{30}O_2$) C, H.

Heterocyclic Steroids. 3. An Androgen Having Three Heteroatoms in Ring A[1a,1b]

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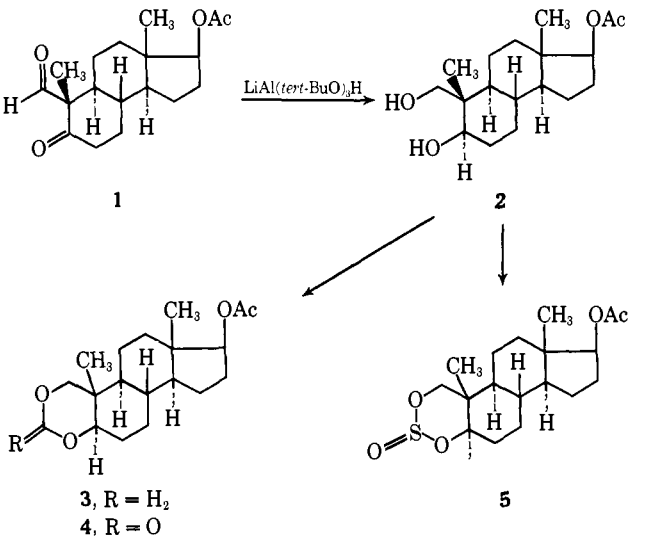
Received February 17, 1971

The synthesis and biological evaluation of 17β-hydroxy-2,4-dioxa-3-thia-5α-androstane 3α-oxide acetate (**5**) is described. Reduction of 17β-hydroxy-1,5-seco-*A*-trisnorandrostane-1,5-dione acetate to the corresponding 1,5-diol followed by treatment with $SOCl_2$ gave **5**. This compd had 50–100% the androgenic-myotrophic effect of testosterone. It is concluded that the activity-engendering effects of A-ring substituents are steric in nature.

In the preceding paper in this series, the activity of oxasteroids having a single heteroatom in the A ring was taken as evidence that the nature of the activity-engendering effect of groups in the A ring of steroids is steric in character. From this it was predicted that any number of heteroatoms could be inserted into the steroid nucleus to provide active compds if the steric characteristics of these atoms were appropriate to this activity. In the present work we have examined the biological consequences of replacing 3 of the 6 atoms in the A ring with heteroatoms.

Treatment of seco steroid **1**[2] with LiAl(*tert*-BuO_3)H gave diol **2**. This compd could be converted into 3 different analogs of dihydrotestosterone, one of which had 3 hetero atoms in the A ring. Thus, treatment of **2** with DMSO[3] gave the acetal **3**. When **2** was refluxed with diethyl carbonate, cyclic carbonate **4** was obtained. Lastly, when **2** was treated with $SOCl_2$, cyclic sulfite **5** was produced.

The structures of all of these substances were established and verified by nmr and mass spectra. Of special interest in this connection are the structures of acetal **3** and cyclic sulfite **5**. The 100-MHz nmr spectrum of **3** (Figure 1) shows the protons at C-1 and C-3 as 2 pairs of doublets resulting from AB splitting typical of such groups. The AB pattern from C-3 protons appears at about 4.7 and 5.1 and requires no special comment. The AB pattern from the C-1 protons, however, is of interest in connection with the corresponding pattern of the C-1 protons of cyclic sulfite **5**. In **3**, the C-1 protons form 2 doublets, one centered at approximately δ 3.3 and the other at approximately 3.85. The coupling constant of 10 Hz as well as the tilt of the peaks clearly indicates that these are protons coupled to each other. Whereas the downfield peaks are sharp, the upfield peaks are broadened. From this



it is clear that the downfield peaks are due to the equatorial 1β-H, and the upfield peaks are due to the axial 1α-H. This is in harmony with the normal axial–equatorial separation of approximately 0.5 ppm for protons in cyclohexane.[4] The axial 1α-H is easily recognizable in this case because the axial 1α-H is spin-coupled to the C-19 angular Me group[5] and therefore the broadened peaks are due to this proton. Turning now to the spectrum of cyclic sulfite **5**, Figure 2, protons at C-1 again form a pair of doublets, in this case centered at approximately δ 3.6 and 4.6. However, now the broadened peak is the downfield peak at δ 4.6 and thus corresponds to the 1α-H. Therefore, the axial proton resonance has been shifted downfield by approximately 1.5 ppm from its expected position 0.5 ppm upfield from the equatorial peak. This can only be due to the deshielding of the 1α-H by the sulfone oxygen of

(1) (a) This investigation was supported in part by Public Health Service Research Grant AM 05016 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service. (b) For Part 2 of this series see G. Zanati and M. E. Wolff, *J. Med. Chem.*, **14**, 958 (1971).

(2) O. R. Rodig and G. Zanati, *J. Org. Chem.*, **33**, 914 (1968).

(3) V. J. Traynelis and W. L. Hergenrother, *ibid*, **29**, 221 (1964).

(4) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden and Day, Inc., San Francisco, Calif., 1964, p 51.

(5) N. S. Bhacca and D. H. Williams, ref 4, p 118.