Synthesis and Bioevaluation of a Series of Fatty Acid Esters of *p*-[*N*,*N*-Bis(2-chloroethyl)amino]phenol

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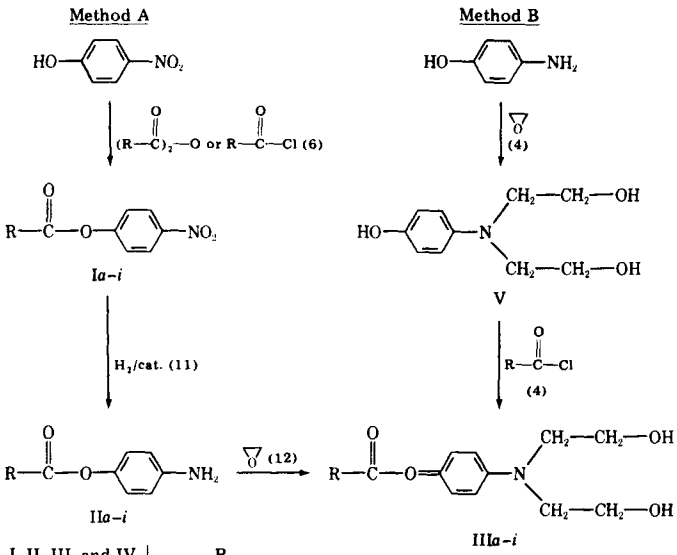
Abstract □ A series of even numbered fatty acid esters (C_2–C_{18}) of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol were synthesized and evaluated as to acute toxicity as well as effectiveness against L-1210 mouse leukemia. The acetate through the decanoate derivatives demonstrated toxicity between 2 and 3 times that of phenol mustard in HA/ICR mice. The less soluble laurate, myristate, palmitate, and stearate derivatives were less toxic. Significant survival times in the leukemia studies (T/C% ≥ 125) were observed for all compounds except the acetate and hexanoate derivatives. The myristate derivative produced the greatest significant increase in survival time, 162%. The palmitate and stearate derivatives produced significant survival at five and four dosage levels, respectively. The butyrate and laurate derivatives produced significant survival at three dosage levels and the octanoate, decanoate, and myristate at two dosage levels.

Keyphrases □ Fatty acid esters—synthesis of fatty acids of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol, toxicity, effectiveness against L-1210 mouse leukemia □ *p*-[*N*,*N*-Bis(2-chloroethyl)amino]phenol—synthesis of fatty acid esters for toxicity and effectiveness against L-1210 mouse leukemia □ Bioevaluation—synthesis of fatty acid esters of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol, toxicity, effectiveness against L-1210 mouse leukemia

Efforts to develop latent forms of phenol mustard, *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol, have resulted in the synthesis of various esters of the phenol (1–3), as well as its ether derivatives (4). A previous study (2) on a series of substituted benzoate esters of phenol mustard suggested that the toxicity of those esters was related to their hydrolysis to the phenolic mustard. They also suggested that antitumor activity in Walker 256 tumor paralleled hydrolysis of the esters to phenol mustard. A retrospective QSAR study on a number of derivatives of aniline mustard has been performed (5). That study supports the concept that, in general, antitumor activity parallels hydrolysis to phenol mustard, as well as toxicity. That evaluation of the aforementioned series of benzoate esters of phenol mustard suggests that the hydrolyzed form of the ester is not the principle antitumor agent.

An earlier report from this laboratory compared the antileukemic activity of the acetate, hexanoate, and decanoate esters of phenol mustard (3). Those results indicated a decrease in toxicity and an increase in antileukemic activity was associated with an increase in the length of the fatty acid side chain. In light of these results, it was felt that an expanded series of fatty acid esters would provide additional information with respect to the effect of chain length on toxicity and antileukemic activity. The specific objectives of this report were to: (*a*) synthesize a series of even numbered fatty acid esters (C_2–C_{18}) of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol, (*b*) determine the acute toxicity as measured by the LD_{50} for each compound studied, and (*c*) determine the effect of each compound on the prolongation of life of L-1210 leukemic mice.

The compounds evaluated in this project were synthesized using either Method A or Method B as shown in Scheme I.



I, II, III, and IV	R
a	CH_3—
b	CH_3—$(CH_2)_2$—
c	CH_3—$(CH_2)_4$—
d	CH_3—$(CH_2)_6$—
e	CH_3—$(CH_2)_8$—
f	CH_3—$(CH_2)_{10}$—
g	CH_3—$(CH_2)_{12}$—
h	CH_3—$(CH_2)_{14}$—
i	CH_3—$(CH_2)_{16}$—

Scheme I

EXPERIMENTAL

Synthesis of the Fatty Acid Esters of *p*-[*N*,*N*-Bis(2-hydroxyethyl)amino]phenol (IIIa–*i*), Method A—*Step I: Synthesis of the p-Nitrophenyl Esters (Ia–i).*—Ninety milliliters of 1,4-dioxane, 10 ml of pyridine, and 13.9 g (0.1 mole) of *p*-nitrophenol were stirred at room temperature until solution of the *p*-nitrophenol was complete. Then, 0.1 mole of either acetic or butyric anhydride, or the appropriate acid chloride for all other derivatives, was added slowly with stirring. The reaction mixture was stirred at room temperature in a glass-stoppered flask for ~2 hr. The volume of the reaction mixture was then reduced ~50% *in vacuo*. The products separated as a solid with the exception of the butyrate, hexanoate, and octanoate derivatives which were oils. The products were removed by filtration or by use of a separatory funnel and washed 3–5 times with a 5% sodium carbonate solution. After drying in a vacuum desiccator, the solid products were recrystallized from absolute ethanol. The oily products were dried *in vacuo* for 30–60 min and the pure products were characterized by physical data (Table I) and IR spectra and NMR spectra[1].

[1] All IR spectra were obtained using potassium bromide pellets or neat on sodium chloride plates using a Beckman Model IR-20A Spectrophotometer. NMR spectra were obtained from solutions of the derivatives in deuterated chloroform using a Hitachi–Perkin-Elmer Model R-24 high resolution spectrometer with tetramethylsilane as the internal standard. The elemental analyses reported were performed by either Galbraith Laboratories, Knoxville, Tenn. or Atlantic Microlab, Inc., Atlanta, Ga. Melting points reported were obtained using a Thomas Hoover capillary melting point apparatus and are uncorrected. All hydrogenation reactions were carried out in a Parr Low-Pressure Hydrogenation Apparatus.

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Table I—Physical Data and Elemental Analysis for the Fatty Acid Esters of *p*-Nitrophenol (I*a–i*) and *p*-Aminophenol (II*a–i*)

Compound Number	mp or bp		Yield, %	Molecular Formula	Calculated, %			Found, %		
	Observed	Lit. Value, (9, 10)			C	H	N	C	H	N
I*a*	76.5°–77°	79°	56	$C_8H_7NO_4$	[b]					
I*b*	185°/760mm[a]	134°/0.8	52	$C_{10}H_{11}NO_4$	[b]					
I*c*	160°/1mm[a]	155°/0.8mm	50	$C_{12}H_{15}NO_4$	[b]					
I*d*	183°/760mm[a]	164°/0.3mm	71	$C_{14}H_{19}NO_4$	[b]					
I*e*	32°–34°	31°	57	$C_{16}H_{23}NO_4$	[b]					
I*f*	44°–45°	44.5°–45°	85	$C_{18}H_{27}NO_4$	[b]					
I*g*	53°–54°	54.5°–55°	83	$C_{20}H_{31}NO_4$	[b]					
I*h*	61.5–63°	62°–64°	61	$C_{22}H_{35}NO_4$	[b]					
I*i*	66.5°–67.5°	66.5°–67°	65	$C_{24}H_{39}NO_4$	[b]					
II*a*	70°–71°	70°–72°	43	$C_8H_9NO_2$	[b]					
II*b*	57°–59°		93	$C_{10}H_{13}NO_2$	67.4	7.26	7.82	66.95	7.17	7.82
II*c*	210°/760mm[a]		48	$C_{12}H_{17}NO_2$	69.56	8.21	6.76	69.36	8.17	6.65
II*d*	35°–36°		87	$C_{14}H_{21}NO_2$	71.48	8.93	5.95	[c]		
II*e*	39°–40°		29	$C_{16}H_{25}NO_2$	73.00	9.50	5.32	73.14	9.62	5.23
II*f*	46°–47°		55	$C_{18}H_{29}NO_2$	74.23	9.96	4.81	74.16	10.02	4.73
II*g*	59°–60°		76	$C_{20}H_{33}NO_2$	75.24	10.35	4.39	75.05	10.18	4.39
II*h*	73°–74.5°		73	$C_{22}H_{37}NO_2$	76.08	10.66	4.03	75.89	10.49	4.00
II*i*	79°–80°		75	$C_{24}H_{41}NO_2$	76.80	10.93	3.73	76.65	11.02	3.69

[a] Temperature/pressure at which fractions used for analysis and subsequent reaction were collected. [b] Characterized using comparative melting or boiling points, IR and NMR spectra. [c] An acceptable elemental analysis could not be obtained, but acceptable products were obtained in the subsequent reaction, Step III, Method A.

Step II: Reduction of the p-*Nitrophenyl Esters*—A solution containing 0.02 mole of the appropriate *p*-nitrophenol ester in 150 ml of absolute ethanol was placed in a reaction bottle. Sixty milligrams (0.0002 mole) of platinum oxide was added and the mixture was shaken under pressure with hydrogen until a pressure drop representing 0.06 mole of hydrogen uptake (4.92 psi) was observed. The catalyst was removed by filtration and the filtrate concentrated *in vacuo*. The product separated from the concentrated solution after cooling to 0°. Recrystallization from absolute ethanol yielded solid derivatives with the exception of the hexanoate ester which was an oil. The pure *p*-aminophenyl esters (II*a–i*) were characterized by physical data (Table I) and IR and NMR spectra.

Step III: Condensation of the p-*Aminophenyl Esters with Ethylene Oxide*—The *p*-[*N,N*-bis(2-hydroxyethyl)amino]phenyl esters (III*a–g*) were formed by dissolving 0.0005 mole of the appropriate *p*-aminophenyl ester in a minimum amount of absolute methanol using a 500-ml round-bottomed reaction flask. Two grams (0.05 mole) of cold ethylene oxide were added to 20 ml of methanol at 0°. The cold ethylene oxide–methanol mixture was added with continuous stirring to the methanol solution of the *p*-aminophenyl ester. The reaction flask was packed in dry ice and allowed to reach room temperature. The methanol and excess ethylene oxide were removed *in vacuo*. The solid products were recrystallized from absolute ethanol. The butyrate and hexanoate derivatives were isolated as semisolids, which gave crystalline solids when recrystallized from acetone–water. Acceptable products were not obtained for the palmitate nor the stearate derivatives (III*h* and III*i*). The pure derivatives were characterized by physical data (Table II) and IR and NMR spectra.

Synthesis of the Fatty Acid Esters of *p*-[*N,N*-Bis(2-hydroxyethyl)amino]phenol (III*a–i*), Method B—The *p*-[*N,N*-bis(2-hydroxyethyl)amino]phenol (V) was prepared using a procedure reported earlier (4). Absolute ethanol (250 ml); powdered KOH (2.8 g); (V) (9.5 g, 0.05 mole); and 0.05 mole of either myristoyl chloride, palmitoyl chloride, or stearoyl chloride were added to a glass 500-ml round bottomed flask, equipped with a magnetic stirrer and a glass stopper. The flask was stoppered and the solution was allowed to stir overnight. The resulting product was removed by filtration, the filtrate reduced *in vacuo*, and the residue dissolved in 100 ml of acetone. To the acetone solution was added 50 ml of water and the suspension was allowed to cool at 5° overnight. The crystals were filtered from the acetone–water solution and combined with the original product. Recrystallization from acetone–water gave analytically pure products (III*g–i*) which were characterized by physical data (Table II) and IR and NMR spectra.

Synthesis of the Fatty Acid Esters of *p*-[*N,N*-Bis(2-chloroethyl)amino]phenol (IV*a–i*)—A solution of 10 ml of chloroform, 0.3 ml of absolute ethanol, and 0.5 ml of thionyl chloride was stirred in a 100-ml round bottomed flask until room temperature was reached. To this solution 0.005–0.01 mole of the appropriate derivative of III was added slowly as the solution was stirred. The reaction flask was fitted with a condensor and a drying tube and packed in dry ice after solution was complete. An additional 1.5 ml of thionyl chloride was added to the cold solution. The reaction mixture was allowed to reach ambient temperature

Table II—Physical Data and Elemental Analysis for the Fatty Acid Esters of *p*-[*N,N*-Bis(2-hydroxyethyl)amino]phenol (III*a–i*) and of *p*-[*N,N*-Bis(2-chloroethyl)amino]phenol (IV*a–i*)

Compound Number	mp	Method	Yield, %	Molecular Formula	Calculated, %			Found, %		
					C	H	N	C	H	N
III*a*	74.5°–76°[a]	A	52	$C_{12}H_{17}NO_4$	60.25	7.11	5.85	60.20	7.18	5.76
III*b*	71.5°–72°	A	48	$C_{14}H_{21}NO_4$	62.92	7.86	5.24	63.06	7.75	5.34
III*c*	52°–53°	A	46	$C_{16}H_{25}NO_4$	65.08	8.47	4.75	64.95	8.51	4.69
III*d*	63°–65°	A	74	$C_{18}H_{29}NO_4$	66.87	8.98	4.33	66.75	8.86	4.41
III*e*	66°–69°	A	25	$C_{20}H_{33}NO_4$	68.38	9.40	3.99	68.52	9.36	4.04
III*f*	73°–76°	A	44	$C_{22}H_{37}NO_4$	69.60	9.76	3.69	69.50	9.57	3.48
III*g*	80°–83°	B	74	$C_{24}H_{41}NO_4$	70.76	10.07	3.44	70.91	10.15	3.47
III*h*	83°–86°	B	86	$C_{26}H_{45}NO_4$	71.72	10.34	3.22	71.52	10.44	3.20
III*i*	88–90°	B	71	$C_{28}H_{49}NO_4$	72.57	10.58	3.02	72.62	10.34	3.06
IV*a*	Oil[b]		32	$C_{12}H_{15}NO_2Cl_2$[c]	50.53	5.61	4.93	50.62	5.61	4.93
IV*b*	50°–52°		42	$C_{14}H_{19}NO_2Cl_2$	55.26	6.25	4.61	55.61	6.52	4.42
IV*c*	40.5°–41°		40	$C_{16}H_{23}NO_2Cl_2$	57.83	6.93	4.22	58.02	6.85	4.12
IV*d*	Oil		33	$C_{18}H_{27}NO_2Cl_2$	60.00	7.50	3.88	59.79	7.51	3.61
IV*e*	Semisolid		22	$C_{20}H_{31}O_2Cl_2$	61.86	7.99	3.61	61.81	8.01	3.47
IV*f*	40°–42°		53	$C_{22}H_{35}NO_2Cl_2$	63.46	8.41	3.37	63.10	8.38	3.54
IV*g*	41°–42.5°		55	$C_{24}H_{39}NO_2Cl_2$	64.86	8.78	3.15	65.06	8.58	3.32
IV*h*	43°–44°		62	$C_{26}H_{43}NO_2Cl_2$	66.10	9.11	2.97	66.40	9.16	2.98
IV*i*	46°–49°		57	$C_{28}H_{47}NO_2Cl_2$	67.20	9.40	2.80	67.11	9.46	2.80

[a] Literature value 82.5°(1). [b] Consistent with literature observation (1). [c] Calculated for the acetate mustard (IV*a*) with one-half mole of water.

and stirred for 24 hr. The mixture was concentrated *in vacuo*. An ether solution of the residue was washed with 5% sodium carbonate or sodium bicarbonate solutions and the ether removed *in vacuo*. The acetate, hexanoate, decanoate, and laurate derivatives were obtained in pure form from absolute ethanol. The acetate (IV*a*) was an oil, the decanoate (IV*e*) a semisolid, and the hexanoate (IV*c*) and laurate (IV*f*) were light brown solids. The butyrate mustard separated from ethyl acetate as a light-brown solid. The octanoate (IV*d*) was isolated as a dark oil from acetone. The myristate (IV*g*), palmitate (IV*h*), and stearate (IV*i*) derivatives were obtained from acetone–water (80:20) as light brown to off-white solids. All pure products were characterized by IR and NMR spectra. Physical data and elemental analysis are given in Table II for all final products.

Biological Evaluation—*Test Animals*—DBA/2 mouse strain[2], BDF_1 mouse strain[2], HA/ICR mouse strain[3], and L-1210 leukemic mice (tumor source)[4] were used.

Instruments—The necessary equipment included an electronic cell counter[5], a channelizer[5], a dilutor[5], an x-y recorder[5], a hemocytometer[6], and a microscope[7].

Materials—Counting diluent[5], red blood cell-lysing reagent[5], crystal violet[8], Giemsa stain[9], isotonic diluting solution[10], trypan blue[11], and Wright's stain[8] were obtained commercially. The ester derivatives of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol were prepared as described in this report, and their physical and analytical data are reported in Table II.

Pharmacological Screen and LD_{50} Evaluation—Five groups of six (HA/ICR) mice each were selected. A gross screen was conducted for acute toxicity, and results were recorded during the 3 hr following injection. The mice were observed and weighed every day for 21 days, and the mortalities were recorded daily. A linear regression and correlation coefficient program (6) and a graphic method (7) were applied to determine the LD_{50} for each drug tested.

Transplantation Procedures and *In Vivo* Determination of Antileukemic Activity Survival Times—Using the procedures described earlier for a series of ethers of phenol mustard (4), the transplantation procedures and *in vivo* determination of antileukemic activity by survival times were conducted. The palmitate (IV*h*) and stearate (IV*i*) derivatives were poorly soluble in propylene glycol; therefore, an equal mixture of polyethylene glycol 400, propylene glycol, and ethanol was used for these two derivatives. This solvent mixture allowed the evaluation of higher dosage levels of IV*h* and IV*i* in the survival studies.

RESULTS AND DISCUSSION

The synthesis of the even numbered fatty acid esters of *p*-[*N*,*N*-bis(2-hydroxyethyl)amino]phenol (III*a–i*) was accomplished *via* either Method A or Method B (Scheme I). The initial synthetic approach (Method A) consisted of an uncomplicated sequence of routine organic synthetic reactions. Using Method A, all of the *p*-nitro intermediates (I*a–i*), all of the *p*-amino intermediates (II*a–i*), and the acetate through the laurate diol intermediates (III*a–f*) were obtained in satisfactory yields. The condensation of ethylene oxide with the *p*-aminophenyl palmitate or stearate led to the isolation of the corresponding *p*-[*N*-(2-hydroxyethyl)amino]phenyl ester. The *p*-aminophenyl myristate gave very poor yields of the diol (≤12%) in addition to the monohydroxy ethyl derivative, when condensed with ethylene oxide. Method B provided an alternate route to intermediates III*g–i*, giving good yields of quality products. Compounds IV*a–i* were obtained following the reaction of thionyl chloride with the diol precursors. Not all of the final products were isolated as crystalline solids but an acceptable elemental analysis was obtained for each compound.

The use of Method A to obtain III*a–f* involved three synthetic steps. Step I, the esterification of *p*-nitrophenol, followed a modification of a procedure reported previously (8) in the synthesis of fatty acid esters of acetaminophen. The replacement of pyridine as a solvent with a mixture of dioxane (90 ml) and pyridine (10 ml) simplified the isolation of the ester products. The yields of the esters (I*a–i*) ranged from 50 to 85% and the observed melting points or boiling points (Table I) were consistent with reported values (9, 10). Step II, the reduction of I*a–i* to II*a–i*, employed the procedure described by Adams and Cohen (11) for the reduction of an aromatic nitro group in the presence of an ester. Yields for Step II ranged from 29 to 93%. The condensation of ethylene oxide with the *p*-aminophenyl esters (II*a–i*), Step III, followed the procedure described by Rao and Price (12) with elimination of the reflux step. This had no effect on the yields, which ranged from 25 to 74% for compounds III*a–f*.

Method B employed a modification of the method described by Wise, *et al.* (4) in the synthesis of a series of alkyl ethers of V. When the myristoyl, palmitoyl, or stearoyl chlorides were added to an alcoholic solution containing the potassium phenolate of Compound V, a quality product was isolated in final yields of 71 to 86% for III*g–i*.

The final nitrogen mustard products (IV*a–i*) were prepared by the method of Benitez *et al.* (13). Although the procedure was conducted at low temperature, tarry crude products were always obtained initially and final yields were relatively low (22–62%). The use of another chlorinating agent, *e.g.*, phosphorus oxychloride or phosphorus pentachloride, proved less satisfactory than thionyl chloride.

Physical data for I*a–i* and II*a–i*, as well as the elemental analyses for II*a–i*, are presented in Table I. The *p*-aminophenyl octanoate failed to yield satisfactory elemental analysis but provided acceptable products from Step III. The physical data and elemental analyses for III*a–i* and the IV*a–i* are shown in Table II. Within each series of compounds there was observed a decrease in the melting points as the chain length of the acid increased to C_6 or C_8, then the melting points increased through the stearate derivative. An exception was noted in the acetate mustard (IV*a*) which was isolated as an oil containing an average of one-half mole of water of hydration. The remainder of the final products (IV*b–i*) followed this melting point trend.

All intermediates and final products were characterized by their IR and NMR spectra. Within each homologous series there were observed the expected characteristic IR and NMR absorptions. The effect of increasing the alkyl chain length produced the only notable spectral differences between members of the same series. The IR spectra of the I*a–i* contained the following characteristic absorptions: aromatic and aliphatic C—H stretching bands between 3100 and 2900 cm^{-1}, a carbonyl absorption band at 1750 cm^{-1}, and an absorption at 875 cm^{-1}, indicating the presence of the nitro group. The C—H stretching bands and the carbonyl band were present in this region for each series of compounds. Reduction of the I*a–i* to II*a–i* resulted in the loss of the nitro group absorption and the appearance of the N—H stretching absorption at ~3400 cm^{-1}. This distinct absorption changed to a broad band in the region of 3400–3200 cm^{-1}, when the II*a–i* were condensed with ethylene oxide, indicating the presence of III*a–i*.

Compounds IV*a–i* were distinguished from the corresponding *p*-bis(2-hydroxyethyl)amino compounds by the absence of the broad —OH band in the IR spectrum. In addition, the final products contained absorption in the region of 650 cm^{-1} that is characteristic for the C—Cl bond. The presence of water was indicated in the acetate mustard by the observation of a broad band from 3500 to 3300 cm^{-1}. Only slight shifts in the carbonyl absorption were observed between the different series of compounds.

The NMR spectra of each series of compounds contained characteristic absorptions for the alkyl side chain. The observed chemical shift values for the alkyl pattern did not demonstrate much difference from one series of compounds to the next. In each series the methyl group of the acetate derivatives appeared as a 3-proton singlet at 2.55. In the butyrate, through the stearate derivatives in each series, the pattern consisted of the following absorptions: 2.45–2.55 δ (t, 2H) for the methylene alpha to the carbonyl; a triplet (3H) centered in the range of 0.85–1.05δ for the terminal methyl group; and a multiplet (which appeared as a singlet for the higher members of each series) centered in the range of 1.5–2.1δ for the remainder of the alkyl chain. The aromatic protons of I*a–i* displayed an AA′XX′ pattern which appeared as two doublets (2H each) centered at 7.25 and 8.2δ. Reduction of I*a–i* to II*a–i* changed the aromatic proton absorption from the AA′XX′ pattern to an AA′BB′ pattern which exhibited two doublets (2H each) centered at 6.5 and 7.0δ. This pattern, which appeared to be a quartet, was essentially the same for the remaining series of compounds (III*a–i* and IV*a–i*). The esters II*a–i* also displayed a singlet at 3.52δ for the two amino protons which disappeared from the spectra of II*a–g* following the condensation with ethylene oxide.

The NH_2 absorption was replaced by the absorption characteristic for the *p*-[*N*,*N*-bis(2-hydroxyethyl)amino] moiety, which in this series includes two triplets (4H each) centered around 3.5 and 3.7δ and the two alcoholic protons, which appeared as a singlet between 3.0 and 4.2δ. The NMR spectra of the products isolated from the condensation with the

[2] Jackson Laboratories.
[3] ARS/Sprague–Dawley.
[4] National Cancer Institute, National Institutes of Health, Bethesda, Md.
[5] Model ZB Counter and accessories, Coulter Electronics.
[6] American Optical Co.
[7] Model RA, Carl Zeis, West Germany.
[8] Matheson, Coleman & Bell.
[9] Fisher Scientific Co.
[10] Microbiological Associates.
[11] Allied Chemical Co.

Table III—Summary of the Bioevaluation Data for the Fatty Acid Esters of *p*-[*N*,*N*-Bis(2-chloroethyl)amino]phenol

Compound Number (Derivative)	LD_{50}[a], μmoles/kg	Dose, mg/kg	Number of Animals	Mean Survival Day (±*SE*) Test Group	Mean Survival Day (±*SE*) Control Group	T/C %[b]
IV*a*,[c]		2.25	6			95
(Acetate)	50.8	2.03	6			106
		1.50	6			115
		1.00	6			108
		0.50	6			102
IV*b*,		75	6	4.0(0.37)	9.33(0.33)	44
(Butyrate)	<82.2	50	5	5.6(0.68)	9.33(0.33)	62
		25	6	10.5(0.56)	9.33(0.33)	113
		10	6	11.83(0.54)	9.33(0.33)	127
		5	6	12.17(0.75)	9.33(0.33)	130
		2	5	12.40(0.68)	9.33(0.33)	133
IV*c*,[c]		2.50	6			98
(Hexanoate)	53.3	2.02	6			112
		1.00	6			117
		0.50	6			113
IV*d*,		25	6	9.83(0.79)	8.60(0.40)	114
(Octanoate)	73.1	10	6	10.33(0.88)	8.60(0.40)	120
		5	5	12.20(0.37)	8.60(0.40)	142
		2	4	11.25(0.48)	8.60(0.40)	131
IV*e*,[c]		2.05	6			112
(Decanoate)	55.4	1.82	6			150
		1.50	6			131
		1.00	6			123
		0.50	6			110
IV*f*,		75	6	9.00(1.21)	9.33(0.42)	97
(Laurate)	>240	50	6	12.00(0.86)	8.8 (0.20)	136
		25	6	11.83(0.87)	8.8 (0.20)	134
		10	6	12.67(1.05)	8.8 (0.20)	144
		5	6	11.33(0.61)	9.33(0.42)	122
IV$_g$		100	6	7.00(1.10)	9.40(0.60)	75
(Myristate)	>225	50	6	13.33(1.31)	9.40(0.60)	142
		25	6	11.50(0.50)	9.40(0.60)	122
		10	6	11.83(0.87)	9.40(0.60)	162
		5	5	11.20(0.49)	9.40(0.60)	119
IV*h*		150	6	12.33(0.76)	8.7 (0.48)	142
(Palmitate)	>211.9	100	5	13.2(0.80)	8.7 (0.48)	152
		75	6	12.83(0.79)	8.7 (0.48)	148
		50	6	12.67(0.49)	9.5 (0.50)	133
		25	6	12.5(1.48)	9.5 (0.50)	132
		10	6	10.33(0.49)	9.5 (0.50)	109
IV*i*,	>200	200	6	13.33(0.67)	9.25(0.48)	144
(Stearate)		150	6	13.00(0.73)	9.25(0.48)	141
		100	6	12.00(0.77)	9.25(0.48)	130
		50	6	12.33(0.61)	9.50(0.56)	130
		25	6	11.67(0.56)	9.50(0.56)	123
		10	6	10.83(0.70)	9.50(0.56)	114
		5	6	10.33(0.49)	9.50(0.56)	109

[a] Administered in propylene glycol. [b] The T/C% represents the ratio of the sum of the number of days the animals in a treated group survived (T) to the sum of the number of days the animals in the control group survived (C) multiplied by 100. [c] A summary of data reported earlier.

p-aminophenyl palmitate and stearate esters, and one of the products from the *p*-aminophenyl myristate ester, indicated that only 1 mole of ethylene oxide had condensed with the amine. These spectra contained one amino proton and the ethylene triplets integrated for only two protons each. The NMR spectra of the final products (IV*a–i*) demonstrated a loss of the two alcoholic protons present in III*a–i*. In addition, the eight ethylene protons appeared as a sharp singlet in the region of 3.5–3.8δ. The aromatic protons and the alkyl side chain absorptions appeared as described previously. The presence of one-half mole of water of hydration in the acetate mustard (IV*a*) was verified by NMR, as well as IR and elemental analysis.

Biological Evaluation—The LD_{50} values for compounds IV*a* and IV*c–e* were determined using probit analysis and linear regression and are presented in μmoles/kg in Table III (6, 7). The data for the butyrate mustard (IV*b*) indicates that its LD_{50} is <25 mg/kg (82.2 μmoles/kg). Determination of exact LD_{50} values for compounds IV*f–i* was restricted by their limited solubility in propylene glycol. The 100 mg/kg dosage level was the highest that could be administered for those compounds in propylene glycol. Only a few isolated deaths occurred in the LD_{50} studies for IV*f* and IV*g*, and no deaths occurred at any dosage levels for IV*h* and IV*i*. The results of the LD_{50} study indicate that IV*b* and IV*d* have a toxicity slightly less than, but comparable to, IV*a*, IV*c*, and IV*e* reported earlier (3). All of these fatty acid ester derivatives (IV*a–i*) appear to be less toxic than the unhindered benzoate esters of a previous report (2).

The acetate through the decanoate mustards (IV*a–e*) demonstrates acute toxicities comparable to, or greater than, that observed for the parent phenolic mustard, *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol, 74.8 (2)–162.4 μmoles/kg in HA/ICR mice[12]. Compounds IV*a–e* are 2–3 times more toxic than the phenolic mustard when LD_{50} values in HA/ICR mice are compared. These esters are more lipophilic in nature and should be more readily absorbed and distributed in the biologic system than the phenolic mustard. This enhanced distribution, coupled with the possible ester hydrolysis to the free phenol, may account for these observations. The lower toxicities observed for compounds IV*f–i* may be related in part to their lower aqueous solubility. All of Compounds IV*a–i* appear to be much more toxic than the comparable series of even numbered ethers (C_2–C_{14}) of phenolic mustard (LD_{50} range 714–>1395 μmoles/kg) recently reported (4).

The effect of compounds IV*a–i* on the prolongation of life of L-1210 leukemic mice was determined. A comparison of the survival of treated groups (T) to untreated control groups (C) was performed. A calculated T/C ratio ≥125% implied that the drug treatment significantly increased the life span. Table III summarizes the T/C% survivals produced at each dosage level for each compound, the number of animals used per dosage level, and the mean survival for each animal group. The doses producing optimum survival for each compound are summarized in Table IV.

Compound IV*g* produced the highest mean survival time (T/C%) in this series, 162% at a dose of 10 mg/kg (22.5 μmoles/kg). Compounds IV*d*, IV*e*, and IV*g* produced significant survival at two dosage levels and compounds IV*b* and IV*f* at three dosage levels each. Compounds IV*h* and

[12] Unpublished data from these laboratories.

Table IV—Summary: Optimum Dose and Survival Time for the Fatty Acid Esters of *p*-[*N*,*N*-bis(2-chloroethyl)amino]phenol

Compound Number (Derivative)	Optimum Dose		T/C%
	mg/kg	μmoles/kg	
IV*a* (Acetate)	1.5	5.4	115
IV*b* (Butyrate)	2.0	6.6	133
IV*c* (Hexanoate)	1.0	3.0	117
IV*d* (Octanoate)	5.0	13.9	142
IV*e* (Decanoate)	1.82	4.7	150
IV*f* (Laurate)	10.0	24.0	144
IV*g* (Myristate)	10.0	22.5	162
IV*h* (Palmitate)	100.0	211.9	152
IV*i* (Stearate)	200.0	400.0	144

IV*i* produced significant survival at five and four dosage levels, respectively. Compounds IV*a* and IV*c* failed to produce significant T/C% survival at any of the dosage levels investigated.

The enzymatic hydrolysis rates for a series of fatty acid esters of acetaminophen have been determined by Bauguess *et al.* (8). Those results indicate the near complete and rapid hydrolysis of the acetate and butyrate esters and a decrease in the rate of hydrolysis for the hexanoate ester which was more rapidly hydrolyzed than the octanoate ester. The rate and completeness of hydrolysis declined from the decanoate through the myristate esters, and only limited hydrolysis could be obtained for the palmitate and stearate esters of acetaminophen. This work by Bauguess, coupled with the suggestion by Hansch *et al.* (5) that the antitumor activity for esters of phenolic mustard is due primarily to the intact ester rather than the hydrolysis product, phenol mustard, provides some insight into the biologic results reported in this paper.

Compounds IV*h* and IV*i* demonstrated low toxicity in the survival studies as well as the toxicity studies. The results for these two compounds are consistent with the expectation of a slow and limited enzymatic hydrolysis rate *in vivo*. The high doses for these compounds required the use of a different solvent mixture due to their limited solubility in propylene glycol. It is feasible that their poor solubility and hydrolysis properties resulted in a continuous and prolonged absorption from the injection site, thus providing significant survival over a greater range of doses. Both IV*f* and IV*g* demonstrated toxicity at the highest respective doses used in the survival study. These two compounds produced significant survival at the medium dosage levels, while activity dropped off at the lowest dose level (5 mg/kg). The toxicity at the higher dosage levels is consistent with an expected increase in hydrolysis of these two compounds, as well as indicating toxicity differences between the leukemic test mice (BDF_1 strain) and the HA/ICR strain used in the LD_{50} determinations. The optimum survival dose level for both IV*f* and IV*g* of 10 mg/kg was much less than IV*h* and IV*i* but was greater than the lower members of the series, IV*a–e*. Optimum survival was observed at lower dosage levels for IV*c*, IV*d*, and IV*e* (Table IV). This observation is consistent with the higher toxicity observed for these compounds, as well as the expected increase in enzymatic hydrolysis when compared to compounds IV*f–i*.

Compound IV*b* produced optimum survival at a slightly higher dose than IV*c* (6.6 *versus* 3.0 μmoles/kg). If the intact ester derivative of phenolic mustard is the active antileukemic molecule, then a higher dose would be required for IV*b* to achieve optimum activity in order to compensate for its anticipated rapid hydrolysis.

Compound IV*a* produced optimum survival at a dose comparable to the optimum dose of IV*b*. The failure of IV*a* to produce significant survival at any dose level suggests that an effective level of IV*a* could not be sustained long enough to be effective against the leukemia, probably due to very rapid hydrolysis *in vivo*. Compound IV*c* also failed to significantly prolong the life of leukemic mice. This observation cannot be related to the expected hydrolysis within the series. However, in the alkyl ether series studied earlier, the *n*-hexyl derivative produced similar survival results in leukemic mice (4).

The possibility that the 6-carbon ether derivative is of appropriate length to interact with the nitrogen atom when in dilute solution, has been suggested. A similar interaction could occur between the terminal methyl of the hexanoate side chain and the nitrogen atom of the mustard moiety. Such an interaction could sterically hinder the participation of the nitrogen atom in the formation of the aziridinium ion intermediate, thus reducing the reactivity of IV*c*. This in turn could reduce its antileukemic effectiveness.

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