

soln was then neutralized to pH 7.0, yielding a ppt of the desired carboxaldehyde which was filtered, washed with H_2O , dried, and recrystd from an appropriate solvent.

Method D. The thiosemicarbazones were prepd by treating a soln of desired carboxaldehyde in EtOH with an aqueous soln of thiosemicarbazide acidified with a few drops of dil AcOH. In some cases final compounds were sufficiently pure and were not recrystd.

1-Formyl-5-bis(β -chloroethyl)aminoisoquinoline Thiosemicarbazone (39). Compd 37 (0.192 g, 1 mmole) was added to a mixt of 0.18 g of $NH(CH_2CH_2Cl)_2 \cdot HCl$ and 0.28 ml of Et_3N in 25 ml of C_6H_6 . The mixt was refluxed for 18 hr and then filtered to remove $Et_3N \cdot HCl$. The solvent was evapd *in vacuo*, and the residue was treated with a soln of thiosemicarbazide in EtOH acidified with concd HCl. The thiosemicarbazone derivative was isolated as the HCl salt.

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Antitumor and Antileukemic Effects of Some Steroids and Other Biologically Interesting Compounds Containing an Alkylating Agent^{†,1}

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p-[*N,N*-bis(2-chloroethyl)amino]phenylacetic acid (BCAPAA) esters and amides of some new steroids and other biologically interesting compounds, two steroid esters of *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyric acid (BCAPBA), and one steroidal nitrosourea were synthesized and tested for antitumor and antileukemic activity.

Two of the best known groups of cancer chemotherapeutic agents are the steroid hormones and the nitrogen mustard class of alkylating agents.² The chemical combination of such compounds as a means of obtaining selective distribution at the tumor site and/or reducing the systemic toxicity of the attached alkylating agents was studied as early as 1952.³ However, until recent reports from a Russian group⁴⁻⁶ and from our laboratory,⁷ this class of compounds had been reported to display only moderate carcinostatic activities.^{3,8} We described the synthesis of a series of steroid esters of *p*-[*N,N*-bis(2-chloroethyl)amino]phenylacetic acid (BCAPAA) and reported that some of these compounds were excellent inhibitors of DMBA-induced mammary adenocarcinoma (13762).⁷ In addition, the steroid BCAPAA esters showed some interesting antileukemic results and appeared to be much less toxic than other commonly used oncolytic agents.⁷ Recently the BCAPAA ester of cholesterol (1a) has been tested clinically,⁹ and the diester of estradiol (2a) is being tested similarly. As a rational extension of this work we have now synthesized: (a) the BCAPAA ester or amide derivative of some new steroids; (b) two steroid esters of the highly active antineoplastic agent *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyric acid (BCAPBA, chlorambucil); (c) one steroidal

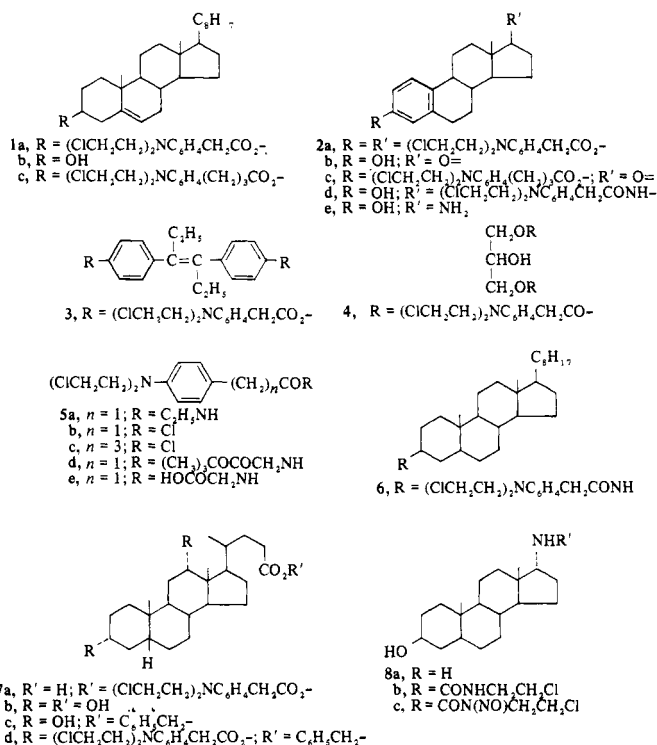
nitrosourea derivative; and (d) a selected group of BCAPAA esters and amides of nonsteroidal alcohols and amines. The latter were prepared in order to determine whether active, nontoxic compounds in this category were feasible. Biological test results of these compounds as well as new results on previously reported compounds⁷ are presented.

Chemistry. The BCAPAA esters 3 and 4 and the BCAPAA amides 5a and 6 were prepared by direct acylation of the appropriate hydroxy or amino compound with *p*-[*N,N*-bis(2-chloroethyl)amino]phenylacetyl chloride (5b). In a like manner the acylation of cholesterol (1b) and estrone (2b) with *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyryl chloride (5c) gave the BCAPBA ester 1c and 2c, respectively. It was not possible to prepare the bis-BCAPAA ester of 3 α ,12 α -dihydroxycholanolic acid (7a) in pure form by direct acylation. The steroid acid (7b) was first converted to its benzyl ester (7c) which was then smoothly acylated with 5b to give 7d. Reductive debenzoylation of 7d using palladium hydroxide on carbon catalyst gave the bis-BCAPAA free acid (7a). The coupling of BCAPAA with estra-1,3,5(10)-3-ol-17 β -ylamine (2e) and *tert*-butyl glycinate in the presence of DCC afforded the amides 2d and 5d, respectively. Treatment of 5d with refluxing CF_3CO_2H gave the glycnamide 5e. The structure assignments were based on the elemental analysis, the ir spectra, which showed typical ester or amide peaks, and the nmr spectra, which showed resonances at δ 3.61–3.67 and 6.10–7.40 ppm characteristic of the chloroethyl and aromatic protons of the

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p-[*N,N*-bis(2-chloroethyl)amino]phenylacyl moieties, respectively.

The reaction of chloroethyl isocyanate with 17 α -amino-3 β -hydroxyandrostane (8a) gave the unsymmetrical urea (8b). Treatment of 8b with HNO₂ afforded the nitroso urea 8c. The higher wave number carbonyl absorption of 8c (1730 cm⁻¹) relative to 8b (1708 cm⁻¹) indicated the completeness of the nitrosation.¹⁰ The nmr spectrum of 8b showed an A₂B₂X pattern for the ClCH₂CH₂NH group, whereas 8c showed an A₂B₂ pattern for the ClCH₂CH₂N(NO) group, thus establishing the point of attachment of the nitroso moiety.¹⁰



Biological Data. The new compounds prepared and reported herein, as well as some of those reported in our previous paper,⁷ have been tested in the 13762 DMBA-induced and transplantable mammary adenocarcinoma (normal female line) (Table I), and a variety of leukemias (Table II), all of which originated in and are maintained in the inbred Fischer/344 rat.^{‡,§}

Steroidal Compounds. The BCAPAA ester of pregnenolone and epiandrosterone exhibited antitumor activity, *i.e.*, inhibition of tumor growth for the 13762 tumor system and increased survival time of the host for the leukemias, similar to other steroid BCAPAA monoesters.⁷ In contrast to the excellent inhibition of tumor growth shown by the bis-BCAPAA derivative of diethylstilbestrol (Table I) and estradiol⁷ in the mammary system, the bis-BCAPAA derivative of 3 α ,12 α -dihydroxycholesterol showed only minimal tumor-growth inhibition and, then, only at a dose level of 20 mg/kg per day given orally. The BCAPAA derivative of estradiol-1,3,5(10)-3-ol-17 β -ylamine which has the oncolytic agent connected through an amide linkage failed to inhibit growth of the mammary tumor when administered orally

Table I. Summary of Antitumor Results Obtained Using 13762 Mammary Tumor of Fisher/344 Rats

R	Position of attachment	X	Activity, ^a dose, mg/kg per day
RXCOCH ₂ C ₆ H ₄ N(CH ₂ CH ₂ Cl) ₂			
Pregnenolone	3 β	O	+++ , 20-40 po +++ , 40 sc
Epiandrosterone	3 β	O	+++ , 10-20 po ++ , 5 po +++ , 5-20 sc +++ , 10 ip
3 α ,12 α -Dihydroxycholesterol	3 α ,12 α	O	+ , 20 po - , 20 sc
Diethylstilbestrol	4,4'	O	+++ , 1.25-10 po +++ , 2.5-10 sc ++ , 1.25 sc +++ , 5.0 ip
Estra-1,3,5(10)-3-ol-17 β -ylamine	17 β	N	- , 1-10 po
H		O	+++ , 2.15-4.29 po +++ , 2.15-4.29 sc
Na ⁺		O ⁻	+++ , 1-4.3 po
C ₂ H ₅		O	+++ , 2.36-6 po +++ , 3.0 sc +++ , 3-4.72 ip
C ₂ H ₅		NH	+++ , 3-12 po +++ , 1.5-6 sc
HOCOCH ₂		NH	+++ , 1.5-6 po +++ , 1.5-6 sc
HOCH(CH ₂) ₂	1,3	O	+++ , 2.5-10 po +++ , 2.5-10 sc
RXCO(CH ₂) ₃ C ₆ H ₄ N(CH ₂ CH ₂ Cl) ₂			
Cholesterol	3 β	O	+++ , 1-10 po
Estrone	3	O	+++ , 0.625-10 po
R-NHCON(NO)CH ₂ CH ₂ Cl			
17 α -Amino-3 β -hydroxyandrostane	17 α		- , 0.1-10 po +++ , 40 sc + , 20 sc ± , 10 sc
RXPO[N(CH ₂) ₂] ₂			
2 α -Methyldihydrotestosterone	17 β	O	- , 5-20 po +++ , 10-20 sc +++ , 20 ip

^aActivity is based on inhibition of tumor growth: - = <35% effective; ± = >35-50%; + = >50-65%; ++ = >65-80%; +++ = >80-100% and over.

at doses of 1-10 mg/kg per day. On the other hand, the BCAPBA esters of cholesterol and estrone exhibited excellent inhibition of tumor growth of the same system. The BCAPBA ester of cholesterol appeared to be more active than its BCAPAA derivative (phenesterin) in the experiments completed. These data show that the activity of the steroidal derivatives is not reserved for the BCAPAA derivative. Unfortunately the physical properties of the BCAPBA derivatives are undesirable; the compounds are oils. The steroid 3-(2-chloroethyl)-3-nitrosoureido derivative 8c, which has the (2-chloroethyl)nitrosoureido moiety connected directly to the steroid nucleus, and the diaziridinyl phosphate from 2 α -methyldihydrotestosterone both showed moderate antitumor activity. The activity of 8c was particularly interesting because in general the steroidal oncolytic agent-steroid link is readily cleavable by hydrolysis or some other *in vivo* process; 8c probably undergoes an *in vivo* decomposition to an active alkylating agent.¹¹

The antileukemic results obtained with three of the steroid BCAPAA derivatives are listed in Table II. All three compounds were highly effective against a variety of rat leukemia systems. It is of particular interest that the BCAPAA ester of testosterone "cured" all the animals inoculated

‡The compounds were tested through the contract programs of the Endocrine Evaluation Branch of the National Cancer Institute. Data reported were obtained in the contract laboratories of Dr. W. F. Dunning, University of Miami, Coral Gables, Fla., and Dr. Arthur E. Bogden, Mason Research Institute, Worcester, Mass.

§The compounds were tested for antitumor and antileukemic activity according to procedures published previously (ref 7).

Table II. Summary of Activity against Various Leukemias of the Inbred Fisher/344 Rats

R	Position of attachment	Leukemic system ^a	Dose, mg/kg per day (no. of doses) ^b	No. of rats T/C ^c	Mean tumor diameter, cm T/C	%	Mean survival, days, T/C (cures) ^d	%	Wt change, g, T/C
RO₂CCH₂C₆H₄N(CH₂CH₂Cl)₂									
Pregnenolone	3β	R3149	10 (10)	10/10	3.2/0.2	6	26.5/17.0	155	9/7
		R3323	10 (10)	10/9			12.0/9.0	121	-18/-10
		R3330	10 (20)	9/9			54.0/16.8 (8)	321	-11/-5
Epiandrosterone	3β	R3323	10 (9)	10/9			19.7/9.6	205	-2/-2
		R3330	10 (20)	10/9			75/16.8 (8)	446	-6/-5
		R3399	10 (14)	10/10	0.0/0.5	0	23.0/10.3 (1)	223	-7/-4
		IRC-741	10 (14)	10/10	0.0/2.6	0	36.0/17.4 (8)	206	-27/-12
Testosterone	17β	R3149	10 (12)	8/8	1.36/1.38	99	19.2/9.5	202	42/17
		R3323	5 (8)	10/9			14.1/9.4	150	3/-4
		R3330	5 (20)	10/9			39/17.7 (9)	220	-3/-10
		R3399	5 (13)	10/9	0.0/2.7	0	27.4/12.8 (1)	214	12/23
		IRC-741	5 (14)	10/10	1.3/1.8	72	63.0/19.1 (2)	330	8/11
		R3432	5 (20)	10/10			e/44.4 (10)	e	13/4
RCOCH₂C₆H₄N(CH₂CH₂Cl)₂									
C ₂ H ₅ O		R3149	5 (5)	10/9	0.0/2.7	0	24.1/11.1 (3)	217	-1/0
		R3323	5 (6)	10/9			52.3/9.9 (7)	528	-19/2
		R3330	10 (6)	9/9			61.6/17.8 (4)	346	-27/10
		R3399	5 (6)	9/10	0.0/1.9	0	35.3/13.1 (5)	269	-4/9
		IRC-741	10 (5)	6/10	0.0/3.1	0	37.3/14.1 (3)	265	-24/10
		R3432	10 (8)	5/10	0.0/3.9	0	65.3/31.8 (2)	205	2/13
C ₂ H ₅ NH		R3149	3 (15)	10/10	0.0/3.4	0	33.3/16.9 (3)	197	-5/9
		R3323	3 (10)	10/9			19.9/10.1	197	-4/-2
		R3330	3 (20)	10/9			e/19.3 (10)	e	-20/-2
			1.5 (20)	10/9			34.2/19.3 (5)	177	-18/-2
		R3399	3 (10)	7/9			27.5/11.0 (3)	250	-1/2
		IRC-741	3 (14)	10/10	0.0/2.7	0	57.8/18.3 (6)	316	-3/-10
		R3432	1.5 (20)	9/10	0.0/2.8	0	e/28.1 (9)	e	0/21
H		R3330	4 (12)	8/9			62.5/19.8 (6)	315	-32/-12
HOCOCH ₂ NH		R3149	3 (14)	8/10			34.8/17.0 (5)	204	-11/-2
		R3330	3 (18)	10/9			e/22.8 (10)	e	-13/-6
		R3323	6 (10)	8/10			26.0/9.6 (5)	270	-35/-22
		R3399	3 (10)	10/9			30/10.2	294	-26/-8
		IRC-741	6 (14)	7/10	0.0/1.1	0	58.7/18.7 (7)	313	-9/1
EtCC ₆ H ₄ O-	4,4'	R3149	5 (14)	10/10	2.4/3.0	80	21.8/17.0	128	-8/1
		R3323	5 (9)	10/9			11.5/9.6	120	-3/-2
EtCC ₆ H ₄ O-		R3330	5 (16)	10/9			19.7/18.3	108	-24/-5
		R3399	5 (14)	10/9	2.1/1.7	123	19.2/24.4	78	-2/9
		IRC-741	5 (14)	10/10	1.9/2.3	80	21.4/19.7	109	-19/-4
		R3432	5 (20)	9/10	0.0/3.2	0	47.3/31.3 (6)	151	-25/6

^aLeukemias: R3149, acute monocytic; R3323, acute monocytic (no solid tumor); R3330, subacute monocytic (no solid tumor); R3399, chronic; R3432, chronic lymphocytic; IRC-741, acute monocytic. ^bThe compounds were administered *via* the subcutaneous route. ^cT/C = treated controls. ^dAnimals that survived over 90 days before reinoculation were classed as cures. ^eAll the treated animals survived over 90 days.

Table III. Esters and/or Amides of *p*-[*N,N*-Bis(2-chloroethyl)amino]phenylacetic Acid and *p*-[*N,N*-Bis(2-chloroethyl)amino]phenylbutyric Acid

RXCO(CH ₂) _n C ₆ H ₄ N(CH ₂ CH ₂ Cl) ₂									
Compound	R	Position	X	n	Method ^a	Recrystn solvent	Yield, % ^b	Mp, °C	Formula ^c
3	Diethylstilbestrol	4,4'	O	1	A	CHCl ₃ -CH ₃ OH	36	154-158	C ₄₂ H ₄₆ Cl ₄ N ₂ O ₄
4	HOCH(CH ₂) ₂	1,3	O	1	A		25	<i>d</i>	C ₂₇ H ₃₄ Cl ₄ N ₂ O ₅
5a	C ₂ H ₅		N	1	A	CH ₂ Cl ₂ -hexane	75	113-114	C ₁₄ H ₂₀ Cl ₂ N ₂ O
5d	(CH ₃) ₃ COCOCH ₂		N	1	B	THF-hexane	96	125-125.5	C ₁₈ H ₂₆ Cl ₂ N ₂ O ₃
6	3β-Aminocholestane	3β	N	1	A	CH ₂ Cl ₂ -hexane	42	132-134	C ₃₉ H ₆₂ Cl ₂ N ₂ O
7d	3α,12α-Dihydroxycholanolic acid benzyl ester	3α,12α	O	1	A		62	<i>d</i>	C ₅₆ H ₇₂ Cl ₄ N ₂ O ₆
2d	Estra-1,3,5(10)-3-ol-17β-ylamine	17β	N	1	B	C ₆ H ₆	71	110-111	C ₃₀ H ₃₈ Cl ₂ N ₂ O ₂
1c	Cholesterol	3β	O	3	C		63	<i>d</i>	C ₄₁ H ₆₃ Cl ₂ NO
2c	Estrone	3	O	3	C		68	<i>d</i>	C ₃₂ H ₃₉ Cl ₂ NO ₃

^aSee Experimental Section. ^bBased on analytically pure material. ^cAnalyzed for C, H, Cl, N (see footnote **). ^dThe product was non-crystalline.

with the R3432 chronic lymphocytic leukemia at a dose level where no toxic deaths were observed.

Nonsteroidal BCAPAA Esters and Amides. Since the original pioneer Russian studies⁴⁻⁶ and our initial studies⁷ were all carried out with steroid esters or amides of BCAPAA, the role of the steroid in affecting the 13762 mammary

adenocarcinoma as well as the rat leukemia systems was of interest. In order to shed some light on this problem, we have prepared and tested some nonsteroidal BCAPAA esters and amides. The free BCAPAA, its sodium salt, ethyl ester, bisglycerol ester, ethylamide, and glycine-amide^{8,12} all have shown antitumor activity comparable

to or greater than that of the steroidal derivatives in initial testing in the DMBA-induced and transplantable mammary adenocarcinoma (Table I) and in a number of rat leukemias (Table II). However, there is a wide difference in toxicity on prolonged administration, some of the nonsteroidal compounds being much more toxic than phenesterin.⁷ For example, phenesterin has been given orally at 10 mg/kg per day for 21 days in a number of the routine testing experiments with the 13762 tumor system without host deaths, while most of the nonsteroidal esters and amides have caused two to six deaths in groups of ten animals when given orally at 4.30 mg/kg per day (unpublished data). The lower dose is equivalent to the amount of alkylating agent in the 10-mg dose of phenesterin. A dosage of 2.15 mg/kg per day of these agents was less toxic; however, one or two deaths per group have not been infrequent. The bis(glycerol) ester appeared to be the least toxic of the BCAPAA nonsteroidal esters and the ethylamide appeared to be less toxic than the glycinamide. Only the ethyl ester of BCAPAA has been evaluated in toxicological studies in dogs by the Chemotherapy Program of the National Cancer Institute; it was found to be too toxic for human administration. It has been mentioned above that both phenesterin and the estradiol alkylating agent are being tried in human breast cancer.

Experimental Section**

***p*-[*N,N*-Bis(2-chloroethyl)amino]phenylacetic Acid Esters and Amides. Method A (Table III).** Stilbestrol, glycerol, C₂H₅NH₂, 3 β -amincholestan-6, 13 and 2 α , 12 α -dihydroxycholelanic acid benzyl ester (7c)^{††} were acylated with *p*-[*N,N*-bis(2-chloroethyl)amino]phenylacetyl chloride in a manner similar to that previously reported for the prep of other BCAPAA esters⁷ to give compounds 3, 4, 5a, 6, and 7d, respectively.^{‡‡}

Method B (Table III). A mixt of 0.5 mmole of estra-1,3,5(10)-triene 3-ol-17 β -ylamine (2e)¹⁴ or *tert*-butyl glycinate, 0.108 g (0.5 mmole) of DCC, and 0.134 g (0.5 mmole) of BCAPAA in 4 ml of CH₂Cl₂ or EtOAc was stirred at 25° for 5 hr. A few drops of AcOH were added, the mixt was filtered, and the solids obtained were washed with CH₂Cl₂ or EtOAc. The filtrate and washings were concd *in vacuo* on a rotary evaporator, and the remaining solid was crystd from the appropriate solvent.

***p*-[*N,N*-Bis(2-chloroethyl)amino]phenylbutyric Acid Esters (Method C, Table III).** A mixt of 20 mmoles of cholesterol or estrone and 7.2 g (20 mmoles) of *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyl chloride hydrochloride (5c)^{§§} in 500 ml of C₆H₆ was refluxed for 72 hr. The soln was concd on a rotary evaporator. The remaining residue from the cholesterol run was chromatographed on alumina using C₆H₆-hexane (3:1) as the eluent to give 1c. The remaining residue from the estrone run was chromatographed on Florisil using C₆H₆ as the eluent to give 2c.

#K. Karpavicius, *et al.*,¹² report that BCAPAA amide of glutamic acid has strong antitumor activity.

**Melting points were determined on a Kofler hot stage microscope using a calibrated thermometer. Uv spectra were measured on a Cary Model 14 spectrophotometer. Nmr spectra were recorded on a Varian Model HA-100 spectrometer (TMS). Ir spectra were measured with a Perkin-Elmer 221 spectrophotometer. Mass spectra were determined on an AEI-MS-902 spectrometer. Microanalyses were carried out by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within $\pm 0.4\%$ of the theoretical values.

††The benzyl ester of 3 α , 12 α -dihydroxycholelanic acid was prepared in 66% yield by refluxing a solution of 3 α , 12 α -dihydroxycholelanic acid and benzyl alcohol in benzene containing a catalytic amount of *p*-toluenesulfonic acid. The water formed was collected in a Dean-Stark trap.

‡‡An equivalent of anhydrous K₂CO₃ was used in the preparation of 4 and 6.

§§The acid chloride 5c was prepared in a manner analogous to the preparation of *p*-[*N,N*-bis(2-chloroethyl)amino]phenylacetyl chloride hydrochloride.⁷

***N*-{[*p*-*N,N*-Bis(2-chloroethyl)amino]phenylacetyl} glycine (5e).** A soln of 0.311 g (0.98 mmole) of 5d in 4 ml of CF₃CO₂H was kept at 25° for 0.5 hr. The solvent was removed *in vacuo* to give a brown solid. Recrystn from an EtOAc and Et₂O mixt gave 0.287 g (88%) of 5e: mp 132–136°. The analytical sample prepd by recrystd from a THF and Et₂O mixt had mp 134–137°. *Anal.* (C₁₄H₁₈Cl₂N₂O₃) C, H, Cl, N.

3 α , 12 α -Dihydroxycholelanic Acid Di-*p*-[*N,N*-bis(2-chloroethyl)amino]phenylacetate (7a). A soln of 10.7 g (10.6 mmoles) of 7d in 200 ml of AcOH contg 1.0 g of palladium hydroxide on powdered charcoal¹⁵ was hydrogenated at atmospheric pressure for 16 hr. The catalyst was removed by filtration, and the filtrate concd by freeze-drying to give 8.0 g (82%) of 7a as a powder: ν_{\max} (CH₂Cl₂) 3500–3000 cm⁻¹ (broad acid OH). *Anal.* (C₂₉H₄₆Cl₄N₂O₆) C, H, Cl, N.

1-(2-Chloroethyl)-3-(3 β -hydroxy-5 α -androstane-17 α -yl)urea (8b). To a stirred, cooled (–10°) soln of 2.0 g (0.65 mmole) of 17 α -amino-3 β -hydroxy-5 α -androstane (8a)¹⁶ in 10 ml of Et₂O was added dropwise 0.7 g (0.69 mmole) of 2-chloroethyl isocyanate.¹⁷ After the addn, the reaction mixt was stirred for 1.5 hr at –10°. The solid that sep'd was filtered, washed with Et₂O, dried, and recrystd from Me₂CO to give 1.64 g (64%) of 8b: mp 184–188°. The analytical sample prep'd by recrystn from Me₂CO had mp 186–188°; ν_{\max}^{KBr} 3340 (NH), 1708 (C=O) and 1650 cm⁻¹ (CNH). *Anal.* (C₂₂H₃₇ClN₂O₂) C, H, Cl, N.

1-(2'-Chloroethyl)-3-(3 β -hydroxy-5 α -androstane-17 α -yl)-1-nitrosourea (8c). To a cooled (0–5°), stirred soln of 0.97 g (2.45 mmoles) of 8b in 60 ml of HCO₂H was added in small portions 3 g of NaNO₂. After 0.5 hr, 60 ml of H₂O was added, and the mixt was stirred at 0–5° for 0.5 hr. The ppt that formed was washed with cold H₂O and dried *in vacuo* (P₂O₅) to give 0.89 g (88%) of 8c. The analytical sample prep'd by recrystn from an C₂H₅OH and H₂O mixt had mp 126–128°; ν_{\max}^{KBr} 1730 (C=O) and 1530 cm⁻¹ (HNC); nmr (CDCl₃ showed) a singlet at δ 0.82 and 0.87 (18- and 19-CH₃), a doublet at 3.52 [-N(NO)-CH₂-] and a doublet at 4.20 ppm (-CH₂Cl). *Anal.* (C₂₂H₃₆ClN₂O₃) C, H, Cl, N.

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