On the Formation of Homo-aza-steroids and Derivatives by Beckmann Rearrangement. Antitumor Activity of Stereoisomers Homo-aza-steroidal Esters

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The Beckmann rearrangement of steroidal oximes is reported. Different methods of esterification of homo-aza-steroids with carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline are reported.

The lipophilic nature of the steroidal hormones is the reason for searching compounds containing as the biological platform the steroid molecule and as the active moiety the alkylating agent, such as nitrogen mustards linked to the steroid with a stable bond or with an easily cleaved ester or ether molecule and different other active species, hopefully to transport and deliver the alkylating agent to the specific target tissue. The concept to design hybrid compounds containing as the biological platform steroidal lactams and as the alkylating congener carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline might form compounds with synergistic activity.

The findings suggest that the conformation of the alkylating agent influences the anticancer activity, while the amide group of the lactam molecule is important for activity in L1210 leukemia.

These hybrid compounds with a modified steroid as the biological platform furnish derivatives with advantages compared to the unmodified steroids with alkylating agents.

Different methods of esterification of homo-aza-steroids with carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline are reported.

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1. Introduction.

Studies have been made recently on the synthetic methods of steroidal bislactam or acetamido-homo-aza-steroids containing the -NHCO- group in the cyclopentanoperhydrophenanthrene skeleton or in the molecular side chain for anticancer testing.

The biological action of steroidal lactams characterized by the -CONH- group may be structurally specific.

The drugs for a particular biological action depends on their chemical reactivity and their spatial arrangement.

The concept of designing and developing steroidal alkylating agents for cancer chemotherapy is due to the lipophilic nature of the cyclopentanoperhydrophenanthrene molecule in the transport and deliver of the congener to the specific target tissue. On the other hand some steroids have the advantage to be active in cancer treatment. Androgens are used to treat patients with advanced breast cancer. Corticosteroids are used in acute lymphocytic leukemia and in combination with oncolytic agents to treat acute myelogenous leukemia, chronic lymphocytic leukemia, multiple myeloma and lymphoma. Progestins are used in advanced progressing endometrial cancer; also, with renal cancer remission with progestin therapy.

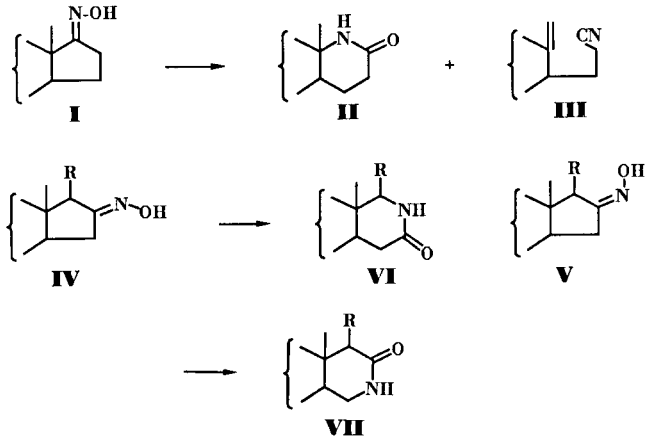
They are occasionally used in advanced breast cancer. Estrogens are used in the palliative treatment of carcinoma of the prostate and in the treatment of postmenopausal patients with advanced breast cancer, while estramustine phosphate (Estracyt) is used as a therapeutic agent in the treatment of prostatic carcinoma.

The concept of the rational approach of hybrid compounds, alkylating agents and steroids such as androgens, corticosteroids, progestins, *etc* utilized in order to lower toxicity and improve specificity of the alkylating agent in cancer treatment.

The resulting hybrid compounds can be divided into two groups, depending on the connecting bond between the active congener and the steroid molecule. A. Alkylating agents linked to the steroid with a stable bond; B. Alkylating agents linked to the steroid with an easily cleaved ester bond.

2. Homo-aza-steroids.

A. D-ring of Homo-aza-steroids.



Beckmann rearrangement of 17-oximinosteroids **I** to the 17α-D-homolactams has been investigated by several

workers [1-4]. This rearrangement beside the lactam **II**, produces the "second order" Beckmann cleavage product **III**.

The rearrangement of 3β-acetoxy-5α-androstan-17-oxime acetate in purified dioxane with etherate solution of boron fluoride gave lactam and the "second order" cleavage product [5]. Similar results have been obtained by other workers, using thionyl chloride as catalyst [6]. Reaction of estrone oxime with dimethyl sulfoxide and dicyclohexylcarbodiimide in the presence of trifluoroacetic acid leads to the formation of lactam and exocyclic nitrile in almost equal amount [7]. Similar results with 6% nitrile were obtained from androsterone oxime upon rearrangement with 4-acetamidobenzenesulfonyl chloride in pyridine [8].

Rearrangement of 16-Oxime Isomers.

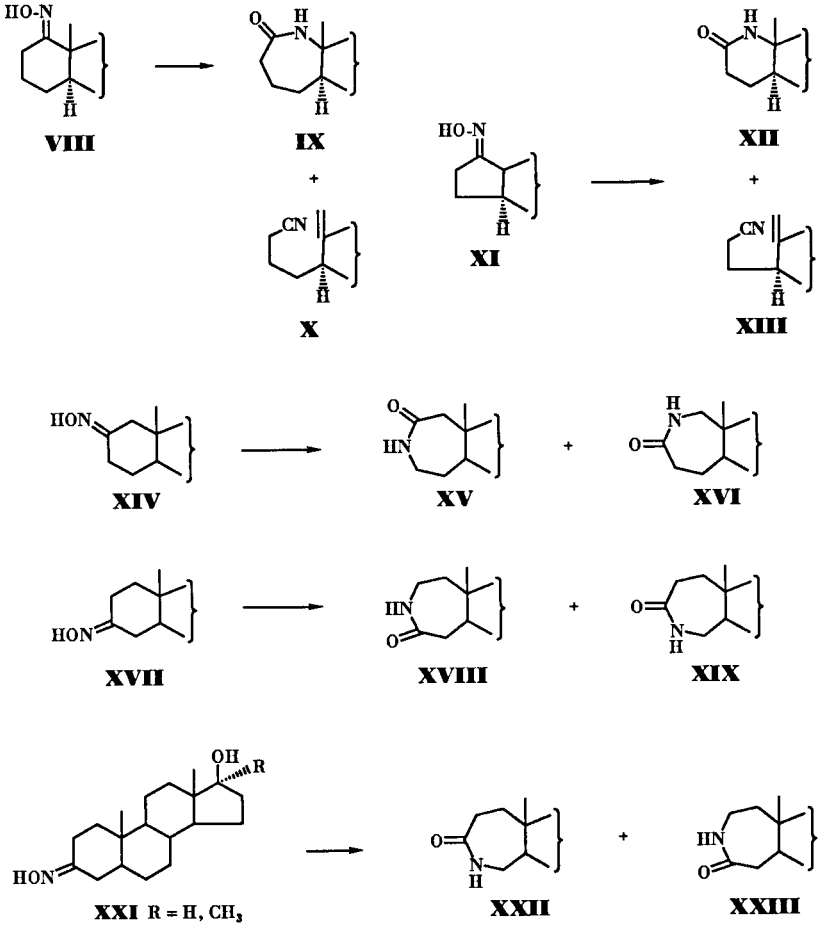
The Beckmann of rearrangement 16-oxime isomers **IV** and **V** give the corresponding lactam isomers **VI** and **VII** [9].

B. A-ring of Homo-aza-steroids.

In the continuing search for modified steroids with hormonal and anti- hormonal activity Shoppee and co-workers [10] in the aza-steroid preparations have examined Beckmann rearrangement of 5α-cholestan-1-one oxime (**VIII**) on treatment with thionyl chloride and they obtained approximately equal amounts of the normal rearrangement product 1-aza-A-homo-5α-cholestan-2-one (**IX**) and the abnormal "second order" cleavage product **X**. The same authors have reported that A-nor-5α-cholestan-1-one oxime (**XI**) on treatment with thionyl chloride furnished 1-aza-5α-cholestan-2-one (**XII**) accompanied by the 1-cyano-1,10-seco-compound **XIII**.

Under the same reaction conditions unresolved 5α-cholestan-2-one oximes (**XIV**) produced the 3-aza-A-homo-5α-cholestan-2-one (**XV**) and 2-aza-A-homo-5α-cholestan-3-one (**XVI**) which were separated by chromatography [10]. Unresolved 5α-cholestan-3-one oximes and 5β-cholestan-3-one oximes produce the 3-aza-A-homo- and 4-aza-A-homo-steroids respectively, which were separated by chromatography. Similar results have been obtained with androstan-3-one oximes [11-12]. Geometrical isomers of 17β-hydroxy-A-nor-5α-androstan-2-one oximes under Beckmann rearrangement produced lactam isomers **XXVI** and **XXVII** respectively [12].

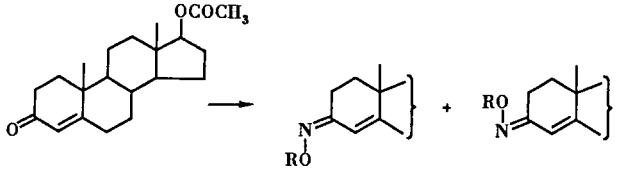
Beckmann rearrangement of α,β-unsaturated ketoximes, such as testosterone ketoximes **XXVIII** and **XXIX**



XXIV **XXVI**

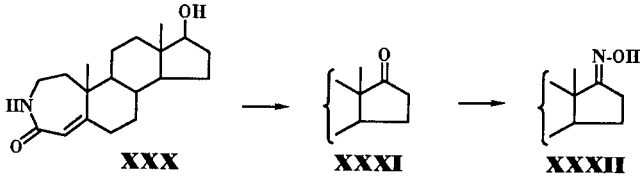
XXV **XVII**

produces the lactam from the syn-isomers [13-14].

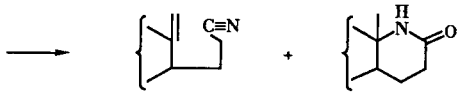


XXVIII, R = H, R = $COCH_3$ **XXIX**, R = H, R = $COCH_3$

C. Synthesis of Diaza-A,D-bis-homo-steroids.



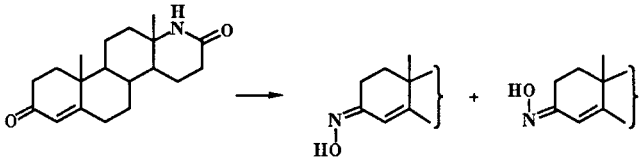
XXX **XXXI** **XXXII**



XXXIII **XXXIV**

The 3-aza-A-homo-4α-androsten-4,17-dione oxime **XXXII** was converted by Beckmann rearrangement to the diaza compound **XXXIV** and the "second order" Beckmann cleavage ω-cyano-olefin **XXXIII** [15].

An alternative method for the preparation of dilactam **XXXIV** was affected from syn-oxime **XXXVI**.

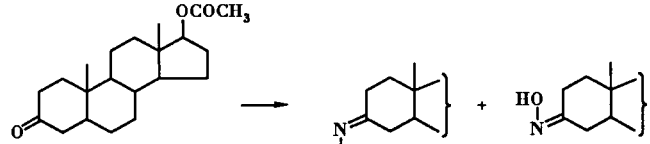


XXXV **XXXVI** **XXXVII**

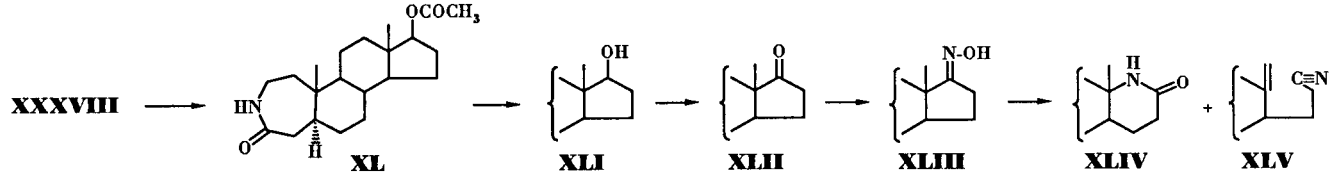
The formation of 3,17α-diaza-A,D-bishomo-5α-androstan-4,17-dione **(XLIV)** and 4,17α-diaza-A,D-bishomo-5α-androstan-3,17-dione **(L)** was affected by Beckmann rearrangement of the corresponding oximes **XLIII** and **XLIX** respectively. Besides the diaza compounds and the "second order" cleavage products were obtained **XLV** and **LI** [16].

D. Synthesis of 17β-Acetamido-3-aza-A-homo-4α-androsten-4-one and 20-Acetamido-3-aza-A-homo-4α-pregnen-4-one.

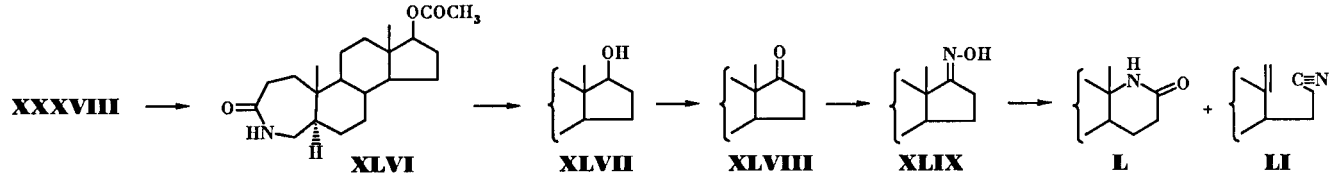
Beckmann rearrangement of pregnenolone oxime formed the 17-acetamido-compound **LII** [17]. The latter hydrolysis and Oppenauer oxidation produced the ketone **LIV**.



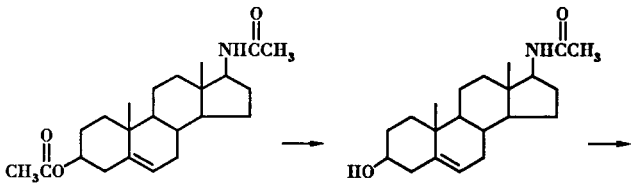
XXXVIII **XXXIX**



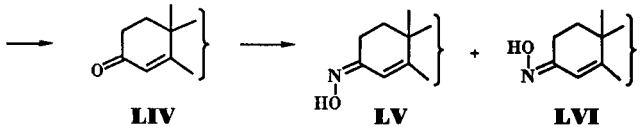
XXXVIII **XL** **XLI** **XLII** **XLIII** **XLIV** **XLV**



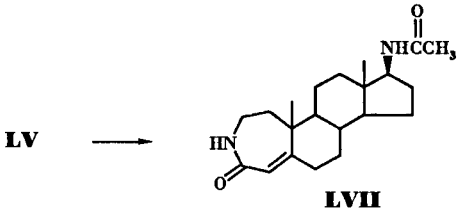
XXXVIII **XLVI** **XLVII** **XLVIII** **XLIX** **L** **LI**



LII LIII



LIV LV LVI

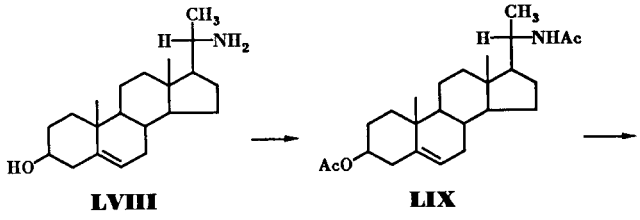


LV LVII

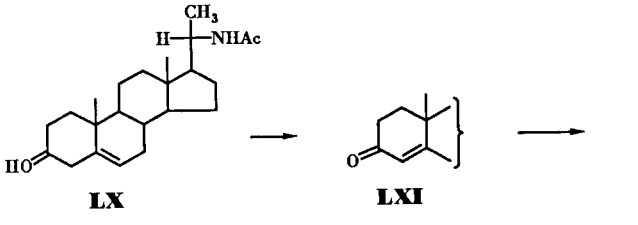
Treatment of compound **LIV** with hydroxylamine hydrochloride in a mixture of pyridine-ethanol produced the corresponding ketoximes.

Beckmann rearrangement of unresolved 17β-acetamido-4-androsten-3-one oximes **LV** and **LVI** with thionyl chloride in dioxane produces lactam **LVII**.

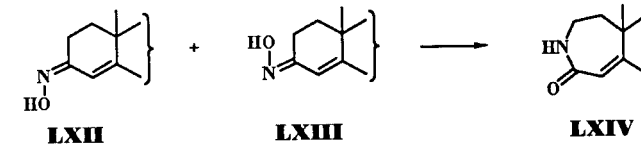
Under similar reaction conditions the 4-pregnen-3-one dioxime produces 17β-acetamido-lactam [18].



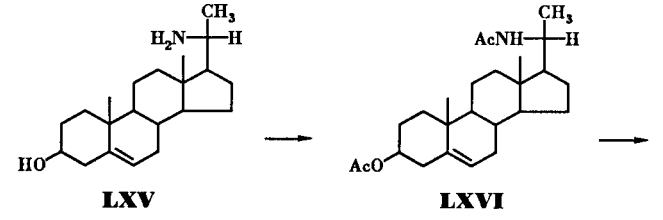
LVIII LIX



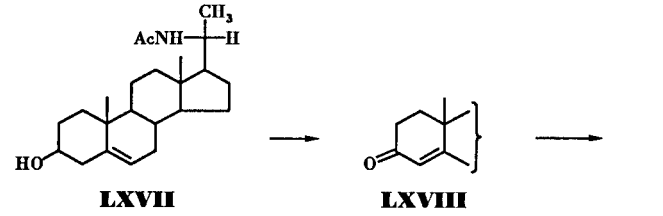
LX LXI



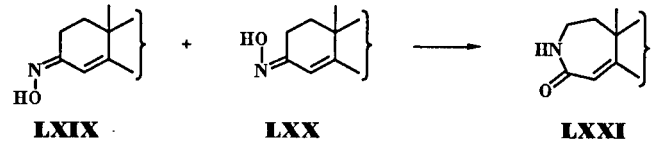
LXII LXIII LXIV



LXV LXVI



LXVII LXVIII



LXIX LXX LXXI

The main methods for synthesizing compounds containing the acetamido groups at C_{20} consists of the reduction of 3β-hydroxy-5-pregnen-20-one oximes followed the separation of the produced epimers and acetylation in the usual manner [19].

Partial hydrolysis then produced 3β-hydroxy-20α-acetamido-5-pregnene (**LX**) and 3β-hydroxy-20β-acetamido-5-pregnene **LXVII**. Oppenauer oxidation produced 20α- and 20β-acetamido-4-pregnen-3-one respectively.

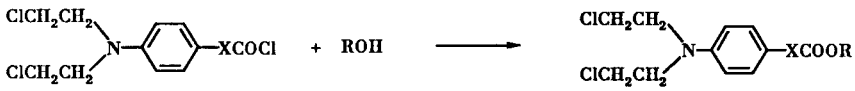
Treatment of the ketones with hydroxylamine hydrochloride in pyridine-ethanol produced a mixture of ketoximes. Beckmann rearrangement of unresolved 20α-acetamido-4-pregnen-3-one oximes **LXII** and **LXXIII** gave the 20α-acetamido-3-aza-A-homo-4α-pregnen-4-one (**LXIV**) and 20β-acetamido-3-aza-A-homo-4α-pregnen-4-one (**LXXI**) from oximes **LXIX** and **LXX**. The general trend is that syn-oximes of α,β-unsaturated ketoximes undergo the rearrangement to lactam [20].

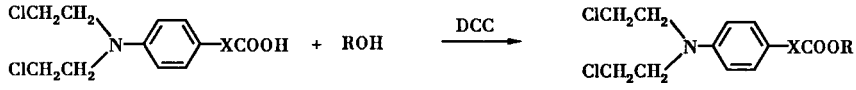
3. Synthesis of Steroidal Esters.

Method A.

The preparation of acid chlorides such as *p-N,N*-bis(2-chloroethyl)aminophenylacetic acid, *p-N,N*-bis(2-chloroethyl)aminophenylbutyric acid, *p-N,N*-bis(2-chloroethyl)-aminothiophenylacetic acid have been prepared by the action of fresh distilled thionyl chloride in a solution of acid in anhydrous benzene [21-23].

The mustard acid chloride was condensed with unmodified or modified steroid alcohols by reflux in anhydrous benzene.



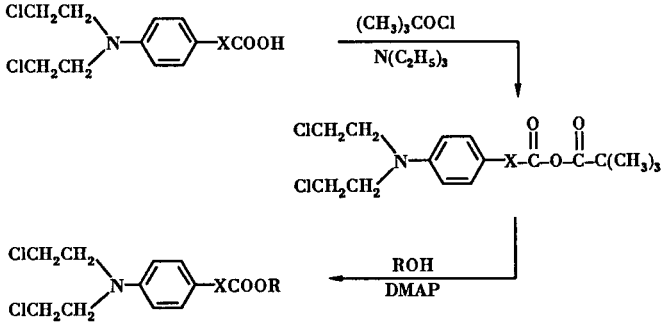


Method B.

Several difficulties during the condensation of carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline with modified steroids prompted to study this condensation reaction in dichloromethane or in pyridine in the presence or not *p*-dimethylaminopyridine with dehydrating agent dicyclohexylcarbodiimide. When dichloromethane was used as solvent, esters were obtained in satisfactory yields, only in the presence of catalyst [24].

Method C.

An improved method for the esterification of carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline with steroidal lactams, involves a mixed anhydrides of the carboxylic acid with trimethylacetyl chloride. The mixed anhydride is treated with steroidal alcohol in the presence of dimethylaminopyridine in toluene [25].



4. Alkylating Agents Linked to the Steroid with an Easily Cleaved Bond.

The elegant work from the Triangle Research Institute [21-22] on the effects of steroidal alkylating agents on experimental animal tumor and leukemia systems, summarized that in general no activity was found in the systems studied with the compounds in which the oncolytic agent and the steroid were connected by a stable bond, which could not be easily cleaved by hydrolytic and/or enzymatic action. The active compounds linked the oncolytic agent to the steroid by a more easily cleaved ester or heterocyclic ether linkage. If the alkylating agent of steroidal ester occupies an axial or equatorial position of the steroid nucleus, the equatorial substituent being the energetically preferred one. Presume is due to the membrane cells penetration and the rate of hydrolysis.

The concept to design, and synthesizing biological platforms aza-steroids, lactams in A- and D-ring of the steroid molecule, is due to their biological action, which characterized by the amide group, may be structural specific and therefore more prolonged as the result of the multiple interactions of such a group with similar groups that exist in proteins and nucleic acids [26-27]. The -NHCO- group of the lactam molecule may be essential for antitumor activity could open enzymatically forming species which could attack the contains of cancer cells [28].

The hybrid compounds have shown better results in experimental animal tumors [29-30] and P388 and L1210 leukemias [31-32] than the corresponding unmodified steroids.

The Table I summarizes the antineoplastic activity of ASE, NSC 290205 [33].

Table I

Anti-neoplastic Activity of ASE, NSC 290205

Tumor system	Best T/C or tumor growth inhibition	Parameter [a]	Assessment [b]	Source of data
L1210 leukemia	182%	ST	+	[31]
P388	286%	ST	+	[31]
Theagenion-Bahner Angiosarcoma	8/8 cures	ST	+	[29]
T8 Guerin tumor	3/3 cures	ST	+	[29]
B16 Melanoma	154%	ST	+	NCI [30]
Lewis lung	131%	ST	-	NCI [30]
$CD8F_1$ Mammary tumor	100%	TI	+	NCI [30]
Colon 26	213%	ST	+	NCI [30]
MXT Mammary tumor	307%	ST	+?	NCI [33]
Colon 38	73%	TI	+	NCI [33]
CX-1 Colon xenograft	+38	*	-?	NCI [33]
LX-1 Lung xenograft	+8	*	+	NCI [33]
MX-1 Breastxenograft	-100	*	+	NCI [33]

[a] ST survival time; TI tumor inhibition: defined in text. [b] ? Denotes inadequate testing.

In a single test, ASE prolonged the median survival of MXT tumor-bearing mice to 87.5 days (controls 28.5 days) when given at 28 mg/kg every 4 days for 3 doses. At this dose there were no toxic deaths recorded. This was the lowest test dose. The next higher dose, 42 mg/kg, resulted in 1/10 toxic deaths with a median survival of 55.5 days.

Tumor growth inhibition of the colon 38 tumor in parallel experiments was 73% and 67% at a dose of 24 mg/kg given twice, 7 days apart. At this dose there were no toxic deaths and little effect on body weight.

MX-1 breast xenograft activity was seen at doses from 25-400 mg/kg when given every 4 days for a total of 3 injections (Q4DX3). Doses of 200 and 400 mg/kg were toxic as reflected in weight differences in animals of -5.3 to -7.4 g compared with controls and 17 of 36 toxic deaths. At 100 mg/kg Q4DX3 or less there was progressively less weight loss and only 3/42 toxic deaths. The T/C values at 100 mg/kg Q4DX3 were -100, -13 and -96 in 3 experiments, respectively, and at 50 mg/kg Q4DX3 -98, -18 and -85 in the same 3 experiments.

The LX-1 lung xenograft was treated in 3 experiments at doses of 25, 50, 100 and 200 mg/kg Q4DX3 with T/C value of 8 and 9, respectively, in the second and third experiments at the highest dose. There was a single toxic death in these 2 groups (12 animals) and weight differences compared to controls of -5.6 and -6.2 g, respectively. All other groups had T/C values of 30.

In a single experiment no activity was shown against the CX-1 colon xenograft. The highest dose used was 100 mg/kg Q4DX4 and no toxicity deaths were seen in any group. Doses of 50 and 100 mg/kg Q4DX4 produced weight changes of -6.0 and 6.0 and -6.8 g, respectively, compared to controls.

This review article aims to give an overview of the experimental animal tumor activity of homo-aza-steroidal esters, in which the alkylating agent is connected by an ester linkage at C-3 in axial and equatorial position, forming the corresponding epimers, while the lactam ring is formed in D-ring of the steroid nucleus and the difference in activity of unmodified and modified steroidal esters. The biochemical pharmacology of these compounds will be reviewed with the emphasis to the amide group for synergistic activity with the congener.

5. On the Activity of Epimers of Homo-aza-Steroidal Esters in Experimental Animal Tumor and Leukemia Systems.

Studying the stereoisomers, 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate (Figure 1) and 3α-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate (Figure 2) against L1210 leukemia, showed the following results. Compound of Figure 2 is less active than compound of Figure 1, presumable that the rate of hydrolysis is faster in the case of the stereoisomer with equatorial alkylating agent, while compound (Figure 3), in which rings A and B are fused cis is active [34].

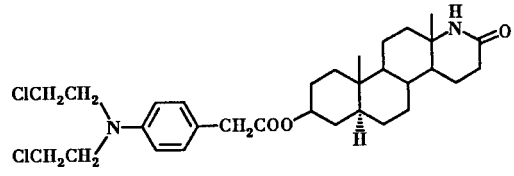


Figure 1. 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-p-*N*,*N*-bis(2-chloroethyl)aminophenylacetate.

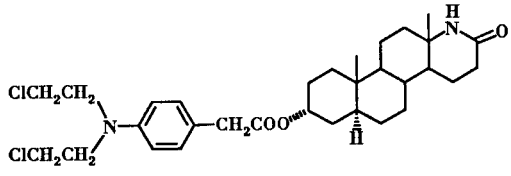


Figure 2. 3α-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate.

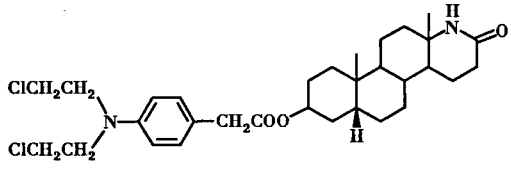


Figure 3. 3β-Hydroxy-13α-amino-13,17-seco-5β-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate.

Stereoisomer Lactam Esters of *p*-*N*,*N*-Bis(2-chloroethyl)-aminophenoxyacetic Acid.

The stereoisomer lactam esters of *p*-*N*,*N*-bis(2-chloroethyl)aminophenoxyacetic acid (Figure 4) and the corresponding 5β (Figure 5) in which the alkylating congener is linked at C-17 of the modified steroid were tested against L1210 leukemia showed moderate difference in activity between them [35]. Evidently the cis or trans fused A/B rings do not affect the activity in L1210 leukemia.

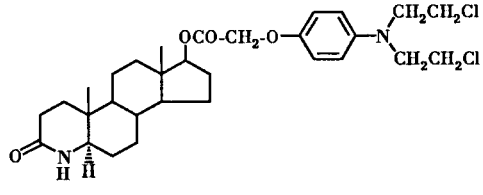


Figure 4. 17β-Hydroxy-4-aza-5α-androstan-3-one-*p*-*N*,*N*-bis(2-chloroethyl)-aminophenoxyacetate.

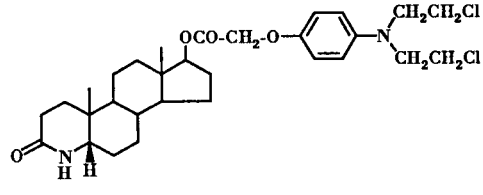


Figure 5. 17β-Hydroxy-4-aza-5β-androstan-3-one-*p*-*N*,*N*-bis(2-chloroethyl)-aminophenoxyacetate.

Steroidal Lactam Esters of Cinnamic Acid Isomers.

In a recent study [36] of homo-aza-steroidal esters of cinnamic acid mustards isomers which have been elected for the reason due to the double bond which is introduced into the acyclic side chain of the alkylating congener and enhanced the pressor effect of these compounds in the *ortho* and *para* position by the formation of changes in resonance through delocalization of π-electron cloud. Compounds (Figures 6-8) are stereoisomers (Figures 9-11). The latter almost inactive in five tumors tested *in vivo*. This probably due to the steric arrangement of the alkylating moiety. The cinnamic acid mustards, *ortho*, *meta* and *para* (*o*-ACA, *m*-ACA, *p*-ACA) are much more toxic than the corresponding esters, while in the study for antitumor activity, the *p*-*N*,*N*-bis(2-chloroethyl)aminocinnamic acid showed activity in EAT and P388 leukemia.

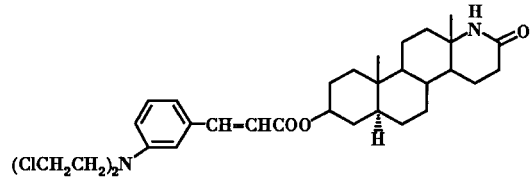


Figure 7. 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*m*-*N*,*N*-bis(2-chloroethyl)aminocinnamate.

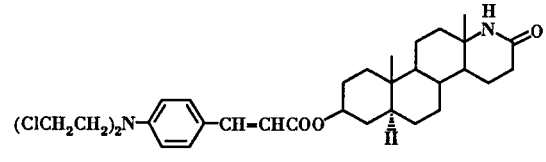


Figure 8. 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminocinnamate.

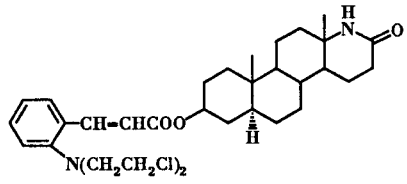


Figure 6. 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,7-lactam-*o*-*N*,*N*-bis(2-chloroethyl)aminocinnamate.

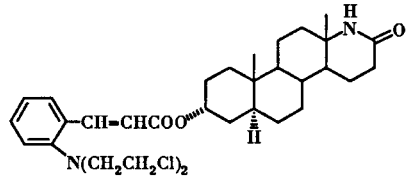


Figure 9. 3α-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*o*-*N*,*N*-bis(2-chloroethyl)aminocinnamate.

Table II

Activity of Phenesterin and ASE in L1210 leukemia [a]

Compound (route of administration)	Schedule [b] Days 1-8 Dose	Days 1-8 ILS	Days 1,5,9 Dose	Days 1,5,9 ILS	Day 1 only Dose	Day 1 only ILS
Phenesterin (ip)	6.7	-7	20	0	20	1.5
	10	0.0	30	0	30	-1.5
	15	0,-1,5	45	-3	45	-3
	22.5	1.5,6				
ASE (ip)	6.7	46	20	57	20	16
	10	46, 74	30	60	30	7
	15	82, -11	45	51	45	-74
	22.5	23, -66				
Phenesterine (sc)	10	-3	20	1.5		
	15	-1.5	30	1.5		
	22.5	-6	45	1.5		
ASE (sc)	10	38	20	38		
	15	56	30	38		
	22.5	10	45	-31		
Phenesterin (oral)	10	-4	20	-3		
	15	16	30	3		
	22.5	0	45	-1.5		
ASE (oral)	10	40	20	59		
	15	46	30	46		
	22.5	-31	45	-18		

[a] L1210 cells $(10)^5$ were injected ip on Day 0; treatment was started 24 hours later using 8 female B6D2F$_1$ mice/group. [b] Dose, mg/kg injection; % ILS calculated as follows: lifespan[1] (treated)-lifespan (controls) x 100/lifespan (controls).

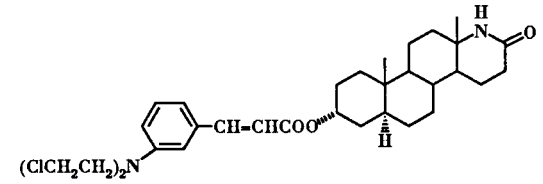


Figure 10. 3α-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*m*-*N*,*N*-bis(2-chloroethyl)aminocinnamate.

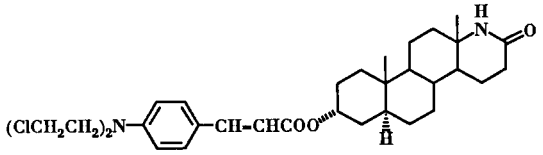


Figure 11. 3α-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminocinnamate.

6. Difference in Activity of Unmodified and Modified Steroidal Esters of Carboxylic Derivatives of *N*,*N*-Bis(2-chloroethyl)aniline.

In view of the importance of steroid containing alkylating agents, it was studied the 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate (Figure 1) in two leukemias and has been compared the results with the extensively studied phenesterin, cholesterol, *p*-bis(2-chloroethyl)-aminophenylacetate (Figure 12).

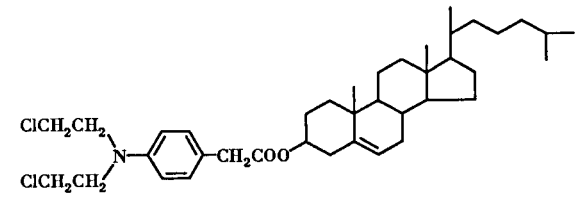


Figure 12. Cholesterol, *p*-*N*,*N*-bis(2-chloroethyl)phenylacetate(Phenesterin).

The cholesterol derivative was inactive in P388 and L1210 leukemia. In contrast, compound of Figure 1 (ASE), produced maximal activity in L1210 leukemia 82%, 56% and 46% ILS by the ip sc and oral routes respectively on a daily treatment schedule in L1210 leukemia and 60%, 38% and 59% ILS by the ip, sc and oral routes respectively, using the day 1, 5 and 9 treatment schedule (Table II) [31]. Maximal activity of 161% ILS on the daily schedule and 186% ILS (with one survivor) on the day 1, 5 and 9 schedule were obtained in P388 leukemia (Table III) [31].

Some years ago Wall and co-workers [21-22] have synthesized steroidal esters of *p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetic acid and *p*-*N*,*N*-bis(2-chloroethyl)aminophenylbutyric acid and tested against on experimental animal mammary tumor and leukemia systems. All the compounds studied in the L1210 leukemia were inactive according to the criteria of the CCNSC. The prepared and tested compounds are reported in Table IV.

Table III

Activity of Phenesterin and ASE in P388 Leukemia [a]

Compound (route of administration)	Schedule [b] Days 1-8 Dose	ILS	Days 1,5,9 Dose	ILS
Phenesterin (ip)	15	-11		
	22.5	-12		
	33.8	-25		
ASE (ip)	6.7	161	15	186
	10	68	22.5	79
	15	-22		

[a] P388 cells (10^6) were injected ip on Day 0; treatment was started 24 hours later using 8 female B6D2F$_1$ mice/group. [b] Dose, mg/kg/injection; ILS, % 1 survivor in this group was not included in the calculation.

Table IV

Steroidal Esters of *N*,*N*-Bis(2-chloroethyl)aminophenylacetic acid and Butyric Acid

R	ROCO(CH$_2$)n Position	n	N(CH$_2$CH$_2$Cl)$_2$
Cholesterol	3β	1	
Desoxycorticosterone	21	1	
Estrone	3	1	
Estradiol	17β	1	
Testosterone	17β	1	
Pregnenolone	3β	1	
Dehydroepiandrosterone	3β	1	
Epiandrosterone	3β	1	
3α,12α-dihydroxycholanic acid	3α,12	1	
Cholesterol	3β	3	
Estrone	3	3	

The activity of 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylbutyrate (Figure 13), lactam of epiandrosterone, which contains a chlorambucil moiety, was consistently slightly less active than that of compound Figure 1 [34].

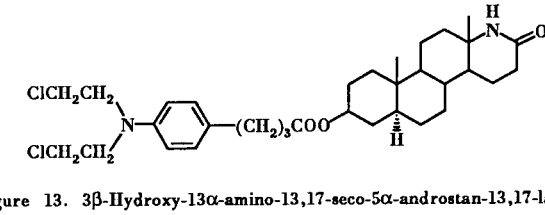


Figure 13. 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylbutyrate.

Compound (Figure 14) is the first A-ring modified steroid tested (lactam of testosterone). Results were favorable with T/C values greater than 200% in L1210 leukemia and up to 528 in P388 leukemia with long-term survivors in two separate experiments using the intermittent treatment schedule [34].

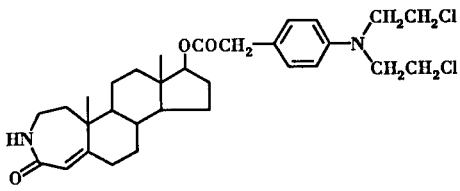


Figure 14. 17β-Hydroxy-3-aza-A-homo-4α-androsten-4-one-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate.

The same lactam with alkylating agent *p*-*N*,*N*-bis(2-chloroethyl)aminophenoxyacetic acid (Figure 15) in duplicate experiments yielded in P388 leukemia T/C values of 225% and 212% at a dose of 400 mg/kg on days 1 and 4 and 1 and 5 [37].

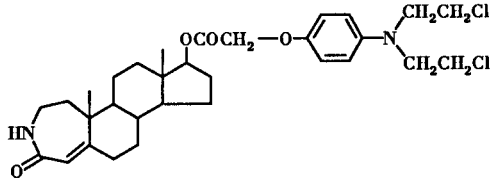


Figure 15. 17β-Hydroxy-3-aza-A-homo-4α-androsten-4-one-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate.

The ester of epiandrosterone lactam of *p*-*N*,*N*-bis(2-chloroethyl)aminophenoxyacetic acid is one of the more active compounds in this series of modified steroidal alkylating agents producing good activity in early and advanced murine leukemias [32].

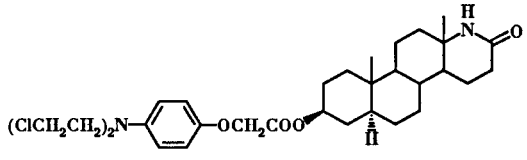


Figure 16. 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenoxyacetate.

Compound of Figure 16 produced in L1210 leukemia maximal activity 103% increase in life span when given by ip route according to the days 1 and 4 treatment schedule. In advanced L1210 leukemia 41% ILS was achieved, while in P388 leukemia 383% ILS was obtained [32]. This compound was active in treatment in Colon 26 tumor giving T/C of 232%. It was also active against melanoma B16 giving 132% T/C [38].

Compound of Figure 16 is one of the more active compounds of this series of modified steroidal alkylating agents. In these studies it has employed activity comparable to ASE in treatment of Colon 26 and melanoma B16.

As part of a continuing program directed toward the development of novel antitumor agents, it was studied the activity of 3β-hydroxy-13α-amino-13,17-seco-5-androsten-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate (Figure 17) (lactam of dehydroepiandrosterone) in P388 and L1210 leukemias [39].

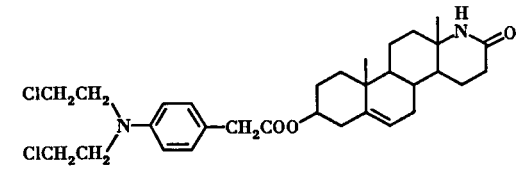


Figure 17. 3β-Hydroxy-13α-amino-13,17-seco-5-androsten-17-oic-13,17-lactam-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate.

The homo-aza-steroidal ester produced maximal activity of 89% ILS when given by the intraperitoneal route according to the days 1, 5 and 9 treatment schedule in L1210. In P388 leukemia 452% ILS was obtained on a 1-day schedule; 276% on a 1, 5 and 9 days and 358% on a 1-8 days schedule.

7. Conclusion.

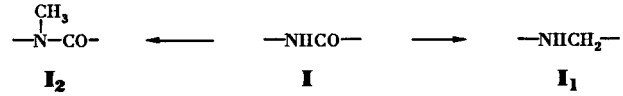
The compounds (Figures 1, 2 and 3) represent three of four possible stereoisomeric permutations at the three and five positions. The difference in these stereoisomers is the potency. Compound (Figure 2) optimally active at about ten times and five the optimal doses of compound (Figure 1) and compound (Figure 3) respectively.

The mustard derivatives of cinnamic acid (Figures 9-11) stereoisomers of (Figures 6-8) were studied against EAT, L1210, P388 and melanoma B16 and it was found that the axial compounds (Figures 9-11) were almost inactive.

Moderate difference in the activity of L1210 and P388 leukemia has been observed in modified steroids (A-ring lactam and alkylating agent at C-17) in which A/B ring is *trans* or *cis* junction.

The steric arrangement of the alkylating agent in axial position is the reason of the rate of hydrolysis and liberation of the alkylating agent in the target tissue. The equatorial ester hydrolyzes faster than the axial.

Most steroidal alkylating agents have been inactive in L1210 leukemia [21], but most homo-aza-steroidal esters of carboxylic derivatives of *N*,*N*-bis(2-chloroethyl)aniline have been active with substitution in either the A-ring or the D-ring of the steroid molecule. The lactam moiety appears to confer this activity. For this purpose two new compounds were synthesized, the 3β-hydroxy-17α-aza-D-homo-5α-androstane-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate and the 3β-hydroxy-*N*-methyl-17α-aza-D-homo-5α-androstan-17-one-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate. In both cases modifications have been affected on the -NHCO- group of compound (Figure 1).



Equation 1

The anticancer activity of compound (Figure 1) is superior to the $\mathbf{I}_1$ and $\mathbf{I}_2$ in L1210 leukemia, P388 leukemia

Ehrlich ascite tumor and Lewis lung carcinoma [28]. Presumable this is due to the possibility of multiple interactions of the -NHCO- lactam group of the steroid nucleus with similar groups which exist in proteins and nucleic acids. There is the possibility that lactam nucleus is transformed by a metabolic process to active species.

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