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A New Steroidal Alkylating Agent with Improved Activity in Advanced Murine Leukemias

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Summary. *The homo-aza-steroidal ester of [p-[bis(2-chloroethyl)amino]phenoxy] acetic acid, 3β-hydroxy 13α - amino - 13,17 - seco - 5α-androstan-17-oic-13,17-lactam-p-bis(2-chloroethyl)aminophenoxyacetate, gave a 100% increase in lifespan over controls in the treatment of L1210 leukemia by IP administration on a days 1 and 4 treatment schedule. This ester gave a maximum activity of 383% increased lifespan over controls in the treatment of P388 leukemia by IP administration on a daily treatment schedule. Activity in advanced L1210 (41% increased lifespan) and P388 leukemias (173% increased lifespan) was maintained, indicating that this compound is the most promising of a number of congeners tested to date.*

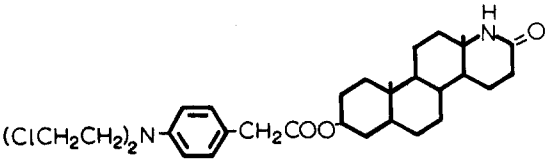

Fig. 1. Chemical structure of 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenylacetate (ASE)

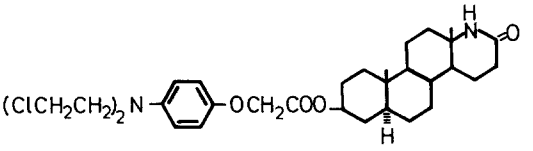

Fig. 2. Chemical structure of 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenoxyacetate (C-15)

Introduction

Recent studies in this laboratory have been concerned with the synthesis and anticancer evaluation of agents synthesized from steroidal lactams or lactones esterified with carboxylic derivatives of *N,N*-bis (2-chloroethyl)aniline [1, 3–5]. These compounds contain a modified steroid as a biological platform for transporting the alkylating agent to the tumor site [6]. Previously we reported on the activity of an ester (Fig. 1) synthesized from 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam and *p*-bis(2-chloroethyl)aminophenylacetic acid (ASE) [2], which showed excellent results in P388 leukemia and satisfactory results in L1210 leukemia [8].

In the course of screening a number of congeners we found that 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenoxyacetate (Fig. 2) has interesting antineoplastic properties and appears to be the most promising congener tested to date.

Assuming hydrolysis in biologic systems, this ester would be transformed to the well-known antitumor aniline derivative, *p*-di-2-chloroethylaminophenol.

Materials and Methods

The compound 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenoxyacetate (C-15) was prepared by heating 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam with *p*-[*N,N*-bis(2-chloroethyl) amino]phenoxyacetylchloride in benzene. C-15 was purified by chromatography on silica gel (eluant, chloroform), and was shown to have a molecular weight of 579 and a melting point of 150°–152° C after recrystallization from ethylacetate-hexane. This compound is soluble in chloroform, dichloromethane, dimethylsulfoxide, and dimethylformamide, but is insoluble in water. Elemental analysis was found to be C, 64.26%; H, 8.10%; N, 4.75% (calculated C, 64.25%;

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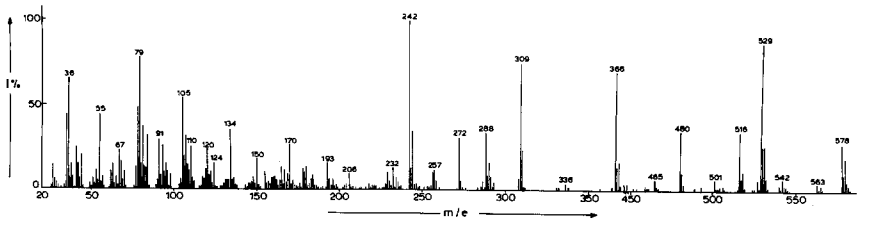

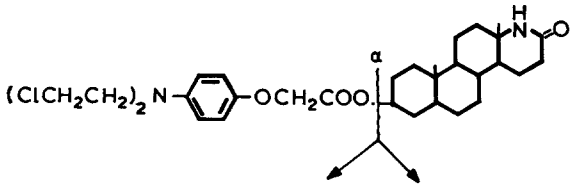

$C_{12}H_{14}NO_3Cl_2^+$ m/e 290 (13.4%) $C_{19}H_{30}NO^+$ m/e 288 (34.1%)

Fig. 3. Mass spectrum of 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenoxyacetate. The mass spectrum of the lactam ester shows a molecular ion M^+ m/e 578 of relatively medium intensity ($C_{31}H_{44}N_2O_4Cl_2$, 28.6%). The ions m/e 563 ($C_{30}H_{41}N_2O_4Cl_2$, 4.5%), m/e 542 ($C_{31}H_{43}N_2O_4Cl$, 7.2%), and m/e 529($C_{30}H_{42}N_2O_4Cl$, 87.4%) arise from the molecular ion M^+ by $\cdot CH_3$, $H^{35}Cl$, and $\cdot CH_2{}^{35}Cl$ elimination. The metastable ion m* (m/e 484.14) indicated the formation of ion m/e 529 (578 $\xrightarrow{m^*}$ 529) by elimination of the $\cdot CH_2{}^{35}Cl$ radical from the molecular ion. The ion m/e 516 ($C_{29}H_{41}N_2O_4Cl$, 35.3%) arises from the molecular ion M^+ by $CH_2 = CHCl \cdot$ radical elimination. The formation of the ions m/e 288 and 290 is due to the breakage of the ester by route α. The most abundant ion, m/e 242, 100%, is formed from m/e 290 with hydrogen transfer and loss of $\cdot CH_2Cl$ radical, as indicated by a metastable peak m*, m/e 201.9

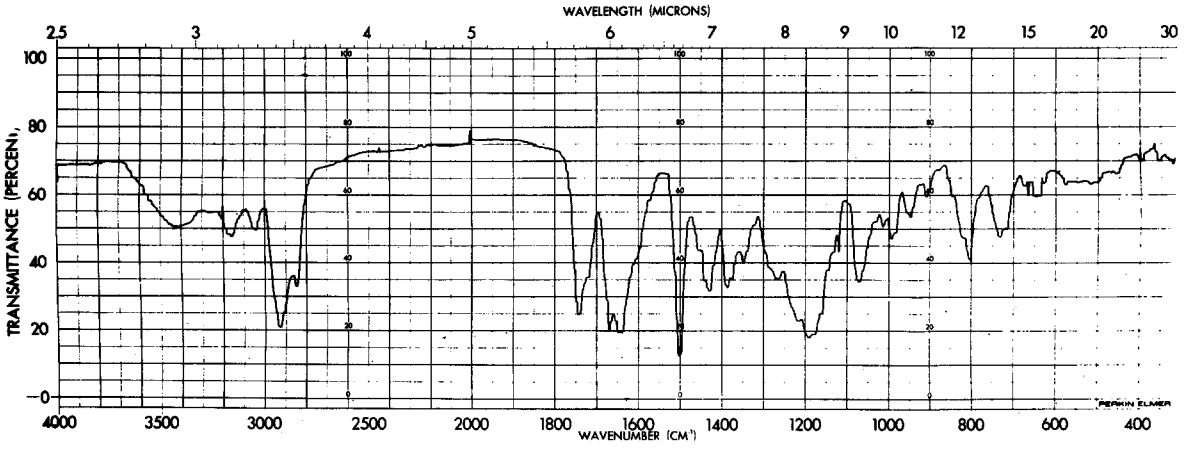

Fig. 4. Infrared spectrum of 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenoxyacetate: NH, at 3160 and 3040 cm^{-1}; COO-, at 1740 cm^{-1}; CONH, at 1670 and 1640 cm^{-1}; aromatic ring, at 800 and 730 cm^{-1}

H, 7.60%; N, 4.83%). Mass, infrared and NMR spectra for C-15 are shown in Figs. 3, 4, and 5, respectively.

C-15 was dissolved in ethanol in a concentration of 10 mg/ml. A small amount of Tween 80 was added (equal to 0.1% of the final suspension), and then saline was added to make a final concentration of 1.5–3.3 mg drug/ml. This produced a stable milky suspension, which was freshly prepared for each separate experiment from the stock chemical.

Drug in doses of 0.13–0.45 ml (10–75 mg/kg/injection) was administered by the IP route according to several schedules (see Table 1).

L1210 leukemia was maintained in our laboratory by weekly IP passage of 10^5 L1210 cells in DBA/2 mice. P388 leukemia was maintained by weekly IP passage of 10^6 P388 cells in DBA/2 mice. C57BL/6 × DBA/2 ($B6D2F_1$) female mice (average weight 21.3 g) or BALB/c × DBA/2 (CD_2F_1) female mice (average weight 20.1 g) were used for the test animals.

For all experiments, mice (uniform as to sex and age) were kept in groups of six or eight in an air-conditioned, light-controlled environment.

Results

Whereas unmodified steroidal esters have generally been reported to be inactive in treatment of L1210 leukemia [7], C-15 produced maximal activity of 103% increase

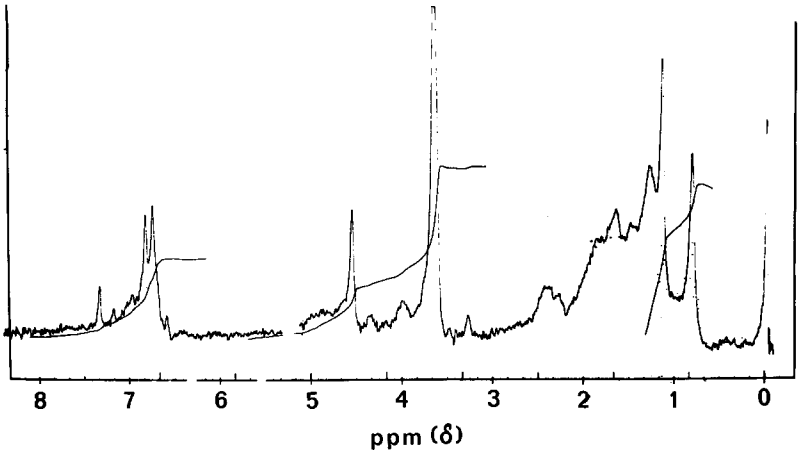

Fig. 5. NMR data of 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenoxyacetate using a Varian Associates A60 instrument: δ 0.82(18-CH_3), 1.13(19-CH_3); δ 4.8 (C_3-H); δ 4.54 (CH_2CO); the aromatic protons at δ 6.8

Table 1. Activity of the homo-aza-steroidal ester of [*p*-[bis(2-chloroethyl) amino]phenoxy]acetic acid (C-15) in treatment of murine leukemias

Tumor								
Ascitic L1210 leukemia			Advanced solid L1210 leukemia			P388 leukemia		
Schedule	Dose[a]	% ILS[b]	Schedule	Dose[a]	% ILS[b]	Schedule	Dose[a]	% ILS[b]
Days 1–8	10	44 ± 7	Days 7 and 11	22.2	10 ± 7	Days 1–8	14.1	254 ± 48
	15	46 ± 8		33.3	17 ± 7		21.1	383 ± 97
	22.5	62 ± 12		50	31 ± 12		31.7	24 ± 17
				75	41 ± 4			
Days 1–7	25	50 ± 7				Days 1, 5 and 9	28	158 ± 23
	33.8	75 ± 7					42	242 ± 35
							63	135 ± 41
Days 1 and 4	25	29 ± 6						
	50	103 ± 10						
Day 1 only	50	47 ± 14				Days 7, 11 and 15	28	80 ± 29
	75	− 10 ± 5					42	173 ± 68
							63	172 ± 89

[a] Dose, mg/kg by IP injection

[b] ILS calculated as follows: $\frac{\text{Lifespan (treated)} - \text{lifespan (controls)} \times 100}{\text{lifespan (controls)}}$

in lifespan (ILS) (Table 1) when given by the IP route according to the days 1 and 4 treatment schedule (25% ILS is usually required as a minimum for activity). In advanced L1210 leukemia (treatment on days 7 and 11) 41% ILS was achieved. In P388 leukemia 383% ILS was obtained on a days 1–8 schedule; 242% ILS on a days 1, 5, and 9 schedule; and 173% ILS on a days 7, 11, and 15 schedule. For perspective, Table 2 lists comparative optimal results obtained in this laboratory with a number of different alkylating agents in the treatment of advanced murine leukemias. All experiments were done under similar conditions in solid L1210 or P388 leukemias according to known or presumed optimum treatment schedules.

Discussion

The modified steroidal alkylating agent we initially reported (ASE) gave a maximum ILS of 82% against early L1210 leukemia and 186% ILS against P388 leukemia. Phenesterin was compared with ASE in the same experiments and was inactive in both tumor systems [8]. Results with C-15 are better than those with ASE in the

Table 2. Comparative activity of alkylating agents in two advanced murine leukemias[a]

Drug	L1210		P388	
	Optimal dose	% ILS	Optimal dose	% ILS
C-15	75	41	42	173
Mechlorethamine	4.5	8	3.9	62
	4.5	10	2.4	20
ThioTEPA	19	75	7.3	82
Mitomycin C	13.5	32	2.6	46
Cyclophosphamide	416	127	218	249
	416	139	286	247
	360	122	230	272
	450	149	164	231
	450	143	242	218

[a] All experiments were performed in B6D2F$_1$ female mice (except C-15 treatment of advanced L1210 leukemia: CD2F$_1$ mice). A minimum of three treatment groups were treated after preliminary experiments indicated a suitable range of doses. No treatment was given before day 7 after tumor inoculation. All drugs were given as single doses except C-15 which was given every 4 days until death, but not more than three times. Optimal results only are listed for each run. % ILS was calculated from the formula given in the footnote to Table 1

treatment of both early L1210 and early P388 leukemias. ASE was not tested against the advanced leukemias, but a number of apparently improved newer congeners have been. ILS values for C-15 are numerically superior to those for all analogs tested to date in both these advanced murine neoplasms. Since treatment of early L1210 or P388 leukemia is analogous to treatment of human malignancies prior to symptomatology, it would appear that the sustained activity of C-15 in advanced disease is a highly desirable characteristic.

Minor structural modification of either the steroid or the mustard moiety of steroidal alkylating agents results in profound changes in antitumor activity of the compounds. At this time it is possible to say that the lactam modification (the homo-aza-steroid) appears to confer activity against L1210 leukemia to many but not all congeners. In view of the importance of L1210 leukemia as a screening system for activity in human neoplasms, such a modification would appear to be highly desirable. Because of the limited number of congeners prepared to date, it is not possible to make detailed conclusions regarding optimal structural arrangements for the mustard moiety. It does appear that the in vivo activity of the predicted hydrolytic product is not the major indicator of relative activity.

C-15 is equal or superior in the treatment of advanced murine leukemias to a number of current clinically useful alkylating agents (Table 2), and has in addition the modified steroid moiety, which may further increase activity in hormonally sensitive tumors. Further trials, preferably in hormonally sensitive animal tumors, are indicated.

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