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Synthesis and Bioevaluation of a Series of Alkyl Ethers of *p*-*N*,*N*-Bis(2-chloroethyl)aminophenol

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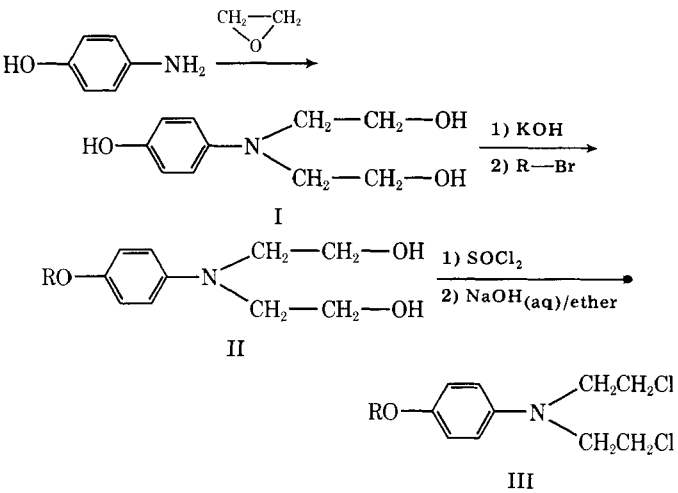
Abstract □ A series of even numbered normal alkyl ethers (C_2–C_{14}) of *p*-*N*,*N*-bis(2-chloroethyl)aminophenol were synthesized and evaluated as to acute toxicity in mice and effects on survival in L-1210 leukemic mice. All of the ether derivatives demonstrated significantly lower acute toxicity than the parent phenol mustard. Significant survival times (≥125%) were obtained with all compounds except the hexyl derivative. The decyl ether produced the greatest significant increase and the ethyl ether the lowest significant increase in mean survival time. Significant survival times were produced at four dosage levels for the butyl, decyl, and dodecyl derivatives, three dosage levels for the octyl and tetradecyl derivatives, and one dosage level for the ethyl derivative.

Keyphrases □ Alkyl ethers—synthesis of alkyl ethers *p*-*N*,*N*-bis(2-chloroethyl)aminophenol □ Bioevaluation—alkyl ethers of *p*-*N*,*N*-bis(2-chloroethyl)aminophenol, toxicity in HA/ICR mice □ Antitumor activity—alkyl ethers of *p*-*N*,*N*-bis(2-chloroethyl)aminophenol, survival in L-1210 leukemic mice

Phenol mustard, *p*-[*N*,*N*-bis(2-chloroethyl)amino]-phenol, has demonstrated a low therapeutic index (1). Less toxic derivatives of phenol mustard have resulted from the synthesis of various esters of the phenol (1–3).



IIa, IIIa: R = CH_3—CH_2—
IIb, IIIb: R = CH_3—$(CH_2)_2$—CH_2—
IIc, IIIc: R = CH_3—$(CH_2)_4$—CH_2—
IId, IIId: R = CH_3—$(CH_2)_6$—CH_2—
IIe, IIIe: R = CH_3—$(CH_2)_8$—CH_2—
IIf, IIIf: R = CH_3—$(CH_2)_{10}$—CH_2—
IIg, IIIg: R = CH_3—$(CH_2)_{12}$—CH_2—

Scheme I

In an effort to develop latent derivatives of phenol mustard, a series of its substituted benzoate esters were studied (1). The results lent support to the hypothesis that hydrolysis of esters of *p*-[*N*,*N*-bis(2-chloroethyl)amino]-phenol to the free phenol mustard is a necessary step for antitumor activity (1). The effectiveness of aniline mustard, *N*,*N*-bis(2-chloroethyl)aniline, in the treatment of advanced plasma cell tumors has been demonstrated (4). The results indicated that aniline mustard was the optimally active compound in this system, and only those compounds that could be metabolized to a phenolic mustard demonstrated activity.

A series of alkyl ethers of phenol mustard should provide potentially latent nitrogen mustard derivatives with variable metabolic routes (5, 6). Such compounds would be expected to demonstrate antineoplastic activity with a reduction in host toxicity. In addition, lipophilicity would increase with the length of the alkyl chain. The specific objectives of this investigation were to: (*a*) synthesize a series of even-numbered, normal alkyl ethers (C_2–C_{14}) 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 Scheme I.

EXPERIMENTAL

Chemistry[1]—p-N,N-*Bis(2-hydroxyethyl)aminophenol* (*I*)—Twenty grams (0.18 mole) of *p*-aminophenol was added to a flask containing 200 ml of absolute methanol and equipped with a reflux condenser. The solution was stirred mechanically until dissolution occurred and then was cooled to 0°. Twenty grams (0.45 mole) of ethylene oxide was added to the cold reaction mixture. Stirring was continued and the reaction was allowed to reach room temperature. The resulting crystals were filtered and recrystallized from ethanol. The product had a melting point of 139.5–141° compared with the reported value of 140° (7).

[1] All IR spectral data were obtained from chloroform solutions of the derivatives on sodium chloride plates using a Beckman Model Acculab-4 spectrophotometer. Nuclear magnetic resonance spectra were obtained from deuterated chloroform solutions of the derivatives using a Hitachi-Perkin-Elmer model R-24 high resolution spectrometer with tetramethylsilane as the internal standard. The reported melting points were obtained using a Thomas Hoover capillary melting point apparatus and are uncorrected. The reported content of hydrogen, carbon, and nitrogen were obtained from analyses performed by Galbraith Laboratories, Knoxville, Tenn. All lyophilization was accomplished using a VirTis Model 10 freeze-dryer.

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Table I—Physical Data and Elemental Analysis for the *p-N,N*-Bis(2-hydroxyethyl)aminophenyl Alkyl Ethers

Compound	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
II*a*	$CH_3—CH_2—$	82	34–35°	$C_{12}H_{19}NO_3$	C 64.00	63.83
					H 8.44	8.39
					N 6.22	6.33
II*b*	$CH_3—(CH_2)_2—CH_2—$	60.5	41–42°	$C_{14}H_{23}NO_3$	C 66.40	66.29
					H 9.09	9.23
					N 5.53	5.43
II*c*	$CH_3—(CH_2)_4—CH_2—$	75	43–44°	$C_{16}H_{27}NO_3$	C 68.33	68.39
					H 9.61	9.85
					N 4.98	4.74
II*d*	$CH_3—(CH_2)_6—CH_2—$	63	47.5–49°	$C_{18}H_{31}NO_3$	C 69.90	70.12
					H 10.03	10.14
					N 4.53	4.38
II*e*	$CH_3—(CH_2)_8—CH_2—$	69	49.5–51°	$C_{20}H_{35}NO_3$	C 71.22	71.00
					H 10.38	10.56
					N 4.15	4.03
II*f*	$CH_3—(CH_2)_{10}—CH_2—$	82	55–56.5°	$C_{22}H_{39}NO_3$	C 72.33	72.49
					H 10.68	10.82
					N 3.83	3.74
II*g*	$CH_3—(CH_2)_{12}—CH_2—$	45	58–59°	$C_{24}H_{43}NO_3$	C 73.28	73.02
					H 10.94	10.87
					N 3.56	3.22

Table II—Physical Data and Elemental Analysis for the *p-N,N*-Bis(2-chloroethyl)aminophenyl Alkyl Ethers

Compound	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
III*a*	$CH_3—CH_2—$	65	Oil	$C_{12}H_{17}Cl_2NO$	C 54.98	54.96
					H 6.49	6.29
					N 5.35	5.27
III*b*	$CH_3—(CH_2)_2—CH_2—$	53	Oil	$C_{14}H_{21}Cl_2NO$	C 57.95	58.14
					H 7.24	7.47
					N 4.83	4.81
III*c*	$CH_3—(CH_2)_4—CH_2—$	63	Oil	$C_{16}H_{25}Cl_2NO$	C 60.40	60.50
					H 7.86	7.80
					N 4.40	4.23
III*d*	$CH_3—(CH_2)_6—CH_2—$	49	Oil	$C_{18}H_{29}Cl_2NO$	C 62.45	62.44
					H 8.38	8.50
					N 4.05	3.99
III*e*	$CH_3—(CH_2)_8—CH_2—$	89	35.5–36.5°	$C_{20}H_{33}Cl_2NO$	C 64.19	64.29
					H 8.83	9.00
					N 3.74	3.62
III*f*	$CH_3—(CH_2)_{10}—CH_2—$	55	44–45°	$C_{22}H_{37}Cl_2NO$	C 65.69	65.54
					H 9.21	9.30
					N 3.48	3.39
III*g*	$CH_3—(CH_2)_{12}—CH_2—$	59	Oil	$C_{24}H_{41}Cl_2NO$	C 66.98	66.73
					H 9.53	9.79
					N 3.26	2.92

Synthesis of p-N,N-*Bis(2-hydroxyethyl)aminophenyl Alkyl Ethers* (*II a–g*)—Ten grams (0.05 mole) of I was dissolved in a reaction flask containing 300 ml of ethanol, 200 ml of water, and 7 g of KOH. This mixture was stirred and heated to reflux. An excess (0.15 mole) of the appropriate alkyl bromide (ethyl, butyl, hexyl, octyl, decyl, dodecyl, or tetradecyl) was introduced and reflux was continued for 24 hr. Excess solvent was removed *in vacuo* and the residue was mixed with ether. The ether solution was filtered to remove potassium bromide. The ethereal filtrate was extracted three times with 10% KOH solution to remove unreacted starting material. The ether layer was evaporated *in vacuo*. With the exception of the butyl, octyl, and tetradecyl derivatives, solid products suitable for analysis were obtained after cooling to 0°. The butyl and octyl derivatives were recrystallized from a methanol–water system, and lyophilized to yield analytically pure product. The tetradecyl derivative was purified by recrystallization from an acetone–water system. The new compounds were characterized by physical data (Table I) and IR and NMR spectra.

Synthesis of p-N,N-*Bis(2-chloroethyl)aminophenyl Alkyl Ethers* (*III* a–g)—Three-tenths of a mole of the appropriate derivative of II was dissolved in a minimum amount of chloroform. Five milliliters of absolute ethanol, then 1 ml of thionyl chloride, was added during continuous stirring. The reaction mixture was cooled to 0° on dry ice, 10 ml of thionyl chloride was added and the solution was allowed to reach room temperature. The mixture was concentrated *in vacuo* to remove solvent and excess thionyl chloride. Upon standing, solid products were obtained for the decyl and dodecyl derivatives. These products were recrystallized from an ethanol–water system and light-colored crystals were obtained. The ethyl, butyl, hexyl, and octyl derivatives were initially isolated as the hydrochloride salt. These products were converted to the free base by mixing with 10% NaOH and extracting with ether. Evaporation of the ether yielded oils suitable for analysis for the ethyl and hexyl derivatives. The butyl and octyl derivatives were recrystallized from an acetone–water system and lyophilized to yield analytical samples. The tetradecyl derivative was initially isolated as the free base and purified by recrystallization from an ethanol–water system. The ethyl, butyl, and octyl derivatives existed as black crystalline solids below 15° and as oils at room temperature. The hexyl derivative was an oil at 0° and room temperature. The tetradecyl derivative existed as a brown semi-solid below 15° and as an oil at room temperature. The final products were characterized by physical data (Table II) and IR and NMR spectra.

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

[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 Assoc.
[11] Allied Chemical Co.

Table III—Summary of the Bioevaluation Data for the *p*-*N*,*N*-Bis(2-chloroethyl)aminophenyl Alkyl Ethers

Compound Number (Derivative)	LD_{50}[a], μmoles/kg	Dose[a], mg/kg	Number of Animals	Mean Survival Day (±*SE*)	Control Mean Survival Day (±*SE*)	T/C, %[b]
III*a* (Ethyl)	1640	25	6	9.83 (0.31)	8.50 (0.34)	116
		50	6	10.00 (0.68)	8.50 (0.34)	118
		75	6	9.33 (0.42)	8.50 (0.34)	110
		100	6	10.67 (0.21)	8.50 (0.34)	126
		125	6	10.17 (0.17)	8.50 (0.34)	120
		150	6	9.33 (0.92)	8.50 (0.34)	110
		200	6	7.33 (0.67)	8.50 (0.34)	86
III*b* (Butyl)	714	25	6	9.50 (0.34)	9.00 (0.37)	106
		50	6	10.00 (0.63)	9.00 (0.37)	110
		75	5	11.40 (0.51)	9.00 (0.37)	127
		100	5	11.60 (0.87)	9.00 (0.37)	129
		125	6	12.50 (0.56)	9.00 (0.37)	139
		150	6	12.83 (0.54)	9.00 (0.37)	143
		200	6	6.50 (0.34)	9.00 (0.37)	72
III*c* (Hexyl)	1069	25	6	10.00 (0.52)	9.33 (0.21)	107
		50	6	10.00 (0.52)	9.33 (0.21)	107
		75	6	10.50 (0.43)	9.33 (0.21)	113
		100	6	10.17 (0.48)	9.33 (0.21)	109
		125	6	10.67 (0.42)	9.33 (0.21)	114
		150	6	9.33 (0.21)	9.33 (0.21)	100
		200	6	9.83 (0.31)	9.33 (0.21)	105
III*d* (Octyl)	>1156	25	5	9.20 (0.66)	7.83 (0.31)	118
		50	6	9.67 (0.33)	7.83 (0.31)	123
		75	6	9.83 (0.40)	7.83 (0.31)	126
		100	5	10.20 (0.37)	7.83 (0.31)	130
		125	6	12.33 (0.80)	9.00 (0.37)	137
		150	6	10.83 (0.79)	9.00 (0.37)	120
		200	6	10.00 (0.37)	9.00 (0.37)	111
III*e* (Decyl)	>1070	25	6	9.33 (0.42)	9.00 (0.37)	104
		50	6	10.33 (0.49)	9.00 (0.37)	115
		75	6	11.67 (0.61)	9.00 (0.37)	130
		100	6	13.17 (0.70)	9.00 (0.37)	146
		125	6	14.67 (0.61)	9.00 (0.37)	163
		150	6	14.50 (0.80)	9.00 (0.37)	161
		200	6	7.67 (0.33)	9.00 (0.37)	85
III*f* (Dodecyl)	>995	25	6	12.33 (0.42)	9.00 (0.37)	137
		50	5	12.60 (0.51)	9.00 (0.37)	140
		75	6	13.83 (0.48)	9.00 (0.37)	154
		100	6	12.33 (0.56)	9.00 (0.37)	137
		125	6	11.00 (0.58)	9.00 (0.37)	122
		150	6	9.67 (0.44)	9.00 (0.37)	107
		200	6	9.83 (0.31)	9.00 (0.37)	109
III*g* (Tetradecyl)	>1395	25	6	10.17 (0.31)	9.00 (0.37)	113
		50	5	10.40 (0.25)	9.00 (0.37)	116
		75	6	11.33 (0.33)	9.00 (0.37)	126
		100	5	12.20 (0.37)	9.00 (0.37)	136
		125	5	11.40 (0.51)	9.00 (0.37)	127
		150	6	11.00 (0.58)	9.00 (0.37)	122
		200	5	9.00 (0.37)	9.00 (0.37)	100

[a] Administered in propylene glycol. [b] The T/C% value 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.

Wright's stain[8] were obtained commercially. The ether derivatives of III were prepared in the laboratory, and their physical and analytical data are reported in Table III.

Pretoxicity Testings—Groups of two healthy (HA/ICR) male mice were injected intraperitoneally with 500[12], 100, 10, 1, or 0.1 mg of test drug/kg in propylene glycol to evaluate the dose range for the pharmacological screen and LD_{50} determination.

Pharmacological Screen and LD_{50} Evaluation—Five groups, each with six HA/ICR mice, 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 (8) and a graphic method (9) were applied to determine the LD_{50} for each drug test.

Transplantation Procedures—Sterile equipment was used under aseptic conditions. DBA/2 (6–9 week old) host mice bearing L-1210 leukemia for 6–7 days were the tumor donors, and were sacrificed under ether vapor, immersed in 0.1% benzalkonium chloride, and swabbed with 70% ethanol. A 10-ml hypodermic syringe equipped with a 20-gauge needle (flushed with heparin at 1000 USP U/ml) was inserted into the abdominal cavity of the mouse and aspirated to obtain the lymphoid leukemia L-1210 cells (10). The leukemia cells were placed in a container over ice. One drop of ascitic fluid was prepared for microscope examination. Cell morphology was determined by staining with Wright's or Giemsa stain to differentiate the leukocytes and lymphoblasts (11). Ascitic fluid containing at least 95% lymphoblasts or lymphocytes was used for transplantation in DBA/2 mice for tumor maintenance and in BDF_1 mice for antileukemic studies. Cell counts and viability were determined using an electronic counter and a hemocytometer, respectively, as described in a recent report (3). The inoculum contained 1×10^5 cells in 0.1 ml. A 1-ml tuberculin syringe equipped with a 25-gauge needle was used to inject 0.1 ml of diluted ascitic L-1210 cells into the DBA/2 or BDF_1 mice. All inoculations of L-1210 cells were made within 1 hr after cell removal from the host mouse to ensure a viable transplant.

In Vivo *Determination of Antileukemic Activities by Survival Times*—On Day 0, the tumor was implanted into BDF_1 male mice; the number of survivors was recorded daily during the test period. The doses used were below the LD_{10} value of each test drug to minimize drug toxicity (12). The test solutions were injected within 15 min after preparation at a dose volume not to exceed 0.01 ml/g of body weight. The test drug solutions were administered intraperitoneally to each test group on Days

[12] Compounds III*d*, III*e*, and III*f* were limited by solubility to an upper dosage of 400 mg/kg.

2 and 5. The experiment was evaluated on the day of death for the last animal in a test group or after 30 days (12).

RESULTS AND DISCUSSION

Chemistry—The starting material (I) was obtained in 90% yields using a modification of the condensation of ethylene oxide with *p*-aminophenol reported previously (7). The ether intermediates (II*a–g*) were then obtained by reacting I with the appropriate alkyl bromide. The conditions for the Williamson synthesis of *p*-nitrophenyl ethers (13) were modified to produce II*a–g*. Because I has a higher pKa than *p*-nitrophenol, more base and a more polar solvent system were used to enhance the formation of the phenolate nucleophile. The reaction proceeded *via* a S_N2 displacement of the bromide by the phenolate nucleophile (14).

The primary isolation and purification step was the formation of the potassium salt of unreacted I and subsequent extraction with ether. Theoretical yields for II*a–g* ranged from 45 to 82% (Table I). The alkyl ethers of I were stable, low-melting solids with solubility in a variety of organic solvents. The IR spectra of these intermediates exhibited characteristic —OH absorption as a broad band between 3600 and 3100 cm^{-1}. The sharp absorption at 1240 cm^{-1} confirmed the presence of the ether function. The aliphatic and aromatic C—H stretching frequencies appeared in the region of 3150–2850 cm^{-1}. The intensity of the aliphatic absorption varied with the size of the alkyl substituent. The aromatic ring stretching occurred at 1520 cm^{-1}. The NMR spectra of these compounds, using $CDCl_3$ as a solvent, contained four distinct absorption patterns. A triplet (2H) (quartet for the ethyl derivative, II*a*) centered at 3.9 ppm, was observed for the methylene group adjacent to the ether oxygen. The remaining methylene groups in the alkyl side chain appeared, in the range of 1.6 to 2.0 ppm, as a multiplet for II*b* and II*c* and as a sharp singlet for the remaining homologs. The terminal methyl group absorbed as a triplet (3H) in the region of 1.2–1.4 ppm. The ethylene bridge yielded two triplets integrating for four protons each. The triplet at 3.3 ppm was assigned to the $—CH_2—$ groups adjacent to the nitrogen and the triplet at 3.7 ppm was assigned to the $—CH_2—$group adjacent to the —OH group. The alcoholic proton absorption appeared as a singlet (2H) in the region of 4.0–4.3 ppm. The four aromatic protons appeared as a typical A_2B_2 multiplet centered in the region of 6.6–6.7 ppm. Analytical purity was determined by elemental analysis (Table I).

The final products (III*a–g*) were obtained using thionyl chloride as the chlorinating agent. The vigor of the reaction was controlled by cooling to <0° and allowing the reaction mixture to slowly reach room temperature. The generation of hydrochloride gas in this reaction led to the formation and isolation of the hydrochloride salts of the ethyl, butyl, hexyl, and octyl derivatives. The IR spectra of the hydrochloride salts contained a strong absorption at 2400 cm^{-1} and medium absorptions at 3620 and 3650 cm^{-1}, confirming the presence of a tertiary amine salt. The NMR spectra of the hydrochloride salts contained a singlet at 7.0 ppm (N—H) and the aromatic protons displayed an AA'BB' pattern which appeared as two doublets centered at 6.8 and 7.7 ppm. Following neutralization with base, the N—H absorptions disappeared from both the IR and NMR spectra and the NMR pattern for the aromatic protons merged into a typical A_2B_2 multiplet centered at 6.6 ppm.

All of the final products (III*a–g*) were isolated and characterized as their free bases in yields ranging from 49 to 89%. The hydrochloride salts were neutralized by extracting ether solutions of the salts with 10% NaOH. Compounds III*a* and III*c* were isolated as oils suitable for analysis following the evaporation of the ether solution. All other products were recrystallized to yield analytically pure oils or solids (Table II). (Compounds III*b* and III*d* were also lyophilized.) The final products (III*a–g*) demonstrated solubility in ether, chloroform, ethanol, and methanol and were nearly insoluble in water. Upon chlorination of the dihydroxy intermediates (II*a–g*), the broad free OH band on the IR spectra disappeared and absorptions characteristic of halogenated hydrocarbons appeared at 1220 and 750 cm^{-1}. The NMR spectra of the nitrogen mustard derivatives (III*a–g*) demonstrated the loss of 2—OH protons. In addition the eight ethylene protons appeared as a sharp singlet in the region of 3.6–3.9 ppm. Other absorptions in the NMR spectra of compounds III*a–g* appeared as described for their precursors (II*a–g*). The purity of the nitrogen mustard products (III*a–g*) was determined by elemental analysis (Table II).

Biological Evaluation—The LD_{50} values for compounds III*a–c* were obtained using probit analysis and linear regression (Table III) (8, 9). Determination of exact LD_{50} values for compounds III*d–g* was restricted by the limited solubility of those compounds in propylene glycol. The LD_{50} values for compounds III*d–g* presented in Table III represent the highest doses that could be administered in propylene glycol. No deaths occurred at those dose levels, therefore, the LD_{50} values are considerably higher. As anticipated, the ether derivatives of phenol mustard demonstrated much less toxicity than reported for phenol mustard or for its unhindered benzoate and fatty acid esters (1, 3). In this series the butyl derivative (III*b*) exhibited the highest toxicity, 714 μm/kg, compared to a 74.8–162.4 μm/kg[13] range for the phenol mustard (1). The remaining compounds, III*a* and III*c–g*, demonstrated LD_{50} values comparable to, or higher than, cyclophosphamide, 1078 μm/kg in HA/ICR mice, an effective antineoplastic agent that is less toxic than clinically useful nitrogen mustards (3).

Table IV—Summary: Optimum Dose and Survival Time for the *p*-*N*,*N*-bis(2-chloroethyl)aminophenyl Alkyl Ethers

Compound Number (Derivative)	Optimum Dose		T/C, %
	mg/kg	μmoles/kg	
III*a* (Ethyl)	100	382	126
III*b* (Butyl)	150	517	143
III*c* (Hexyl)	125	393	114
III*d* (Octyl)	125	361	137
III*e* (Decyl)	125	335	163
III*f* (Dodecyl)	75	187	154
III*g* (Tetradecyl)	100	233	136

The effect of compounds III*a–g* 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 dose 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.

The decyl derivative (III*e*) produced the highest mean survival time (T/C% values) in the series, 163% at a dose of 335 μm/kg (125 mg/kg) as illustrated in Table IV. The ethyl derivative (III*a*) demonstrated the lowest significant optimum T/C% values in this series, 126% at 382 μm/kg (100 mg/kg). This was the only dose level at which significant survival (≥125%) was obtained for the ethyl derivative (III*a*). The butyl (III*b*), decyl (III*e*), and dodecyl (III*f*) derivatives produced significant survival at four dosage levels each and the octyl (III*d*) and tetradecyl (III*g*) derivatives at three dosage levels. Only the hexyl derivative (III*c*) failed to produce a significant T/C% value at any of the dosage levels investigated.

The differences in the observed toxicities and T/C% survival times may possibly be attributed to differences in both the metabolism and lipophilic character of the compounds studied. The ethyl ether of *p*-nitrophenol has been shown to undergo *O*-dealkylation both at a rapid rate *in vitro* and to a great extent (78%) *in vivo* (5). Conjugation of the phenolic mustard resulting from the *O*-dealkylation of III*a* would result in enhanced elimination, lower toxicity, and reduced antineoplastic activity. The butyl ether of *p*-nitrophenol has been shown to undergo *O*-dealkylation at a much slower rate and to a lesser extent than the ethyl ether (5). The (ω-1)-hydroxylation pathway has been reported to be a primary metabolic route for the butyl ether of *p*-nitrophenol in rabbits (6). Longer chain alkyl ethers of *p*-nitrophenol undergo *O*-dealkylation at a negligible rate and to a lesser extent *in vivo* and the (ω-1)-hydroxylation pathway is probably most important in their metabolism (5, 6). The lower LD_{50} value and the activity observed against L-1210 mouse leukemia for the butyl derivative (III*b*) may be attributed to the role of these metabolic pathways, as well as the solubility of the parent compound and the (ω-1)-hydroxy metabolite. The observation of lower toxicity for the longer chain ether derivatives, hexyl (III*c*)–tetradecyl (III*g*), is consistent with the expectation of negligible *O*-dealkylation for any of them. For the octyl (III*d*)–tetradecyl (III*g*) derivatives, the lower toxicity, coupled with an increase in the lipid solubility of both the parent compounds and their (ω-1)-hydroxy metabolites, should contribute to the observed antileukemic activity.

The lack of a significant prolongation of life in leukemic mice, at any of the dosage levels evaluated for the hexyl derivative (III*c*), cannot be explained on the basis of expected metabolism nor by the anticipated solubility properties of the compound and its (ω-1)-hydroxy metabolite. The role played by a complex combination of these factors may be important and could serve as the basis for a future investigation. An additional possibility is that in an aqueous solution of these compounds, a freely rotating alkyl sidechain could fold back and assume a conformation that would allow interaction between the sidechain and the ring and/or

[13] Unpublished data from these laboratories.

the bis(2-chloroethyl)amino moiety. In an attempt to gain further insight into this consideration, stereomodels and atomic models were constructed for the final products (IIIa–g). From both model types it appeared that the hexyl side chain was the appropriate length to allow the terminal methyl group to sterically affect the nitrogen atom. The ethyl and butyl side chains were too short for such an interaction and side chains longer than hexyl appeared subject to repulsion by the chloroethyl groups, thus, reducing their steric interaction with the nitrogen atom. Such an interaction between the nitrogen atom and the hexyl side chain could sterically hinder the participation of the nitrogen atom in the formation of the aziridinium ion intermediate. Such an interaction could reduce the reactivity of the hexyl derivative (IIIc) which would in turn reduce its antileukemic effectiveness and contribute to its relatively low toxicity.

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Comparison of the Absorption, Excretion, and Metabolism of Suxibuzone and Phenylbutazone in Humans

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Abstract □ The absorption, excretion, and metabolism of a single oral dose of suxibuzone, a new nonsteroidal anti-inflammatory agent, in healthy male volunteers were compared with those of phenylbutazone. After oral administration of either suxibuzone or phenylbutazone, phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone were found in the plasma; phenylbutazone was the main metabolite of suxibuzone and phenylbutazone. In the urine, *p*-γ-dihydroxyphenylbutazone and several glucuronide conjugates also were found. Spectrometric and/or enzymatic analysis showed that these glucuronide conjugates were suxibuzone glucuronide, 4-hydroxymethylphenylbutazone glucuronide, 4-hydroxymethyloxyphenbutazone glucuronide, oxyphenbutazone glucuronide, and phenylbutazone glucuronides (two types: *O*-glucuronide and C-4-glucuroxide) after suxibuzone administration, and oxyphenbutazone glucuronide and phenylbutazone glucuronide after phenylbutazone administration. The conjugates specific to suxibuzone administration, suxibuzone glucuronide, 4-hydroxymethylphenylbutazone glucuronide, and 4-hydroxymethyloxyphenbutazone glucuronide, were excreted in the first 6 hr urine. These findings and the pharmacokinetics of these metabolites in the plasma and urine show that suxibuzone is a prodrug of phenylbutazone.

Keyphrases □ Suxibuzone—*in vivo* absorption, excretion, and metabolism compared to phenylbutazone, humans □ Phenylbutazone—*in vivo* absorption, excretion, and metabolism compared to suxibuzone, human □ Pharmacokinetics—suxibuzone and phenylbutazone, *in vivo* humans □ Anti-inflammatory agents—suxibuzone and phenylbutazone, *in vivo*, humans

Suxibuzone is a derivative of phenylbutazone, in which the proton at the C-4-position of the pyrazolidine ring is replaced by a β-carboxypropionyloxymethyl group. The outstanding feature of the drug is that it has extremely low ulcerogenicity (1, 2) although its anti-inflammatory, analgesic, and antipyretic properties are as strong as those of an equimolar dosage of phenylbutazone (3).

This feature of suxibuzone can be understood by comparing the biological fates of suxibuzone and phenylbutazone; however, there have been no studies on metabolism of suxibuzone in humans. Therefore, the present study compared the metabolic pathways and pharmacokinetics of suxibuzone and phenylbutazone in humans.

EXPERIMENTAL

Materials—Suxibuzone[1], phenylbutazone[1], oxyphenbutazone[1], and γ-hydroxyphenylbutazone[1] were used as received. 4-Hydroxymethylphenylbutazone[2] was synthesized and purified.

Chromatography—HPLC was performed using a μ-Bondapak C_{18} column (30 × 0.4-cm i.d.)[3], which was fitted with a 254-nm UV detector. For low-resolution liquid chromatography, a column (20 × 2.5 cm) packed with Amberlite XAD-2 resin (coarse grade 35–50 mesh)[4] or Dowex-1 resin (200–400 mesh)[5] was used.

GC was carried out under the following conditions: column 5% Silicon GE SE-30 on Chromosorb W AW-DMCS 60–80 mesh[6], 2 m × 3-mm i.d.; column temperature, 275°; nitrogen flow rate, 60 ml/min; and detector, flame ion detector.

TLC was carried out using commercial silica gel plates[7] with the following solvent systems (SS):

[1] S. A. Esteve Laboratory, Barcelona, Spain.
[2] Taiho Pharmaceutical Co., Research Laboratory, Tokushima, Japan.
[3] Waters Associates, Milford, Mass.
[4] Rohm and Haas Co.
[5] Dow Chemical Co.
[6] Shimadzu, Kyoto, Japan.
[7] Kieselgel 60 F_{254}, Precoated, 0.25-mm thick, Merck, West Germany.

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