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Steroids and Steroidases. 10.[1] Studies on Some Potentially Antitumor Active Androstane Compounds Containing C-17 Nitrogen Mustard Functions

J. BRYAN JONES,* DAVID J. ADAM, AND JEFFREY D. LEMAN[2]

Lash Miller Chemical Laboratories, Department of Chemistry, University of Toronto, Toronto 181, Ontario, Canada

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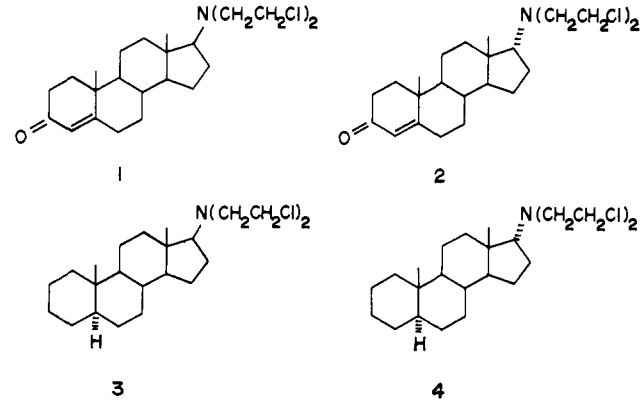
Various approaches to 17α- and 17β-$N(CH_2CH_2Cl)_2$ androstane compounds have been evaluated and of the routes explored, those involving $N(CH_2CH_2OH)_2$ derivatives proved the most reliable. Using androstanes with no functionality other than the mustard moiety, and also the more sensitive compounds containing oxygen and/or ethylenic groups in ring A, a detailed evaluation of the merits and disadvantages of a range of reagents and procedures for effecting the final, critical, chlorination reaction has been made. The data obtained are considered to be relevant to nonaromatic mustards in general and thus provide a guide for the selection of the most appropriate chlorinating conditions for variously functionalized steroidal and aliphatic mustards. An analysis of the factors to be considered in designing antitumor active steroid mustards has been carried out. No cytotoxic activity was detected when representative mustards were tested against DMBA-induced mammary tumors in Sprague–Dawley rats.

During the past decade or so a significant effort has been expended by several research groups in attempts to find steroidal N mustard derivatives which possessed clinically useful antitumor properties. The justifications for expecting the steroid–mustard combination to be a fruitful one were multiple[3] and they included the expectation that a lipophilic steroid carrier molecule would aid transport of the mustard moiety and that the use of hormonally active steroids for this purpose might direct the mustard to specific target tissues. Even though the observation that treatment of breast cancer with testosterone and the thiophosphoethylenimine mustard, Thiotepa, administered together was more effective than when either compound was used alone[4] had provided an important stimulus to the search for antitumor steroid hormone–mustard combinations, at the time we began this study (1966) very few examples had been reported[5,6] in which the biologically important C-3 O functions of the hormone had not been modified or eliminated.[7] Accordingly, since the antitumor activities observed for the majority of the C-3 modified steroidal N mustards which had been evaluated up to that time had proved somewhat disappointing we decided to investigate androgen–N mustard combinations in which the C-3 O function was maintained as a CO or OH group.

The C-17 epimers of the testosterone-related mustards **1** and **2** were selected as the initial synthetic targets. Mustard derivatives in which the amino N is bonded directly to the steroid nucleus have been prepared by several routes.[3,12] However, comparisons of



the experimental data reported showed that many of the synthetic steps were plagued by low yields and by other problems and that for those syntheses involving $N(CH_2CH_2OH)_2$ intermediates the final chlorination stage appeared to be a particularly sensitive one. In addition, preliminary investigations on oxygenated and unsaturated steroid derivatives[13] had indicated that chlorination of the diol precursors of **1** and **2** would be further complicated by the presence of the Δ^4-3-keto system. In view of this rather discouraging picture it was decided to carry out model investigations on the androstane mustards **3** and **4**, for which no interfering functionalities were present in the steroid, in order to evaluate the best methods for the introduction of the C-17 mustard functions desired and in order to delineate the optimum chlorination conditions in the final step of those routes involving $N(CH_2CH_2OH)_2$ intermediates.[14]

The synthetic schemes followed are outlined in Scheme I. Conversion of 5α-androstan-17β-ol† (**5**) to 17β-amino-5α-androstane (**8**) *via* the intermediate ketone **6** and oxime **7**[16] followed by reaction with ethylene

(1) For part 9, see J. B. Jones and D. C. Wigfield, *Can. J. Chem.*, **47**, 4459 (1969).

(2) Abstracted mainly from the Ph.D. thesis of J.D.L., University of Toronto, Toronto, Ontario, 1969. First presented in part at the C.I.C. Conferences, Montreal, May 1969, and Toronto, May 1970.

(3) W. C. J. Ross, "Biological Alkylating Agents," Butterworths and Co., Ltd., London, 1962.

(4) G. W. Watson and R. L. Turner, *Brit. Med. J.*, 1315 (1959).

(5) S. H. Burstein and H. J. Ringold, *J. Org. Chem.*, **26**, 3084 (1961).

(6) E. Cioranesan and D. Raileanu, *Acad. Rep. Pop. Rom., Stud. Cercet. Chim.* **10**, 295 (1962); *Chem. Abstr.*, **59**, 4439 (1963).

(7) Several further examples have subsequently been reported.[8-11]

(8) I. N-Duvaz, A. Carmbanis, and E. Tarnauceanu, *J. Med. Chem.*, **10**, 172 (1967).

(9) H. Zimel, *Proc. 5th Int. Congr. Chemother.*, 247 (1967).

(10) E. L. Foster and R. T. Blickenstaff, *J. Med. Chem.*, **11**, 1106 (1968).

(11) M. E. Wall, G. S. Abernathy, F. I. Carroll, and P. J. Taylor, *J. Med. Chem.*, **12**, 810 (1969).

(12) For a complete review of steroidal N mustards including synth methods, and their activities see ref 2. A selective, but representative, summary of most of the important data and leading references is contd in ref 11.

(13) J. B. Jones, D. J. Adam, J. E. Hawkins, J. D. Leman, and G. C. Niece, unpublished results.

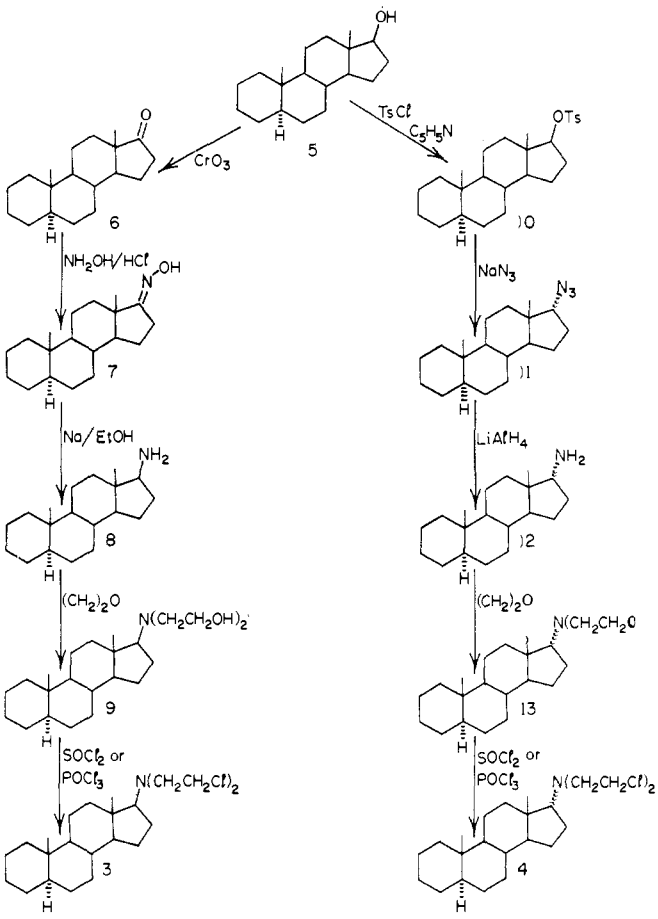
(14) Although **3** and **4** were not expected to exhibit appreciable antineoplastic activities, the possibility of their doing so was finite since the carrier moiety, 5α-androstane, is weakly androgenic.[15]

(15) R. I. Dorfman, W. H. Rooks, J. B. Jones, and J. D. Leman, *J. Med. Chem.*, **9**, 930 (1966); A. Segaloff and R. B. Gabbard, *Endocrinology*, **71**, 949 (1962).

† The systematic nomenclature used throughout is based on the IUPAC recommendations for steroids.

(16) *Cf.* G. R. Pettit, R. L. Smith, A. K. D. Gupta, and J. L. Accolowitz, *Can. J. Chem.*, **46**, 501 (1967), and ref therein.

SCHEME I



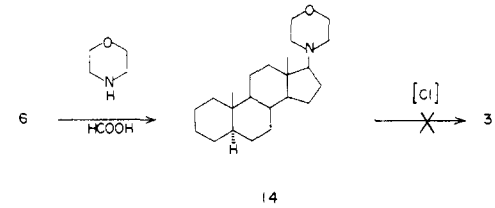
oxide[3,17] gave 17β-bis(2-hydroxyethyl) amino-5α-androstane (**9**) in 60% overall yield. In the 17α series, the preparation of the $N(CH_2CH_2OH)_2$ derivative **13** from **5** *via* the tosylate **10**, the azide **11**, and the amine **12**[18] proceeded equally smoothly in 41% overall yield.[19]

The most widely used chlorinating agent for the conversion of hydroxyethylamines to the corresponding mustards has been $SOCl_2$.[3,12] Disappointingly, when the 17β compound **9** was treated with reagent grade $SOCl_2$ in $CHCl_3$ in a standard way[17] an unidentifiable black tar resulted[21] even at room temp. However, using carefully purified (with triphenyl phosphite[22]) $SOCl_2$ in anhy EtOH-free $CHCl_3$ at 50° no discoloration occurred and the 17β-mustard **3** was obtained in 50% yield. Treatment of the 17α-precursor **13** with the same reagent afforded 17α-bis(2-chloroethyl)amino-5α-androstane (**4**) in 48% yield in an equally facile reaction[23] both mustards being isolated initially as their HCl salts.

Since the lowest yield step in both the 17β and 17α series was the final one, other chlorinating agents were surveyed in an attempt to improve the yield of this critical stage. Triphenylphosphine, and phosphorus trisdimethylamide (both in CCl_4),[25] and $AlCl_3$[26] were investigated but were not successful in effecting the conversion of **9** into **3**. In contrast, chlorinations of **9** and **13** with neat, freshly distilled $POCl_3$ proved to be quite satisfactory. Although the yields (50–55%) obtained of the mustard derivatives do not represent an appreciable improvement over those of the $SOCl_2$ reactions, $POCl_3$ is much the preferred reagent for the preparation of **3** and **4** since it obviates the necessity for rigorous purification of chlorinating agent and solvent. In addition the reaction mixtures are more stable and do not discolor even when heated at 100° for 1 hr, and isolation and purification of the mustards are much simpler than for the $SOCl_2$ reactions.

Owing to the multiplicity of steps involved in the reaction schemes of Scheme I the overall yields of the 17β- and 17α-mustards (~33 and ~22%, respectively) from the starting alcohol **5** were not as high as could be wished. Accordingly, attention was turned to the development or application of other routes to **3** and **4** in which the number of synthetic steps required was reduced to a minimum. The first, and most direct, method considered is outlined in Scheme II. Condensation of morpholine with the 17-keto function of **6** under Leuckart–Wallach conditions to give the 17β derivative **14** was expected to be facile[18] and in view of the report[27] on the cleavage of some aromatic morpho-

SCHEME II



(17) *Cf.* G. V. Rao and C. C. Price, *J. Org. Chem.*, **27**, 205 (1962), and ref therein.

(18) *Cf.* M. Davis, E. W. Parnell, and J. Rosenbaum, *J. Chem. Soc. C*, 1045 (1967), and ref therein.

(19) The C-17 stereochemistries assigned to the amino derivatives **8**, **9**, **12**, and **13** and to the azide **11** are implicit from their mode of synthesis and for all other 17-amino derivatives described in this paper, the reactions used in the prepn of the compds allow the C-17 configuration to be predicted with equal confidence. However, in all such cases the assigned geometry was confirmed by examination of the C-17 proton peak in the pmr spectra. For most of the 17β-amino derivatives the C-17α hydrogen peak appeared as a triplet (sometimes poorly defined) whereas the C-17β proton of the epimeric series was an apparent doublet.[20]

(20) A. A. Patchett, F. Hoffman, F. F. Giarusso, H. Schwan, and G. E. Arth, *J. Org. Chem.*, **27**, 3822 (1962).

(21) This situation is not without its precedents in the aliphatic N mustard literature.[3,12]

(22) L. Friedman and W. P. Wetter, *J. Chem. Soc. A*, 36 (1967).

(23) Comparisons of the mass and pmr spectra of the mustards **3** and **4** with those of their precursor diols **9** and **13** proved particularly valuable during these chlorination studies. In addn to the distinguishing C-17 proton peaks[19] the resonances of CH_2 adjacent to N and O, respectively, were also characteristic and usually appeared as structured "triplets". For the CH_2 of **3** and **4** the chemical shift differences between the 2 "triplets" was significantly less than for those in the spectra of their preceding bis(hydroxyethyl)amines. This decrease in triplet separation on formation of the mustard proved to be a general and sensitive criterion and it was subsequently used to diagnose the presence and concn of mustards in the often very crude mixts obtd from the many reactions carried out during the delineation of optimum chlorination condns and in surveying the efficacy of various chlorinating agents. The $^{35}Cl:^{37}Cl$ dependant patterns in the parent ion region of the mass spectra were also quant characteristic and were used extensively as a reliable indicator of the presence of the bis(2-chloroethyl)amino function. For example, for **3** the mass spectrum showed parent ions at *m/e* 399 ($C_{23}H_{39}N^{35}Cl_2$), 401 ($C_{23}H_{39}N^{35}Cl^{37}Cl$), and 403 ($C_{23}H_{39}N^{37}Cl_2$). Furthermore the ratio of the intensities of the signals (57:38:7) is in good agreement with the theor ratio (57:37:6) for a natural abundance 2 Cl system.[24]

(24) J. H. Beynon, "Mass Spectrometry and Its Applications to Organic Chemistry," Elsevier and Co., Amsterdam, 1960, pp 294–300.

(25) I. M. Downie, J. B. Holmes, and J. B. Lee, *Chem. Ind. (London)*, 900 (1966); J. B. Lee, *J. Amer. Chem. Soc.*, **88**, 3440 (1966); I. M. Downie, J. B. Lee, and M. F. S. Matough, *Chem. Commun.*, 1350 (1968).

(26) J. Broome, B. R. Brown, and G. H. R. Summers, *J. Chem. Soc.*, 2071 (1957).

(27) E. Cerkovnikov and P. Stern, *Ark. Kemi.*, **18**, 12 (1946); *Chem. Abstr.*, **42**, 1938 (1946).

lines with HBr to give $N(CH_2CH_2Br)_2$ derivatives we were hopeful that similar chlorinative cleavage of the ether function of **14** might be possible.

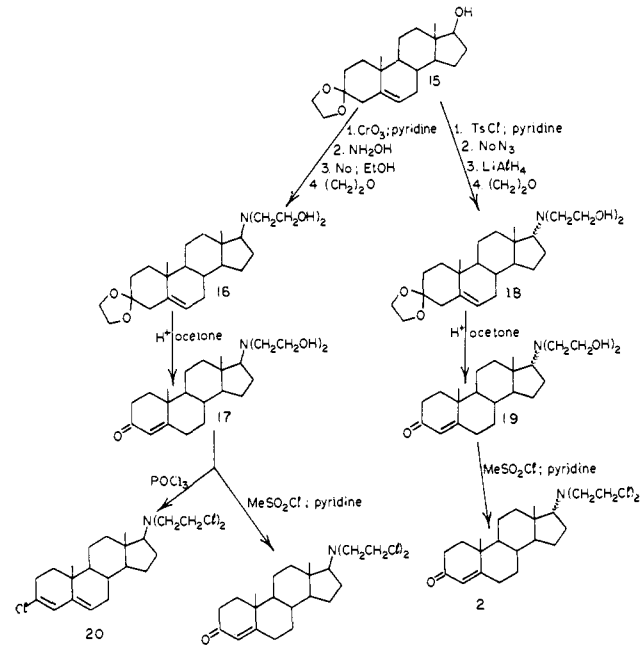
When 5α-androstan-17-one (**6**) was treated with morpholine and HCO_2H, the 17β-morpholino androstane **14** was obtained as the sole product in 65% yield. Very few data are available on methods for cleaving the ether linkage of morpholine rings and the strongly acidic[27] and oxidative[28] methods known to be effective were considered too vigorous to be applicable to the synthesis of sensitive mustards such as **1**. Of the potentially useful methods which have been used for ether cleavage[29] triphenylphosphine dihalides appeared to be the most attractive of the mild reagents but, disappointingly, treatment of **14** with the dichloride or dibromide under a variety of conditions[30] was completely unsuccessful as a mustard forming process and no identifiable products, other than triphenylphosphine oxide, were isolated.[31]

At this stage in the investigation, Duranleau[33] reported that although acidic or oxidative cleavages of morpholine rings were satisfactory for introducing the mustard function into aromatic compounds the methods were not applicable to the corresponding aliphatic systems. In view of this further evidence of the instability of aliphatic morpholines and of the problems anticipated with the remaining mild ether cleavage possibilities[29] as a result of interference by the basic and nucleophilic morpholino N, further studies on this approach to mustards were postponed.

Several attempts were also made to improve the routes to the bis(hydroxyethyl)amines **9** and **13**. The facile synthesis of **14** under Leuckart–Wallach conditions suggested that a similar reductive amination of 5α-androstan-17-one (**6**) with diethanolamine and HCO_2H might produce **9** in a 1-step process. However, even under sealed tube conditions at 225° for 2 days a trace (tlc) only of the required amine was produced. The preparation of the 17α epimer **13** by displacement of the 17β-tosyl group[17,34] of **10** with diethanolamine was also attempted, but again only a trace of the desired product was produced.[35] Direct conversions of the 17β-tosylate **10** to the mustard **4**, and by reductive amination of the ketone **6** to **3** utilizing $HN(CH_2CH_2Cl)_2$ itself, were not investigated because of the marked instability of the latter compound[36] at the elevated reaction temps which would have been required.

Since of all the approaches to the mustards **3** and **4**, only those outlined in Scheme I had been successful, the same reaction schemes were applied toward the synthesis of the Δ⁴-3-keto mustards **1** and **2** as summarized in Scheme III. In the 17β series, the $N(CH_2CH_2OH)_2$

SCHEME III



intermediates **16** and **17** were obtained from the ketal **15** in 23 and 18% overall yields, respectively, and the 17α epimers **18** and **19** in 13 and 7%, respectively.[37]

The initial attempts to prepare the N mustards from both the ketalized and Δ⁴-3-keto bis(2-hydroxyethyl)-amino compounds **16–19** were extremely discouraging. Most of the chlorination survey reactions were carried out on the 17β compounds **16** and **17** and when these were treated with $SOCl_2$ either neat or in $CHCl_3$ or C_6H_6 solution no identifiable products could be isolated from the black reaction mixtures under conditions that had proven quite satisfactory for the preparation of the model androstane mustards **3** and **4**. It was suspected that HCl produced during the reaction was responsible for the problems encountered but rapid discoloration of the reaction mixtures occurred even when pyridine, $NaHCO_3$, and pinene[34] were added to remove the acid *in situ*. PCl_5[3] was also ineffective as a chlorinating agent.

$POCl_3$, the most satisfactory chlorinating agent for the preparation of the androstane mustards **3** and **4**, did not prove as successful a reagent when applied to the preparation of the C-3 oxygenated and unsaturated mustards **1** and **2**. A broad spectrum of conditions was surveyed during attempts to convert **16** and **17** to the corresponding mustards but with the ketal **16** only intractable gums were obtained. However, when the Δ⁴-3-keto diol **17** was heated with $POCl_3$ under reflux, very little color developed in the reaction mixture and the product exhibited the characteristic mustard trip-

(28) H. B. Henbest and A. Thomas, *J. Chem. Soc.*, 3032 (1957).

(29) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. 1, Wiley, New York, N. Y., 1967; Vol. 2, 1969; R. Burwell, *Chem. Rev.*, **54**, 615 (1954).

(30) A. G. Anderson and F. J. Freener, *J. Amer. Chem. Soc.*, **86**, 5037 (1964).

(31) Many nucleophiles, including primary amines, effect displacement of halide from such pentavalent P compds[32] and it was suggested that the morpholine N was interfering with the desired reaction by such a process.

(32) A. J. Kirby and S. G. Warren, "The Organic Chemistry of Phosphorus," Elsevier & Co., London, 1967, pp 250–273.

(33) R. G. Duranleau, Ph.D. thesis, Virginia Polytechnic Institute, Blacksburg, Virginia 24061, 1967.

(34) G. C. Hazen, Ph.D. thesis, University of Michigan, Ann Arbor, Michigan 48104, 1951.

(35) Although the negative result was disappointing in view of the success achieved earlier with the related azide reaction (Scheme I) and with other amines[18] it was not entirely unexpected since the basicity and nucleophilicity of the amine is known to be critical.[18]

(36) G. R. Pettit and J. A. Settepani, *J. Org. Chem.*, **27**, 2962 (1962), and ref therein.

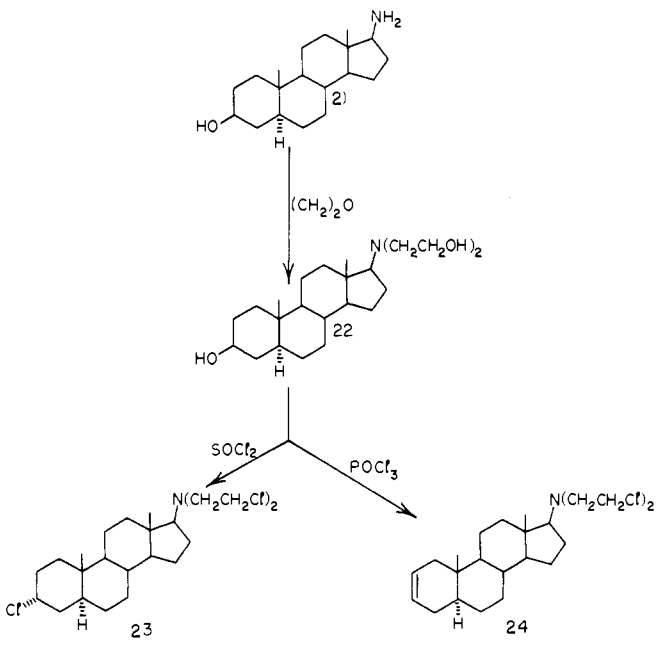
(37) These relatively low overall yields emphasize the disadvantages inherent in the multiplicity of steps required to elaborate the mustard function by the routes of Schemes I and III. The yields of each individual step were in fact very good (>75%) in most reactions and seldom fell below 60% even for the most unfavorable stages.

lets[23] at δ 2.98 and 3.51 ppm in the pmr spectrum. However, that the mustard was not the hoped for Δ^4-3-ketone **1** was demonstrated by the absence of CO ir absorption. The characteristic Δ^4-3-keto C-4 olefinic resonance at δ 5.78 ppm had also disappeared from the pmr spectrum and instead, 2 new 1-proton vinylic peaks were visible at δ 5.38 and 6.06 ppm. These data, coupled with the structured uv absorption maximum at 242 nm, which is characteristic of $\Delta^{3,4}$-3-chlorosteroids,[38] indicated the product to be the 3-chloro-3,5-diene mustard **20**.[39]

Successful chlorinations of the epimeric diols **17** and **19** to give the elusive androst-4-en-3-one mustards **1** and **2**, respectively, were finally accomplished with MsCl in pyridine.[41] The reactions were not as clean as might have been desired and plc of the black reaction mixtures was necessary before **1** and **2** were obtained in 25% and 21% yields from their respective MsCl reactions.

During the early stages of this work we had been fortunate to have a quantity of 17β-amino-5α-androstan-3-ol (**21**) in hand from a previous, but unrelated, investigation and advantage had been taken of the availability of this material to work out optimum conditions for the C-17 amine alkylations with ethylene oxide reactions applied subsequently to **8** and **12**, and to their 3,3′-ethylenedioxy-5-ene analogs of Scheme III. From this model study the bis(2-hydroxyethyl)amine **22** had been obtained in 70% yields (Scheme IV) and since it

SCHEME IV



was available in reasonable quantity its conversion to a mustard was explored even though it was appreciated that selective formation of the $N(CH_2CH_2Cl)_2$ group, without concomitant chlorination of the C-3 OH, was unlikely to be achieved.

Chlorination of **22** with purified $SOCl_2$ in pure $CHCl_3$ was achieved fairly readily, but not selectively, to give the trichloro mustard **23** in low (25%) yield. As the reaction had been carried out in nonbasic solution the introduction of the C-3 Cl was assumed initially to have proceeded with retention of the β configuration.[42] However, examination of the pmr spectrum indicated that inversion at C-3 during chlorination had in fact occurred to give the 3α derivative as shown in structure **23**.[43] This conclusion was based on the well-documented[44] characteristic differences between C-3 axial and equatorial proton resonances of such compounds. In the spectrum of the trichloro mustard obtained the C-3 proton appeared as a narrow, but only poorly defined, triplet centered at δ 4.48 ppm which was identical in pattern and chemical shift with that of the 3β proton of 3α-chloro-5α-androstan-17-one.[13] In contrast, the 3α-H of 3β-chloro-5α-androstan-17-one was observed as a broad septet centered at δ 3.84 ppm when the spectrum was recorded under identical conditions.[13]

Treatment of the triol **22** with the more preferred reagent $POCl_3$ effected mustard formation smoothly and in good yield. However, somewhat unexpectedly, elimination occurred during the reaction, and the product isolated was 17β-(2-chloroethylamino)-5α-androst-2-ene (**24**). The presence of the olefinic bond was indicated by the spectroscopic data and the possibility that the product was the isomer with the double bond in the less stable Δ^3 position, or was a mixture of ring A olefins, was eliminated by comparing the olefinic regions of its pmr and ir spectrum with those of a sample of 5α-androst-2-en-17-one obtained in connection with other studies.

Whereas dehydration of steroidal tertiary alcohols with $POCl_3$ is well established[45] basic solvents are generally employed and trans-diaxial eliminations are favored. The latter stereochemical requirement is not satisfied by the 3β-OH group nor by the intermediate phosphate ester presumed to be formed initially with retention of configuration. However, precedents do exist for eliminations involving equatorial phosphate groups[46] and, furthermore, the possibility of formation of the 3α-chloride (through participation of the C-17-NH_2 group as suggested in the $SOCl_2$ reaction[43]) followed by dehydrochlorination cannot be discounted.

Review of Chlorination Procedures.—The synthetic studies have shown that approaches involving $N(CH_2CH_2OH)_2$ derivatives provide the most reliable general routes to steroidal N mustards where bonding of the mustard *via* a C–N bond is required. The data accumulated have also shown the final chlorination step to be the most critical one. In view of the difficulties which have been encountered with this reaction in these and previous studies a summary of our conclusions regarding the merits and disadvantages of the

(38) R. Deghenghi and R. Gaudry, *Can. J. Chem.*, **40**, 818 (1962).

(39) The formation of $\Delta^{3,5}$-3-chlorosteroids from Δ^4-3-ketones in the presence of other chlorinating agents, such as oxalyl chloride[38] and C_6H_5COCl,[40] has been reported.

(40) L. Ruzicka and W. H. Fischer, *Helv. Chim. Acta*, **19**, 806, 1371 (1936).

(41) J. De Graw and L. Goodman, *J. Org. Chem.*, **27**, 1395, 1728 (1962); Z. B. Papanastassiou, R. J. Bruni, and E. White, *J. Med. Chem.*, **10**, 701 (1967).

(42) C. A. Bunton, "Nucleophilic Substitution at a Saturated Carbon," Elsevier, London, 1963, p 101.

(43) The C-3 inversion observed on chlorination of **22** under conds normally leading to retention of configuration can be rationalized if the C-17 amino functions of **22** and **23** are sufficiently basic to cause S_N2 substitution to replace S_Ni displacement as the dominant mechanistic pathway.[42]

(44) N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, London, 1964, pp 47–49.

(45) J. L. Beton, T. G. Halsall, E. R. H. Jones, and P. C. Phillips, *J. Chem. Soc.*, 753 (1957), and ref therein.

(46) H. B. Henbest and W. R. Jackson, *J. Chem. Soc.*, 954 (1962), and ref therein.

various reagents surveyed is given below in the hope that it might prove useful in guiding the future selections of the most appropriate chlorination conditions for variously functionalized steroidal or aliphatic mustard precursors.

(1) When functional groups other than $N(CH_2CH_2OH)_2$ are absent, $POCl_3$ is the preferred chlorinating agent. Highly purified $SOCl_2$ in pure $CHCl_3$ is also satisfactory. These 2 reagents should also be suitable when an isolated or nonconjugated double bond is present.

(2) When ketone or α,β-unsaturated ketone functions are present, MsCl in pyridine is the reagent of choice. $POCl_3$ may convert an enone into a chlorodiene system; $SOCl_2$ is not recommended owing to the extensive decomposition which may well occur.

(3) Mustard precursors containing primary or secondary OH functions may undergo chlorination with inversion with the $SOCl_2$ reagent, and elimination is a possibility when $POCl_3$ is used. Some preliminary data on tertiary alcohols[13] indicate that elimination will occur with $SOCl_2$, $POCl_3$, or MsCl.

(4) $AlCl_3$ and triphenylphosphine or phosphorus trisdimethylamide in CCl_4 were not effective as chlorinating agents.

Biological Data.—Since it was hoped that the androgen-like steroid carriers of the mustards prepared would confer breast tissue specificity properties on the compounds, androgen regressable, DMBA-induced mammary tumors in female Sprague–Dawley rats were selected for the initial evaluation of three representative mustards **1, 20,** and **24.**

None of the compounds was lethal nor caused any obvious distress when injected sc into mice as a suspension in 2% gelatin at a level of 50 mg/kg. The LD_{50} of **1** was ~100 mg/kg in these toxicity tests but **20** and **24** were well tolerated even at this dose level.

In the preliminary survey carried out, groups of 3 rats bearing well-developed and progressively growing breast tumors were injected sc with mustards **1**, **20**, and **24** as 2% gelatin suspensions 3 times weekly for 3–4 weeks. A control group of 7 rats was used. The doses were gradually increased during this period from 30 to 40 mg/kg for **1**, 40 to 80 mg/kg for **20**, and 40 to 100 mg/kg for **24**. Disappointingly, the Δ^4-3-ketone **1** and the 3-chloro-$\Delta^{3,5}$-diene **20** were found to be totally without effect on the rate of growth or proliferation of the tumors and the Δ^2-mustard **24** induced only a moderate regression (estimated from 3-dimensional measurements of all tumors with calipers) in the size of 2/3 tumors. At the end of the experiment the rats were sacrificed and portions of the tumors were examined histologically. No evidence of tumor destruction attributable to the treatments was detectable although the 2 tumors which decreased in size during administration of **24** showed areas of regression comparable with those seen after ovariectomy or androgen treatment.

In view of the total absence of any evidence of cytotoxic activity for any of the mustards **1**, **20**, and **24**, more detailed studies on larger groups of rats were not considered justified.

Overall Evaluation of the Potential of Nitrogen Mustards.—The discouraging nature of the above results prompted us to reevaluate the total literature on steroid mustards in an attempt to ascertain whether or not the effort required to prepare such compounds was justified from an anticancer point of view. The data available showed clearly that (a) with very few exceptions the attachment of $N(CH_2CH_2Cl)_2$ directly to the steroid nucleus was relatively ineffective, (b) aromatic mustard moieties are better than aliphatic, presumably since they are less reactive and thus are less likely to be intercepted before reaching the target site, (c) appropriate steroid structures *are* effective carriers, (d) if the mustard–steroid link is readily cleavable by hydrolysis or other possible *in vivo* processes, the chance of activity is increased, and (e) although hormone-like carriers are not essential they do impart increased selectivity of action.

However, most of the steroid–mustards evaluated have proved inactive and in general it must be concluded that they have demonstrated very little potential from the clinical point of view. The exceptions to this general observation are the very promising activities observed for a series of steroid ester–aromatic mustard compounds studied first by Degteva and Larionov[47] and then more extensively by Wall and his coworkers.[11] The results of the latter authors are particularly encouraging and it is largely on the basis of their work that any optimism for eventual clinical applications of steroid–mustards rests.

Experimental Section

Apparatus and Materials.—Mp's were detd on a Fisher-Johns block and are cor. Ir spectra were recorded on a Perkin-Elmer 237B or a Perkin-Elmer 257 spectrophotometer, and uv spectra were measured on a Unicam SP 800A instrument. Pmr spectra were detd in $CDCl_3$ with TMS as the internal standard on a Varian A-60 or HA-100 instrument. Glc anals were performed on an F and M 400 biomedical unit equipped with 3.8% SE 30 on Diatoport S, 2% XE-60 on Chromosorb G, and 1% QF-1 on Chromosorb G columns. Column chromatographic sepns were effected with Fisher Scientific Co. neutral Al_2O_3 which had been deactivated by shaking with 2% by wt of H_2O or with Fisher Scientific Co. Florisil. Tlc and plc were performed on silica gel G and the compds were visualized with I_2 vapor.

All solvents were redistd before use. $CHCl_3$ was shaken with half its vol of H_2O, dried over anhyd $CaCl_2$ for 24 hr, and then distd. $SOCl_2$ was shaken with one-sixth its vol of triphenylphosphite[22] and was then fractionally distd. The process was repeated, and the colorless liq obtd was stored at −20° in the dark. $POCl_3$ was distd and stored at −20° in the dark.

Unless otherwise stated, all compds described herein were purified until they were at least homogeneous to tlc and where applicable, glc anal.

Spectral data were as expected for all compds and only where the data are of special diagnostic value[23] have details been reported.

17β-Amino-5α-androstane (8).—5α-Androstan-17β-ol (**5**), mp 168–169° (lit.[48] mp 165.5–166.5°), was obtd by redn of 17β-hydroxyandrost-4-en-3-one (testosterone)[48,49] and its oxidn with Jones' reagent in freshly distd Me_2CO gave 5α-androstan-17-one (**6**), mp 124.5–125° (lit.[50] mp 119–121°), in 82% yield. Subsequent treatment of **6** with $NH_2OH \cdot HCl$ in aq EtOH–pyridine soln afforded 17-hydroxyimino-5β-androstane (**7**) (97% yield), mp 175–177° (lit.[51] mp 173–176°).

A soln of the above oxime **7** (0.4 g, 1.4 mmoles) in abs EtOH (20 ml) was heated under reflux while freshly cut Na (2.4 g, 0.1 g-atom) was added in small portions during 2 hr.[16] Heating

(47) S. A. Degteva and L. F. Larionov, *Vop. Onkol.*, **12**, 51 (1966), and ref therein.

(48) W. V. Ruyle, A. E. Erickson, A. Lovell, and E. M. Chamberlin, *J. Org. Chem.*, **25**, 1260 (1960).

(49) F. L. Weisenborn and H. E. Applegate, *J. Amer. Chem. Soc.*, **81**, 1960 (1959).

(50) C. W. Shoppee, R. H. Jenkins, and G. H. Summers, *J. Chem. Soc.*, 3048 (1958).

(51) C. W. Shoppee and J. C. P. Sly, *ibid.*, 345 (1959).

was continued for a further 2 hr, and the soln was then dild carefully with warm (60°) H_2O (60 ml) and kept overnight at 20°. The resulting ppt was filtered, washed with H_2O, and recrystd from Me_2CO to give 0.36 g (86%) of 17β-amino-5α-androstane (**8**), mp 131–134° (lit.[51] mp 138–141°).

17β-Bis(2-hydroxyethyl)amino-5α-androstane (9).—A soln of 17β-androstane (**8**, 200 mg, 0.73 mmole) in the min amt of $CHCl_3$ (*ca.* 10 ml) was added rapidly with stirring to a cold (0°) soln of ethylene oxide (4 ml, 81 mmoles) in MeOH (8 ml). The reaction flask was then tightly stoppered and stirring was continued for a further 1 hr at 0°. After keeping for a further 2 days at 20° the reaction mixt was refluxed for 8 hr using an Me_2CO–CO_2 cooling system to condense the ethylene oxide. The solvent was then removed by rotary evapn, and the residue obtained was recrystd from MeOH to yield **9** as colorless plates (230 mg, 87%): mp 151–153°; pmr δ 2.53–3.02 (m, **7**, CH and NCH_2CH_2OH) and 3.64 ppm ("t," 4, J = 6.0 Hz, NCH_2CH_2OH).[52] *Anal.* ($C_{23}H_{41}NO_2$) C, H, N.

17β-Bis(2-chloroethyl)amino-5α-androstane (3). (a) With $SOCl_2$ as Chlorinating Agent.—To a stirred soln of 17β-bis(2-hydroxyethyl)amino-5α-androstane (**9**, 100 mg, 0.28 mmole) in $CHCl_3$ (5 ml) cooled in an ice bath was added slowly a soln of $SOCl_2$ (0.6 ml, 8.3 mmoles) in $CHCl_3$ (2.5 ml). Stirring at 0° was continued for 1 hr, and the mixt was then gradually warmed up to 60° and maintained at that temp overnight. After cooling to 5°, the reaction mixt was poured into cold (5°) H_2O (25 ml) and washed with cold (5°) satd aq $NaHCO_3$ soln (1 × 10 ml). The aq layer was extd with $CHCl_3$ (2 × 10 ml), and the combined $CHCl_3$ exts were washed with cold (5°) H_2O (2 × 10 ml) and then dried ($MgSO_4$). On removal of the solvent the mustard was obtained as a yellow solid (55 mg, 50%). Recrystn of a sample from Me_2CO yielded **3** as a cryst powder: mp 82–82.5°; pmr δ 2.94 ("t," 4, J = 8.0 Hz, NCH_2CH_2Cl) and 3.48 ppm ("t," 4, J = 8.0 Hz, NCH_2CH_2Cl); mass spectrum (70 eV) m/e (rel intensity) 399 (57), 401 (38), 403 (7).

(b) With $POCl_3$ as Chlorinating Agent.—A soln of the diol **9** (150 mg, 0.41 mmole) in $POCl_3$ (5 ml) was heated at 95–100° for 1 hr. The excess $POCl_3$ was then removed by vacuum distn, and the colorless oil which resulted was taken up in C_6H_6 (20 ml). The C_6H_6 was then removed by vacuum distn. This latter cycle of operations was repeated until the odor of $POCl_3$ could no longer be detected in the reaction flask. The resulting gum was redissolved in C_6H_6 (20 ml), and the soln was washed with cold satd $NaHCO_3$ soln (1 × 10 ml) and dried ($MgSO_4$). Evapn of the solvent gave **3** as a pale yellow solid (91 mg, 55%). The mustard was dissolved in the min amt of anhyd Et_2O, and HCl was bubbled through the soln. The hydrochloride was obtained as a fine powder (85 mg), mp 151–153°.[53] *Anal.* ($C_{23}H_{40}Cl_2N$) C, H, N, Cl.

17α-Azido-5α-androstane (11).—To a soln of 5α-androstane 17β-tolnene-*p*-sulfonate[54] (0.50 g, 1.4 mmoles) in dry *N*-methyl-2-pyrrolidone (10 ml) under N_2 was added cryst NaN_3 (0.43 g, 6.6 mmoles).[18] While still under N_2 the mixt was heated at 150° for 5 hr and was then cooled and poured into H_2O (60 ml). The aq soln was extd with Et_2O (4 × 30 ml), and the Et_2O exts were then washed with H_2O and dried ($MgSO_4$). Evapn afforded a yellow solid which was recrystd from MeOH to give **11** as plates (245 mg, 80%), mp 61.8–62.5°. *Anal.* ($C_{19}H_{31}N_3$) C, H, N.

17α-Amino-5α-androstane (12).—A soln of 17α-azido-5α-androstane (**11**, 100 mg, 0.34 mmole) in anhyd Et_2O (4.5 ml) was added slowly with stirring to a slurry of LAH (100 mg, 2.46 mmoles) in anhyd Et_2O (10 ml).[18] After the mixt had been heated under reflux for 3 hr moist ether was added until gas evoln ceased. H_2O (10 ml) and 5% NaOH soln (10 ml) were then added, and the Et_2O layer was removed by decantation. The remaining aq mixt was stirred further to effect soln of the Al salts and then reextd with Et_2O (3 × 20 ml). The combined Et_2O exts were dried ($MgSO_4$) and evapd to give the 17α-amine **12** as a clear, colorless gum (77 mg, 84%) which solidified upon standing.

17α-Bis(2-hydroxyethyl)amino-5α-androstane (13) was prepd from 17α-amino-5α-androstane (**12**, 1.0 g, 3.6 mmoles) and ethylene oxide (18 ml, 0.36 mole) according to the method described above for 17β-bis(2-hydroxyethyl)amino-5α-androstane (**9**). The crude product obtained after work-up was recrystd from hexane to give the diol **13** as plates (1.15 g, 87%): mp 148–149°; pmr δ 2.81 ("t," 4, superimposed on C-17 H signal, J = 5.9 Hz, NCH_2CH_2OH), and 3.58 ppm ("t," 4, J = 5.9 Hz, NCH_2CH_2OH). *Anal.* ($C_{23}H_{41}NO_2$) C, H, N.

17α-Bis(2-chloroethyl)amino-5α-androstane (4). (a) With $SOCl_2$ as Chlorinating Agent.—17α-Bis(2-hydroxyethyl)amino-5α-androstane (**13**, 100 mg, 0.38 mmole), dissolved in $CHCl_3$ (5 ml), and $SOCl_2$ (0.6 ml, 8.3 mmoles) in $CHCl_3$ (2.5 ml) were treated and worked-up as described for the 17β-mustard **3**. The resulting brown oil was dissolved in $CHCl_3$, treated with Norite, and filtered. Removal of the solvent gave **4** as a white solid (52 mg, 47%): mp 163–163.5°; pmr δ 2.93 (structured "t," 4, J = 8 Hz, NCH_2CH_2Cl) and 3.42 ppm (structured "t," 4, J = 8 Hz, NCH_2CH_2Cl); mass spectrum (70 eV) m/e (rel intensity) 399 (57), 401 (32), 403 (7).

(b) With $POCl_3$ as Chlorinating Agent.—$POCl_3$ (5 ml) and 17α-bis(2-hydroxyethyl)amino-5α-androstane (**13**, 150 mg, 0.41 mmole) were treated and worked-up as described above for **3**. Compd **4** was obtd as a white solid (83 mg, 50%) and was converted to the hydrochloride (70 mg), mp 154–155°, as described previously. *Anal.* ($C_{23}H_{40}Cl_3N$), C, H, N; Cl calcd, 24.34; found, 23.47.[53]

17β-Morpholino-5α-androstane (14).—A mixt of 5α-androstan-17-one (1 g, 3.6 mmoles), HCO_2H (1 ml, 30 mmoles), and morpholine (3 ml, 34 mmoles) was heated in a sealed tube at 170–180° for 15 hr and was then poured into H_2O and filtered. Recrystn from Me_2CO gave **14** as plates (0.82 g, 65%), mp 154–155°. *Anal.* ($C_{23}H_{39}NO$) C, H, N.

3,3′-Ethylenedioxy-17-hydroxyiminoandrost-5-ene.—17β-Hydroxyandrost-4-en-3-one (testosterone) was reacted with $HO(CH_2)_2OH$[55] to give the hydroxyketal **15**, mp 183–184° (lit.[55] mp 182–184°), in 46% yield. Oxidn of **15** was effected with CrO_3 in pyridine[29] to give 80% of 3,3′-ethylenedioxyandrost-5-en-17-one, mp 196–197° (lit.[55] mp 197–198°). The latter oxoketal (500 mg, 1.5 mmoles) was dissolved in pyridine (5 ml), and a soln of $NH_2OH \cdot HCl$ (440 mg, 70 mmoles) in 90% aq EtOH (5 ml) was added. The mixt was refluxed for 5 hr and was then poured into H_2O (100 ml) and extd with $CHCl_3$ (3 × 50 ml). The dried ($MgSO_4$) $CHCl_3$ exts were evapd, and the residue was recrystd from MeOH contg a trace of pyridine to give 313 mg of 3,3′-ethylenedioxy-17-hydroxyiminoandrost-5-ene as needles, mp 245–246°. *Anal.* ($C_{21}H_{31}NO_3$), C, H, N.

17β-Amino-3,3′-ethylenedioxyandrost-5-ene.—3,3′-Ethylenedioxy-17-hydroxyiminoandrost-5-ene (500 mg, 14.5 mmoles) was reduced with Na (3 g) in EtOH as described above in the prepn of 17β-amino-5α-androstane (**8**). 17β-Amino-3,3′-ethylenedioxyandrost-5-ene was obtained as prisms (269 mg, 56%) from C_6H_6–hexane, mp 183–184°. *Anal.* ($C_{21}H_{39}NO_2$) C, H, N.

17β-Bis(2-hydroxyethyl)amino-3,3′-ethylenedioxyandrost-5-ene (16).—17β-Amino-3,3′-ethylenedioxyandrost-5-ene (3 g, 0.09 mole) in the min vol of $CHCl_3$ and ethylene oxide (27 ml, 0.54 mole) were allowed to react using the procedure described for the conversion of **8** to **9**. The ketalized diol **16** recrystd from MeOH contg a trace of pyridine as lustrous plates (2.97 g, 72%): mp 188–189°; pmr δ 2.83 ("t", 4, J = 5 Hz, NCH_2CH_2OH) and 3.68 ("t," 4, J = 5 Hz, NCH_2CH_2OH). *Anal.* ($C_{25}H_{41}NO_4$) C, H, N.

17β-Bis(2-hydroxyethyl)aminoandrost-4-en-3-one (17).—The above ketal **16** (105 mg, 2.5 mmoles) and hydrated TsOH (70 mg) were kept in Me_2CO–$CHCl_3$ (1:1, 12 ml) soln for 12 hr at 20°. The soln was then poured into satd aq $NaHCO_3$ (50 ml), and the mixt was extd with $CHCl_3$ (5 × 20 ml). The combined $CHCl_3$ exts were dried ($MgSO_4$) and evapd to give quant the Δ^4-3-ketone **17** which on recrystn from cyclohexane afforded needles (68 mg): mp 127–128.5°; pmr δ 2.82 "t," 4, J = 5 Hz, NCH_2CH_2OH) and 3.66 ("t," 4, J = 5 Hz, NCH_2CH_2OH). *Anal.* ($C_{23}H_{37}NO_3$) C, H, N.

(52) The CH_2 adjacent to N and those adjacent to O or Cl in 2-hydroxyethylamines and 2-chloroethylamines, respectively, do not constitute a true A_2X_2 system. Hence, distorted and structured triplets and other more complex absorptions are often observed for these protons as the system varies from an A_2X_2 to an A_2B_2 case. Although the appearance of the resonance patterns is thus directly related to the basicity of the N atom in these molecules, the patterns are always symmetrical about their midpoints. In the spectra of the derivs prepd during this investigation distortion of the CH_2 triplets is not appreciable in most cases and these patterns have been described as triplets (designated as "t" if not *true* triplets) and the apparent coupling constants quoted as if the patterns observed were truly of the A_2X_2 type.

(53) Neutralization of the hydrochloride with aq $NaHCO_3$ regenerated the parent mustard and repeated cycling through the hydrochloride formation–neutralization procedure finally afforded an anal. sample.

(54) J. Elks and C. W. Shoppee, *J. Chem. Soc.*, 241 (1953).

(55) H. J. Daulen, B. Loken, and H. J. Ringold, *J. Amer. Chem. Soc.*, **76**, 1359 (1954).

17β-Bis(2-chloroethyl)aminoandrost-4-en-3-one (1).—To a soln of 17β-bis(2-hydroxyethyl)aminoandrost-4-en-3-one (**17**, 170 mg, 0.45 mmole) in pyridine (1 ml) was added MsCl (0.08 ml, 1.05 mmoles) during 2 min using a cold H_2O bath to prevent the temp of the reaction mixt from rising above 90°. The resulting brown soln was heated at 80–100° for a further 20 min and, after cooling to 10°, it was dild with H_2O (3 ml). The aq supernatant was decanted from the pptd gum and was extd with $CHCl_3$ (3 × 10 ml). The combined exts were then added to the residual gummy material, and the resulting soln was dried ($MgSO_4$). Evapn of the solvent and plc on silica gel of the residue yielded the mustard **1** as a colorless gum[56] (47 mg, 25%): pmr δ 2.96 ("t," 4, J = 6.0 Hz, NCH_2CH_2Cl) and 3.50 ("t," 4, J = 6.0 Hz, NCH_2CH_2Cl). An Et_2O soln of the product was treated with HCl and the hydrochloride was obtained as a fine white powder[55] (35 mg): mp 123–124°; mass spectrum (70 eV) m/e (rel intensity) 411 (57), 413 (37), 415 (7).

3-Chloro-17β-bis(2-hydroxyethyl)aminoandrosta-3,5-diene (20) from the Reaction of 17 with $POCl_3$.—$POCl_3$ (4 ml) and **17** (200 mg, 0.53 mmole) were refluxed for 30 min, and the reaction mixt was then worked-up as for the previous $POCl_3$ reactions (*e.g.*, **9** → **3**). The yellow, oily product obtained was recrystd from Me_2CO to give **20** as a yellow powder[56] (99 mg, 43%): mp 145–147°; pmr δ 2.98 ("t," 4, J = 6.5 Hz, NCH_2CH_2Cl) and 3.52 ("t," 4, J = 6.5 Hz, NCH_2CH_2Cl); uv max (MeOH) 242 (log ε 4.4), 236 (s) and 250 nm (s). The 3-chloro mustard **20** was converted to its hydrochloride,[55] mp 173–174.5°, as described for **3**.

3,3′-Ethylenedioxyandrost-5-ene 17β-Toluene-*p*-sulfonate.—3,3′-Ethylenedioxyandrost-5-en-17β-ol[55] (**15**, 1.1 g, 30 mmoles) was dissolved in dry pyridine (5 ml), and TsCl (1.06 g, 60 mmoles) was added at 20° with stirring. The reaction mixt was kept at 20° for 2 days after which time satd aq $NaHCO_3$ (50 ml) was added, and the mixt extd with $CHCl_3$ (2 × 25 ml). The $CHCl_3$ exts were dried ($MgSO_4$) and evapd, and the resulting solid was recrystd from MeOH to give the product as needles (1.2 g, 80%), mp 154–155°. *Anal.* ($C_{28}H_{38}O_5S$) C, H.

17α-Azido-3,3′-ethylenedioxyandrost-5-ene.—The above tosylate (15 g, 30 mmoles) and NaN_3 (13.8 g, 210 mmoles) were allowed to react in dry *N*-methyl-2-pyrrolidone as described for the conversion of **10** into **11**. The 17α-azido-3,3′-ethylenedioxyandrost-5-ene obtained (10.8 g, quant) was recrystd from MeOH contg a trace of pyridine as prisms (7.5 g), mp 157–158°. *Anal.* ($C_{21}H_{31}N_3O_2$) C, H, N.

17α-Amino-3,3′-ethylenedioxyandrost-5-ene.—A soln of the above 17α-azide (10.8 g, 30 mmoles) in dry Et_2O (450 ml) was reduced with a slurry of LAH (10.7 g, 280 mmoles) in dry Et_2O (1 l.). The procedure used was as described for the prepn of 17α-amino-5-androstane (**12**) and 17α-amino-3,3′-ethylenedioxyandrost-5-ene, prisms from hexane, mp 143–148°, was isolated in 45% yield (4.6 g).

The amine itself was rather unstable and was further characterized as the mono toluene-*p*-sulfonamide deriv (obtained by treatment with TsCl in pyridine), which recrystd from MeOH contg a trace of pyridine as needles, mp 230–231°. *Anal.* ($C_{28}H_{39}NO_4S$) C, H, N, S.

17α-Bis(2-hydroxyethyl)amino-3;3′-ethylenedioxyandrost-5-ene (**18**).—The general procedure described for the alkylation of **8** was employed using the above 17α-amino-Δ⁵-3-ketal (400 mg, 1.2 mmoles) and ethylene oxide (6 ml, 120 mmoles). Compd **18** was produced and was recrystd from hexane contg a trace of pyridine to give needles (257 mg, 52%): mp 140.5–141°; pmr δ 2.82 ("t," 4, J = 5 Hz, NCH_2CH_2OH) and 3.63 ("t," 4, J = 5 Hz, NCH_2CH_2OH). *Anal.* ($C_{25}H_{41}NO_4$) C, H, N.

17α-Bis(2-hydroxyethyl)aminoandrost-4-en-3-one (**19**).—The protecting ketal group of **18** (105 mg, 2.5 mmoles) was removed as described for the 17β analog **16**. Compd **19**, prisms from hexane, mp 129–131°, was isolated in 56% yield (53 mg);[56] pmr δ 2.84 ("t," 4, J = 5.5 Hz, NCH_2CH_2OH) and 3.62 ("t," 4, J = 5.5 Hz, NCH_2CH_2OH).

17α-Bis(2-chloroethyl)aminoandrost-4-en-3-one (**2**).—17α-Bis(2-hydroxyethyl)aminoandrost-4-en-3-one (**19**, 100 mg, 0.27 mmole) and MsCl (0.05 ml, 0.66 mmole) were allowed to react as described above for the prepn of **1** to give 24 mg (21%) of the 17α-mustard 2; pmr δ 2.72–3.17 (m, 4, NCH_2CH_2Cl) and 3.46 (structured "t," 4, J = 7 Hz, NCH_2CH_2Cl); mass spectrum (70 eV) m/e (rel intensity) 411 (57), 413 (40), 415 (10). The small amt of material available precluded further purification of this somewhat unstable mustard.[56]

17β-Bis(2-hydroxyethyl)amino-5α-androstan-3β-ol (**22**).—17β-Amino-5α-androstan-3β-ol (**21**, 500 mg, 1.7 mmoles), mp 157–159° (lit.[16] mp 158–160°), was prepd by the method of Pettit and coworkers[16] and was treated with ethylene oxide (8.5 ml, 170 mmoles) as described in detail for **8**. The diol **22** obtd was recrystd from a large vol of MeOH to give needles (416 mg, 65%), mp 195–196°. The insoly of **22** precluded the recording of a routine pmr spectrum. *Anal.* ($C_{23}H_{41}NO_3$) C, H, N.

17β-Bis(2-chloroethyl)amino-3α-chloro-5-α-androstane (**23**) **from the Reaction of 22 with $SOCl_2$.**—Treatment of the triol **22** (250 mg, 0.65 mmole) with $SOCl_2$ (2 ml, 28 mmoles) in the usual way (*cf.* **9** → **3**) gave the 3α-chloro mustard **23**, plates (70 mg, 25%) from Me_2CO: mp 122.5–124.5°; pmr δ 2.91 ("t," 4, J = 7 Hz, NCH_2CH_2Cl) and 3.46 ("t," 4, J = 7 Hz, NCH_2CH_2Cl). *Anal.* ($C_{23}H_{38}Cl_3N$) C, H, Cl, N.

17β-Bis(2-chloroethyl)amino-5α-androst-2-ene (**24**) **from the Reaction of 22 with $POCl_3$.**—17β-Bis(2-hydroxyethyl)amino-5α-androstan-3β-ol (300 mg, 0.79 mmole) was treated with $POCl_3$ (15 ml) as described for **9** → **3** and 17β-bis(2-chloroethyl)aminoandrost-2-ene was obtained as a yellow oil[56] (170 mg, 54%): pmr δ 3.02 ("t," 4, J = 6.1 Hz, NCH_2CH_2Cl) and 3.52 ("t," 4, J = 6 Hz, NCH_2CH_2Cl). The oil was dissolved in anhyd Et_2O and HCl was bubbled through the soln to give the hydrochloride as a fine powder (140 mg): mp 146–147.5°; mass spectrum (70 eV) m/e (rel intensity) 397 (57), 399 (39), 401 (7).

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(56) Great difficulty was encountered in the purification of several of the N mustards prepd during this study and acceptable elemental anal. data were not obtainable for 17β-bis(2-chloroethyl)aminoandrost-4-en-3-one (**1**), 17α-bis(2-chloroethyl)aminoandrost-4-en-3-one (**2**), 3-chloro-17β-bis(2-chloroethyl)aminoandrosta-3,5-diene (**20**), and 17β-bis(2-chloroethyl)aminoandrost-2-ene (**24**). The purification procedures surveyed included column chromatography on Al_2O_3 and Florisil, plc on silica and recrystn of the mustards and their HCl salts from a variety of org solvents. Furthermore, the mustards were found to decomp on XE-60, QF-1, and SE-30 columns during glc analysis. In this context, it should be noted that Peck, *et al.*,[57] reported that analytical laboratories encounter great difficulty in total halogen analyses on N mustards and their hydrochlorides due to the lability of both types of Cl atoms. The 17α-mustard precursor **19** also proved too unstable for a satisfactory elemental anal. to be obtd.

(57) R. M. Peck, R. K. Preston, and H. J. Creech, *J. Amer. Chem. Soc.*, **76**, 3984 (1959).