phenyl Sulfone.—A solution of 2.5 g (5.7 mmoles) of N-(3-chloronaphthoquinonyl) diaminodiphenyl sulfone in 40 ml of 97% HCO_2H was heated under reflux for 2.0 hr, an equal volume of H_2O was added to the hot solution, and the bright orange crystalline precipitate was collected and washed (H_2O, MeOH, Et_2O); yield 2.6 g, mp 244–248° dec. The compound was recrystallized by solution in 100 ml of DMF (dissolved at room temperature) and adding an equal volume of H_2O. The product (2.4 g) separated as glistening orange platelets, mp 244–248°. See Table I.

N-Acetyl-N′-(3-chloro-1,4-naphthoquinonyl) Diaminodiphenyl Sulfone.—N-(3-Chloronaphthoquinonyl) diaminodiphenyl sulfone (0.3 g, 1.25 mmoles) was suspended in 20 ml of Ac_2O and then warmed on the steam bath. The red crystalline suspension dissolved and an orange crystalline product precipitated. After cooling to room temperature, the crystals were collected by filtration and washed (MeOH, Et_2O); yield 0.29 g. The crystals were recrystallized from a THF and H_2O mixture to give 0.27 g of product. See Table I.

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Single Crystal Studies of Chemotherapeutic Agents. I. The Structure of 1-(2-Chloroethyl)-3-(5-carbamoylimidazol-4-yl)-Δ^2-1,2,3-triazolinium Chloride[1]

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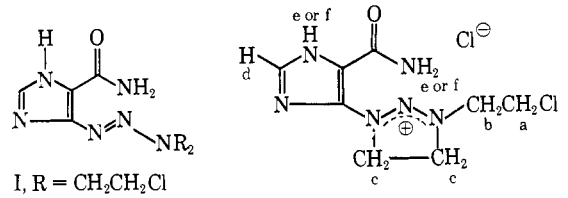
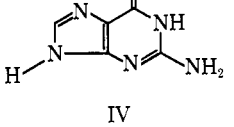
The nitrogen mustard 5(4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(5)-carboxamide (I) is a promising anticancer agent.[3] Other related triazenes, particularly the dimethyl compound II, also show anticancer activity to a lesser extent.[4] Unfortunately, the nitrogen mustard I undergoes a spontaneous



I, R = CH_2CH_2Cl
II, R = CH_3

III



IV

transformation at room temperature in the solid state to form an inactive isomer, which was recognized as a quaternary ammonium chloride by Shealy, *et al.*[5]

They further suggested that it might be an aziridinium, piperazinium, or *v*-triazolinium salt. We wish to report the identification of the transformation product, by X-ray crystal structure analysis as well as nmr and mass spectral studies, as 1-(2-chloroethyl)-3-(5-carbamoylimidazol-4-yl)-Δ^2-1,2,3-triazolinium chloride (III). This shows that the transformation reaction is a novel means of formation of the *v*-triazoline ring system.

Bond lengths and angles between nonhydrogen atoms at the present stage of refinement (R = 0.10) are shown in Figure 1. The two rings, amide group, and α carbon of the chloroethyl group are roughly coplanar, the maximum deviation of any atom from the plane being 0.22 Å. The bond lengths and angles in the triazolinium ion show that it has the symmetrical resonance-stabilized form. The chloroethyl group has a *gauche* conformation. The hydrogen atoms have been located in a three-dimensional difference synthesis, and their positions confirm the tautomeric form ascribed to the imidazole ring, and the existence of an intramolecular N–H···N hydrogen bond of length 2.98 Å between the amide N and the N^2 of the triazoline ring.

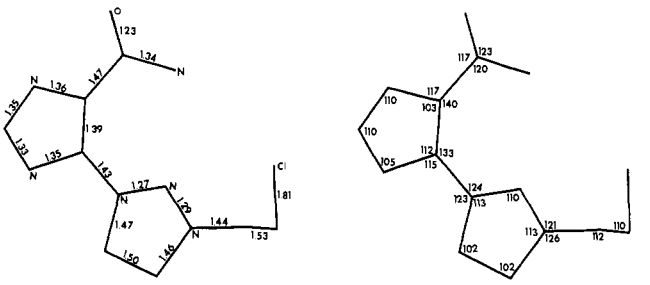


Figure 1.—Bond lengths and angles in the 1-(2-chloroethyl)-3-(5-carbamoylimidazol-4-yl)-Δ^2-1,2,3-triazolinium ion.

The nmr spectrum agrees well with this structure, both in the ratios of the various types of hydrogens and in the observed splittings. The fact that all CH_2 protons occur downfield from the usual CH_2 range is in accord with this structure, since b and c are next to a positive N, while protons of type a are adjacent to Cl.

This investigation further allows us to draw inferences concerning the mode of action of the drug, based on the stereochemistry of the inactive compound. The substituent groups on the imidazole ring are almost coplanar with it, and so arranged that the amide N and N^2 of the triazoline ring face each other at a distance of about 3 Å. An intramolecular H bond between these two atoms holds the molecule in this configuration. If we assume that this arrangement also occurs in the parent compound, the structural similarity to guanine (IV) becomes immediately apparent. The C(2)–N(1) bond of guanine has been replaced by this N–H···N hydrogen bond. The peripheral pattern of H-bond donors and acceptors, required by guanine's role in the nucleic acids and determining the specificity of enzymes, remains unchanged except for the modification of N^2 to a nitrogen mustard. The fact that the anticancer activity remains, although reduced, when these 2-chloroethyl groups are replaced by other substituents, supports the conclusion that the activity is related to the structure of the remainder of the molecule. The over-all implication is that I may bind,

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and subsequently bond, by means of the alkylating groups, to, for example, the active site of enzymes for which guanine is a substrate.

Experimental Section

Nmr.—The nmr of III was determined in DMSO-d_6 with Me_4Si as the internal standard. H were assigned as shown in Table I. Upon addition of D_2O the peaks at δ 7.50 and 8.0 disappeared.

TABLE I

ASSIGNMENT OF HYDROGENS

Type H	δ value	Ratio	Splitting
a	3.95	2	Distorted triplet
b	4.35	6	Distorted triplet
c	4.60		Distorted triplet
d	7.60	1	Singlet
e	7.50 or 8.0	?	Broad humps
f	7.50 or 8.0	?	Broad humps

Mass Spectrum.—The mass spectral fragmentation pattern showed the molecular ion peak to occur at m/e 242, which corresponds to the positive ion III.

Crystal Data and Structure Determination.—The transformation product was recrystallized (MeOH) to give thick tabular yellow crystals (001) with $a = 12.80$, $b = 7.41$, $c = 12.42$ Å; $\beta = 90°44'$; $V = 1178$ Å^3; D_m (flotation) 1.58; $Z = 4$; D_x 1.573; absorption coefficient for CuKα radiation, 49.5 cm^{-1}. The crystal size was 0.2 × 0.2 × 0.2 mm. Intensity data were measured with a Picker four-circle automatic diffractometer in θ–2θ mode to a maximum 2θ of 130°. Systematic extinctions for $0k0$ (k odd) and $h0l$ (l odd) indicated, unambiguously, the monoclinic space group $P2_1/c$. The structure was solved using Patterson and heavy-atom methods and refined by block diagonal least squares to give an agreement factor R of 0.20. Further anisotropic full-matrix least-squares refinement has reduced the R factor to 0.10 and permitted the determination of H-atom positions by the difference Fourier method.

3-Aza-A-homoandrostenes

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One of the views on the mechanism of steroid hormone action is that steroids exert their effect at the level of DNA control of RNA synthesis.[1] The messenger RNA molecules thus are the templates for *de novo* enzyme synthesis and it is these enzymes which regulate the process resulting in the observed physiological effects.[2] The interaction between steroid and DNA[3-5] and steroid and protein[6] is well established. In fact, Ts'o and Lu[7] have demonstrated that the coil form of DNA has a higher affinity for steroids than the helical form. These types of findings prompted Ringold[8] to postulate an α-face adsorption for androgen molecules. In contrast, Wolff and coworkers[9] have proposed two discrete areas of adsorption: the β face for rings A, B, and C and the α face for ring D. Both of these authors[8,10] have indicated a β-face adsorption for progestational agents.

We were interested in exploring these findings using the androgen, ethisterone, as our starting compound for molecular modification. The progestational activity of this compound was of interest to us because although only one-third as active as progesterone, it exhibited oral activity. Alteration of the A ring of ethisterone to a seven-membered ring containing nitrogen would increase the π-bonding characteristics of the molecule and could conceivably enhance the nonbonded adsorption to the receptors. Hence, ethisterone was converted to its acetate by the procedure of Yamada[11] and transformed to compounds **1**–**5** (Scheme I) by methods described in the Experimental Section.

The progestational activity of these compounds was determined by the Clauberg test[12] and the endometrial response was scored according to the index of McPhail.[13] Ethisterone showed a McPhail index of 0.8 at a 5-mg dose, whereas both **2** and **3** exhibited 0 response at the same dose levels. These results, though limited in nature, perhaps do point out that in the androgen molecule the expansion of the A ring alters the site of π complex with the receptor (β face) and thus decreases the activity. Compounds **4** and **5** were also subjected to androgen–anabolic assay[14] and were found to have only 5% the activity of methyltestosterone. The decrease in activity can probably be attributed to steric effects in ring A (β face) and at C-17 (α face). No data are available at present on the mode of action of these aza steroids, but it is possible that these compounds may act at the protein level by inducing a modification in the enzyme which regulates the physiological effect.

Experimental Section

All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. The uv and ir data were obtained on a Cary Model 11 and Beckman IR-5 spectrophotometers, respectively. The nmr spectra were determined on a Varian A-60 spectrometer in $CDCl_3$ using TMS as an internal standard (0 ppm). All parts per million values are the center of the signals. Microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical value.

3-Oximino-17α-ethynyltestosterone Acetate (1).—A solution containing 2.0 g of 17α-ethynyltestosterone acetate, 1.0 g of $HONH_3^+Cl^-$, and 10 ml of pyridine was heated on a steam bath for 0.5 hr. The mixture was poured into 300 ml of ice–water and the solid thus precipitated was collected by filtration. Recrystallization (MeOH-H_2O) gave 1.95 g (94%) of **1**: mp 183–185°; $[\alpha]D$ +75.2°; λ_{max}^{KBr} 2.92, 3.04, 5.69, and 6.1 μ; λ_{max}^{EtOH} 238 mμ; pmr ($CDCl_3$), 0.91 (C-18), 1.1 (C-19), 2.03 (C-17, OAc), 2.57 (C-17, C≡CH), 5.81 (C-4 *syn*), and 6.5 ppm (C-4 *anti*). *Anal.* ($C_{23}H_{31}NO_3$) C, H, N.

Preparative tlc of the oxime isomers was carried out on silica gel G (750-μ thickness) and estimated by titrating (with Radiometer) each eluent (*syn* and *anti*) with 0.5 N $HClO_4$. The

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