

Steroids and Steroidases. 10.¹ Studies on Some Potentially Antitumor Active Androstane Compounds Containing C-17 Nitrogen Mustard Functions

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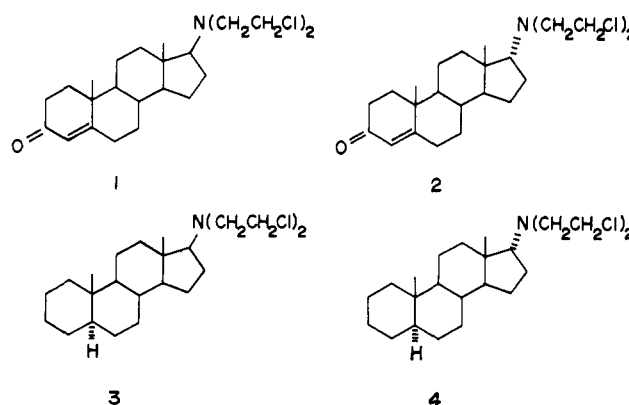
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Various approaches to 17 α - and 17 β -N(CH₂CH₂Cl)₂ androstane compounds have been evaluated and of the routes explored, those involving N(CH₂CH₂OH)₂ derivatives proved the most reliable. Using androstanes with no functionality other than the mustard 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 multiple³ 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, Thiotepe, administered together was more effective than when either compound was used alone⁴ 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(CH₂CH₂OH)₂ intermediates the final chlorination stage appeared to be a particularly sensitive one. In addition, preliminary investigations on oxygenated and unsaturated steroid derivatives¹³ 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(CH₂CH₂OH)₂ intermediates.¹⁴

The synthetic schemes followed are outlined in Scheme I. Conversion of 5 α -androstane-17 β -ol† (**5**) to 17 β -amino-5 α -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. Cioranescu 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 synthesis 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.

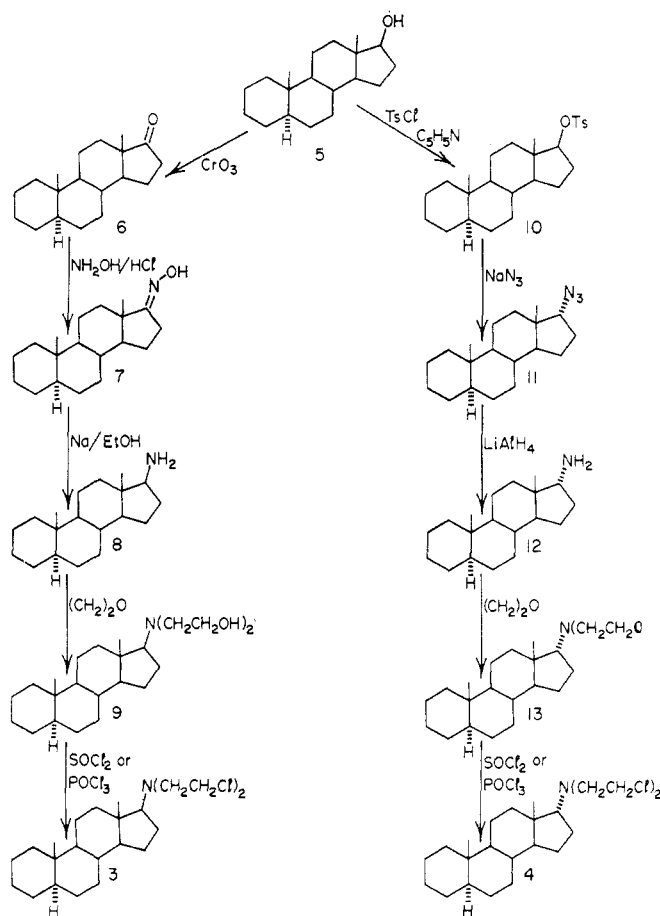
(14) Although **3** and **4** were not expected to exhibit appreciable antineoplastic activities, the possibility of their doing so was finite since the carrier moiety, 5 α -androstane, is weakly androgenic.¹⁵

(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 $\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$ derivative 13 from 5 via the tosylate 10, the azide 11, and the amine 12¹⁸ proceeded equally smoothly in 41% overall yield.¹⁹

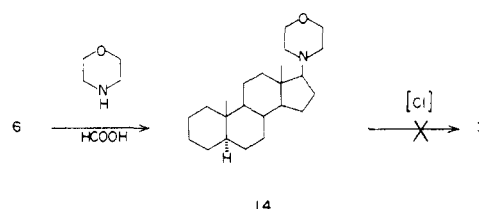
The most widely used chlorinating agent for the conversion of hydroxyethylamines to the corresponding mustards has been SOCl_2 .^{3,12} Disappointingly, when the 17β compound 9 was treated with reagent grade SOCl_2 in CHCl_3 in a standard way¹⁷ an unidentifiable black tar resulted²¹ even at room temp. However, using carefully purified (with triphenyl phosphite²²) SOCl_2 in anhyd 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 reac-

tion²³ 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

SCHEME II



sation of morpholine with the 17-keto function of 6 under Leuckart-Wallach conditions to give the 17β derivative 14 was expected to be facile¹⁸ and in view of the report²⁷ on the cleavage of some aromatic morpho-

(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 peaks¹⁹ 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 ($\text{C}_{25}\text{H}_{40}\text{N}^{35}\text{Cl}_2$), 401 ($\text{C}_{25}\text{H}_{40}\text{N}^{35}\text{Cl}^{37}\text{Cl}$), and 403 ($\text{C}_{25}\text{H}_{40}\text{N}^{37}\text{Cl}_2$). 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).

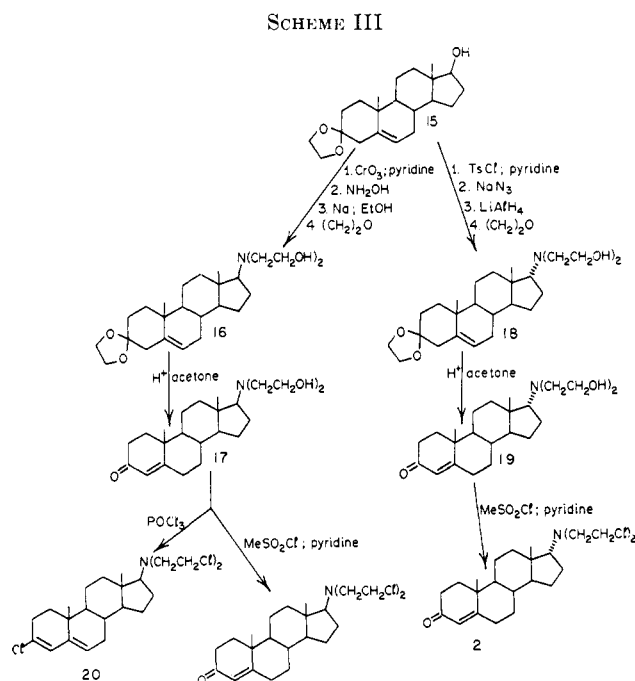
lines with HBr to give $N(\text{CH}_2\text{CH}_2\text{Br})_2$ derivatives we were hopeful that similar chlorinative cleavage of the ether function of **14** might be possible.

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 acidic²⁷ and oxidative²⁸ 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 cleavage²⁹ 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 conditions³⁰ 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, Duranleau³³ 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 possibilities²⁹ 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 $\text{HN}(\text{CH}_2\text{CH}_2\text{Cl})_2$ itself, were not investigated because of the marked instability of the latter compound³⁶ 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 Δ^4 -3-keto mustards **1** and **2** as summarized in Scheme III. In the 17β series, the $N(\text{CH}_2\text{CH}_2\text{OH})_2$



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 Δ^4 -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 pinene³⁴ were added to remove the acid *in situ*. PCl_3 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 Δ^4 -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 compounds³² 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 amines¹⁸ it was not entirely unexpected since the basicity and nucleophilicity of the amine is known to be critical.¹⁸

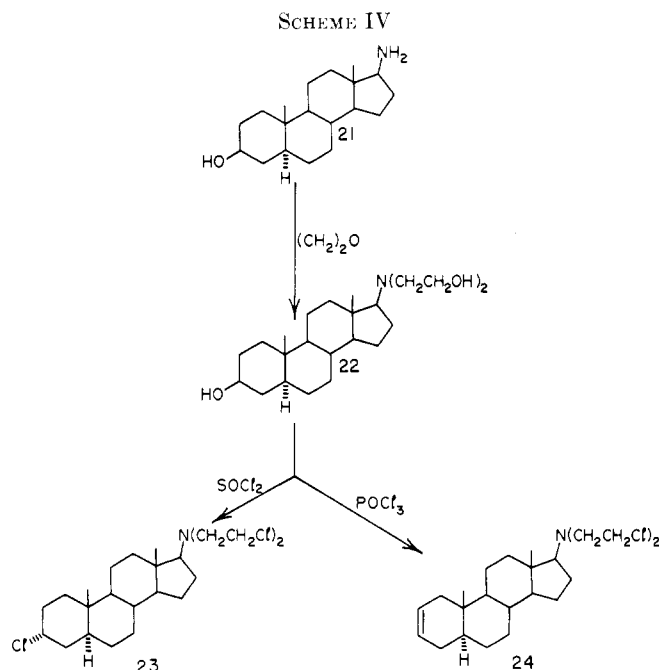
(36) G. R. Pettit and J. A. Settepani, *J. Org. Chem.*, **27**, 2962 (1962), and ref therein.

(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.

lets²³ 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 17 β -amino-5 α -androst-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



was available in reasonable quantity its conversion to a mustard was explored even though it was appreciated that selective formation of the N(CH₂CH₂Cl)₂ group, without concomitant chlorination of the C-3 OH, was unlikely to be achieved.

(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 chloride³⁸ and C₆H₅COCl,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).

Chlorination of **22** with purified SOCl₂ in pure CHCl₃ 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 3 α derivative as shown in structure **23**.⁴³ This conclusion was based on the well-documented⁴⁴ 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 3 β proton of 3 α -chloro-5 α -androst-17-one.¹³ In contrast, the 3 α -H of 3 β -chloro-5 α -androst-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 POCl₃ effected mustard formation smoothly and in good yield. However, somewhat unexpectedly, elimination occurred during the reaction, and the product isolated was 17 β -(2-chloroethylamino)-5 α -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 5 α -androst-2-en-17-one obtained in connection with other studies.

Whereas dehydration of steroidal tertiary alcohols with POCl₃ is well established⁴⁵ basic solvents are generally employed and trans-diaxial eliminations are favored. The latter stereochemical requirement is not satisfied by the 3 β -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 groups⁴⁶ and, furthermore, the possibility of formation of the 3 α -chloride (through participation of the C-17-NH₂ group as suggested in the SOCl₂ reaction⁴³) followed by dehydrochlorination cannot be discounted.

Review of Chlorination Procedures.—The synthetic studies have shown that approaches involving N(CH₂CH₂OH)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

(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 conditions 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 alcohols¹³ 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 -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 Larionov⁴⁷ 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 anal. 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 triphenylphosphite²² 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 value²³ have details been reported.

17 β -Amino-5 α -androstande (8).—5 α -Androstan-17 β -ol (**5**), mp 168–169° (lit.⁴⁸ mp 165.5–166.5°), was obtd by redn of 17 β -hydroxyandrost-4-en-3-one (testosterone)^{48,49} and its oxidn with Jones' reagent in freshly distd Me_2CO gave 5 α -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-5 β -androstande (**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°) H₂O (60 ml) and kept overnight at 20°. The resulting ppt was filtered, washed with H₂O, and recrystd from Me₂CO to give 0.36 g (86%) of 17 β -amino-5 α -androstan-8), mp 131–134° (lit.⁵¹ mp 138–141°).

17 β -Bis(2-hydroxyethyl)amino-5 α -androstan-8) (9).—A soln of 17 β -androstan-8), 200 mg, 0.73 mmole) in the min amt of CHCl₃ (ca. 10 ml) was added rapidly with stirring to a cold (0°) soln of ethylene oxide (4 ml, 81 mmole) 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 Me₂CO–CO₂ 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 NCH₂CH₂OH) and 3.64 ppm ("t," 4, J = 6.0 Hz, NCH₂CH₂OH).⁵² Anal. (C₂₃H₄₁NO₂) C, H, N.

17 β -Bis(2-chloroethyl)amino-5 α -androstan-8) (3). (a) **With SOCl₂ as Chlorinating Agent.**—To a stirred soln of 17 β -bis(2-hydroxyethyl)amino-5 α -androstan-8) (9, 100 mg, 0.28 mmole) in CHCl₃ (5 ml) cooled in an ice bath was added slowly a soln of SOCl₂ (0.6 ml, 8.3 mmole) in CHCl₃ (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°) H₂O (25 ml) and washed with cold (5°) satd aq NaHCO₃ soln (1 \times 10 ml). The aq layer was extd with CHCl₃ (2 \times 10 ml), and the combined CHCl₃ exts were washed with cold (5°) H₂O (2 \times 10 ml) and then dried (MgSO₄). On removal of the solvent the mustard was obtained as a yellow solid (55 mg, 50%). Recrystn of a sample from Me₂CO yielded 3 as a cryst powder: mp 82–82.5°; pmr δ 2.94 ("t," 4, J = 8.0 Hz, NCH₂CH₂Cl) and 3.48 ppm ("t," 4, J = 8.0 Hz, NCH₂CH₂Cl); mass spectrum (70 eV) m/e (rel intensity) 399 (57), 401 (38), 403 (7).

(b) **With POCl₃ as Chlorinating Agent.**—A soln of the diol 9 (150 mg, 0.41 mmole) in POCl₃ (5 ml) was heated at 95–100° for 1 hr. The excess POCl₃ was then removed by vacuum distn, and the colorless oil which resulted was taken up in C₆H₆ (20 ml). The C₆H₆ was then removed by vacuum distn. This latter cycle of operations was repeated until the odor of POCl₃ could no longer be detected in the reaction flask. The resulting gum was redissolved in C₆H₆ (20 ml), and the soln was washed with cold satd NaHCO₃ soln (1 \times 10 ml) and dried (MgSO₄). Evapn of the solvent gave 3 as a pale yellow solid (91 mg, 55%). The mustard was dissolved in the min amt of anhyd Et₂O, and HCl was bubbled through the soln. The hydrochloride was obtained as a fine powder (85 mg), mp 151–153°.⁵³ Anal. (C₂₃H₄₀Cl₂N) C, H, N, Cl.

17 α -Azido-5 α -androstan-8) (11).—To a soln of 5 α -androstan-17 β -toluene-*p*-sulfonate⁶⁴ (0.50 g, 1.4 mmole) in dry *N*-methyl-2-pyrrolidone (10 ml) under N₂ was added cryst NaN₃ (0.43 g, 6.6 mmole).¹⁸ While still under N₂ the mixt was heated at 150° for 5 hr and was then cooled and poured into H₂O (60 ml). The aq soln was extd with Et₂O (4 \times 30 ml), and the Et₂O exts were then washed with H₂O and dried (MgSO₄). Evapn afforded a yellow solid which was recrystd from MeOH to give 11 as plates (245 mg, 80%); mp 61.8–62.5°. Anal. (C₁₉H₃₁N₃) C, H, N.

17 α -Amino-5 α -androstan-8) (12).—A soln of 17 α -azido-5 α -androstan-8) (11, 100 mg, 0.34 mmole) in anhyd Et₂O (4.5 ml) was added slowly with stirring to a slurry of LAH (100 mg, 2.46 mmole) in anhyd Et₂O (10 ml).¹⁸ After the mixt had been heated under reflux for 3 hr moist ether was added until gas evoln ceased. H₂O (10 ml) and 5% NaOH soln (10 ml) were then added, and the Et₂O layer was removed by decantation.

(52) The CH₂ adjacent to N and those adjacent to O or Cl in 2-hydroxyethylamines and 2-chloroethylamines, respectively, do not constitute a true A₂X₂ system. Hence, distorted and structured triplets and other more complex absorptions are often observed for these protons as the system varies from an A₂X₂ to an A₂B₂ 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 prep'd during this investigation distortion of the CH₂ 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 A₂X₂ type.

(53) Neutralization of the hydrochloride with aq NaHCO₃ 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).

The remaining aq mixt was stirred further to effect soln of the Al salts and then reextd with Et₂O (3 \times 20 ml). The combined Et₂O exts were dried (MgSO₄) and evapd to give the 17 α -amine 12 as a clear, colorless gum (77 mg, 84%) which solidified upon standing.

17 α -Bis(2-hydroxyethyl)amino-5 α -androstan-8) (13).—A prep'd from 17 α -amino-5 α -androstan-8) (12, 1.0 g, 3.6 mmole) and ethylene oxide (18 ml, 0.36 mole) according to the method described above for 17 β -bis(2-hydroxyethyl)amino-5 α -androstan-8) (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, NCH₂CH₂OH), and 3.58 ppm ("t," 4, J = 5.9 Hz, NCH₂CH₂OH). Anal. (C₂₃H₄₁NO₂) C, H, N.

17 α -Bis(2-chloroethyl)amino-5 α -androstan-8) (4). (a) **With SOCl₂ as Chlorinating Agent.**—17 α -Bis(2-hydroxyethyl)amino-5 α -androstan-8) (13, 100 mg, 0.38 mmole), dissolved in CHCl₃ (5 ml), and SOCl₂ (0.6 ml, 8.3 mmole) in CHCl₃ (2.5 ml) were treated and worked-up as described for the 17 β -mustard 3. The resulting brown oil was dissolved in CHCl₃, 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, NCH₂CH₂Cl) and 3.42 ppm (structured "t," 4, J = 8 Hz, NCH₂CH₂Cl); mass spectrum (70 eV) m/e (rel intensity) 399 (57), 401 (32), 403 (7).

(b) **With POCl₃ as Chlorinating Agent.**—POCl₃ (5 ml) and 17 α -bis(2-hydroxyethyl)amino-5 α -androstan-8) (13, 150 mg, 0.41 mmole) were treated and worked-up as described above for 3. Compd 4 was obt'd as a white solid (83 mg, 50%) and was converted to the hydrochloride (70 mg), mp 154–155°, as described previously. Anal. (C₂₃H₄₀Cl₂N), C, H, N; Cl calcd, 24.34; found, 23.47.⁵³

17 β -Morpholino-5 α -androstan-8) (14).—A mixt of 5 α -androstan-17-one (1 g, 3.6 mmole), HCO₂H (1 ml, 30 mmole), and morpholine (3 ml, 34 mmole) was heated in a sealed tube at 170–180° for 15 hr and was then poured into H₂O and filtered. Recrystn from Me₂CO gave 14 as plates (0.82 g, 65%); mp 154–155°. Anal. (C₂₃H₃₉NO) C, H, N.

3,3'-Ethylenedioxy-17-hydroxyiminoandro-5-ene.—17 β -Hydroxyandro-4-en-3-one (testosterone) was reacted with HO(CH₂)₂OH⁵⁵ to give the hydroxyketal 15, mp 183–184° (lit.⁵⁶ mp 182–184°), in 46% yield. Oxidn of 15 was effected with CrO₃ in pyridine²⁹ to give 80% of 3,3'-ethylenedioxyandro-5-en-17-one, mp 196–197° (lit.⁵⁵ mp 197–198°). The latter oxoketal (500 mg, 15 mmole) was dissolved in pyridine (5 ml), and a soln of NH₂OH·HCl (440 mg, 70 mmole) in 90% aq EtOH (5 ml) was added. The mixt was refluxed for 5 hr and was then poured into H₂O (100 ml) and extd with CHCl₃ (3 \times 50 ml). The dried (MgSO₄) CHCl₃ exts were evapd, and the residue was recrystd from MeOH contg a trace of pyridine to give 313 mg of 3,3'-ethylenedioxy-17-hydroxyiminoandro-5-ene as needles, mp 245–246°. Anal. (C₂₁H₃₁NO₃) C, H, N.

17 β -Amino-3,3'-ethylenedioxyandro-5-ene.—3,3'-Ethylenedioxy-17-hydroxyiminoandro-5-ene (500 mg, 14.5 mmole) was reduced with Na (3 g) in EtOH as described above in the prep'n of 17 β -amino-5 α -androstan-8). 17 β -Amino-3,3'-ethylenedioxyandro-5-ene was obtained as prisms (269 mg, 56%) from C₆H₆–hexane, mp 183–184°. Anal. (C₂₁H₃₃NO₂) C, H, N.

17 β -Bis(2-hydroxyethyl)amino-3,3'-ethylenedioxyandro-5-ene (16).—17 β -Amino-3,3'-ethylenedioxyandro-5-ene (3 g, 0.09 mole) in the min vol of CHCl₃ 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, NCH₂CH₂OH) and 3.68 ("t," 4, J = 5 Hz, NCH₂CH₂OH). Anal. (C₂₅H₄₁NO₄) C, H, N.

17 β -Bis(2-hydroxyethyl)aminoandro-4-en-3-one (17).—The above ketal 16 (105 mg, 2.5 mmole) and hydrated TsOH (70 mg) were kept in Me₂CO–CHCl₃ (1:1, 12 ml) soln for 12 hr at 20°. The soln was then poured into satd aq NaHCO₃ (50 ml), and the mixt was extd with CHCl₃ (5 \times 20 ml). The combined CHCl₃ exts were dried (MgSO₄) 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, NCH₂CH₂OH) and 3.66 ("t," 4, J = 5 Hz, NCH₂CH₂OH). Anal. (C₂₃H₃₇NO₃) C, H, N.

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

17 β -Bis(2-chloroethyl)aminoandrost-4-en-3-one (1).—To a soln of 17 β -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 H₂O 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 H₂O (3 ml). The aq supernatant was decanted from the pptd gum and was extd with CHCl₃ (3 \times 10 ml). The combined exts were then added to the residual gummy material, and the resulting soln was dried (MgSO₄). Evapn of the solvent and plc on silica gel of the residue yielded the mustard **1** as a colorless gum⁵⁶ (47 mg, 25%): pmr δ 2.96 ("t," 4, J = 6.0 Hz, NCH₂CH₂Cl) and 3.50 ("t," 4, J = 6.0 Hz, NCH₂CH₂Cl). An Et₂O soln of the product was treated with HCl and the hydrochloride was obtained as a fine white powder⁵⁶ (35 mg): mp 123–124°; mass spectrum (70 eV) m/e (rel intensity) 411 (57), 413 (37), 415 (7).

3-Chloro-17 β -bis(2-hydroxyethyl)aminoandrost-3,5-diene (20) from the Reaction of 17 with POCl₃.—POCl₃ (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 POCl₃ reactions (e.g., **9** \rightarrow **3**). The yellow, oily product obtained was recrystd from Me₂CO to give **20** as a yellow powder⁵⁶ (99 mg, 43%): mp 145–147°; pmr δ 2.98 ("t," 4, J = 6.5 Hz, NCH₂CH₂Cl) and 3.52 ("t," 4, J = 6.5 Hz, NCH₂CH₂Cl); uv max (MeOH) 242 (log ϵ 4.4), 236 (s) and 250 nm (s). The 3-chloro mustard **20** was converted to its hydrochloride,⁵⁶ mp 173–174.5°, as described for **3**.

3,3'-Ethylenedioxyandrost-5-ene 17 β -Toluene-*p*-sulfonate.—3,3'-Ethylenedioxyandrost-5-en-17 β -ol⁵⁶ (**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 NaHCO₃ (50 ml) was added, and the mixt extd with CHCl₃ (2 \times 25 ml). The CHCl₃ exts were dried (MgSO₄) and evapd, and the resulting solid was recrystd from MeOH to give the product as needles (1.2 g, 80%), mp 154–155°. Anal. (C₂₈H₃₈O₅S) C, H, N.

17 α -Azido-3,3'-ethylenedioxyandrost-5-ene.—The above tosylate (15 g, 30 mmoles) and NaN₃ (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 17 α -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. (C₂₇H₃₁N₃O₂) C, H, N.

17 α -Amino-3,3'-ethylenedioxyandrost-5-ene.—A soln of the above 17 α -azide (10.8 g, 30 mmoles) in dry Et₂O (450 ml) was reduced with a slurry of LAH (10.7 g, 280 mmoles) in dry Et₂O (1 l.). The procedure used was as described for the prepn of 17 α -amino-5-androstane (**12**) and 17 α -amino-3,3'-ethylenedioxyandrost-5-ene, prisms from hexane, mp 143–148°, was isolated in 45% yield (4.6 g).

(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 17 β -bis(2-chloroethyl)aminoandrost-4-en-3-one (**1**), 17 α -bis(2-chloroethyl)aminoandrost-4-en-3-one (**2**), 3-chloro-17 β -bis(2-chloroethyl)aminoandrost-3,5-diene (**20**), and 17 β -bis(2-chloroethyl)aminoandrost-2-ene (**24**). The purification procedures surveyed included column chromatography on Al₂O₃ 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.*,⁴⁷ 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 17 α -mustard precursor **19** also proved too unstable for a satisfactory elemental anal. to be obt'd.

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

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. (C₂₈H₃₈NO₅S) C, H, N, S.

17 α -Bis(2-hydroxyethyl)amino-3,3'-ethylenedioxyandrost-5-ene (18).—The general procedure described for the alkylation of **8** was employed using the above 17 α -amino- Δ^5 -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, NCH₂CH₂OH) and 3.63 ("t," 4, J = 5 Hz, NCH₂CH₂OH). Anal. (C₂₈H₄₄NO₄) C, H, N.

17 α -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 17 β 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, NCH₂CH₂OH) and 3.62 ("t," 4, J = 5.5 Hz, NCH₂CH₂OH).

17 α -Bis(2-chloroethyl)aminoandrost-4-en-3-one (2).—17 α -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 17 α -mustard **2**; pmr δ 2.72–3.17 (m, 4, NCH₂CH₂Cl) and 3.46 (structured "t," 4, J = 7 Hz, NCH₂CH₂Cl); 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

17 β -Bis(2-hydroxyethyl)amino-5 α -androst-3 β -ol (22).—17 β -Amino-5 α -androst-3 β -ol (**21**, 500 mg, 1.7 mmoles), mp 157–159° (lit.¹⁶ mp 158–160°), was prepd by the method of Pettit and coworkers¹⁶ and was treated with ethylene oxide (8.5 ml, 170 mmoles) as described in detail for **8**. The diol **22** obt'd was recrystd from a large vol of MeOH to give needles (416 mg, 65%), mp 195–196°. The insolv of **22** precluded the recording of a routine pmr spectrum. Anal. (C₂₈H₄₄NO₃) C, H, N.

17 β -Bis(2-chloroethyl)amino-3 α -chloro-5 α -androstane (23) from the Reaction of 22 with SOCl₂.—Treatment of the triol **22** (250 mg, 0.65 mmole) with SOCl₂ (2 ml, 28 mmoles) in the usual way (cf. **9** \rightarrow **3**) gave the 3 α -chloro mustard **23**, plates (70 mg, 25%) from Me₂CO: mp 122.5–124.5°; pmr δ 2.91 ("t," 4, J = 7 Hz, NCH₂CH₂Cl) and 3.46 ("t," 4, J = 7 Hz, NCH₂CH₂Cl). Anal. (C₂₈H₃₈Cl₂N) C, H, Cl, N.

17 β -Bis(2-chloroethyl)amino-5 α -androst-2-ene (24) from the Reaction of 22 with POCl₃.—17 β -Bis(2-hydroxyethyl)amino-5 α -androst-3 β -ol (300 mg, 0.79 mmole) was treated with POCl₃ (15 ml) as described for **9** \rightarrow **3** and 17 β -bis(2-chloroethyl)aminoandrost-2-ene was obtained as a yellow oil⁵⁶ (170 mg, 54%): pmr δ 3.02 ("t," 4, J = 6.1 Hz, NCH₂CH₂Cl) and 3.52 ("t," 4, J = 6 Hz, NCH₂CH₂Cl). The oil was dissolved in anhyd Et₂O 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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