Preparation of 4-[p-[Bis(2-chloroethyl)amino]phenyl]butyramides as Potential Antitumor Agents I

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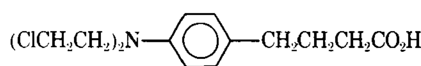
Abstract □ Six of the title compounds were synthesized and submitted for antitumor evaluation. Three of them exhibited significant activity in initial screening tests by the National Cancer Institute.

Keyphrases □ Chlorambucil—synthesis and screening of related 4-[p-[bis(2-chloroethyl)amino]phenyl]butyramides as potential antitumor agents □ 4-[p-[Bis(2-chloroethyl)amino]phenyl]butyramides—synthesized and screened as potential antitumor agents □ Antitumor agents, potential—synthesis and screening of 4-[p-[bis(2-chloroethyl)amino]phenyl]butyramides

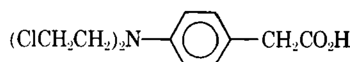
Chlorambucil (I), 4-[p-[bis(2-chloroethyl)amino]phenyl]butyric acid, has been shown to exhibit marked antitumor activity, primarily by virtue of its properties as an alkylating agent. It has found extensive clinical utilization in the treatment of chronic lymphocytic leukemia (1, 2) and ovarian carcinoma (3, 4) as well as in conjunction with other drugs (*e.g.*, 5–7). Chlorambucil exists predominately as the carboxylate ion at physiological pH; under these conditions the nitrogen mustard group has been shown to be significantly more reactive than when the molecule is in the undissociated form (8).

DISCUSSION

Linford (9, 10) postulated that the greater activity of chlorambucil in basic media involves interaction of the chloroethyl groups with the carboxylate groups of the chlorambucil molecule. This interaction would increase the reactivity of the nitrogen mustard group, thus explaining the effect of the length of the alkanoic acid chain and its point of attachment to the benzene ring. This is also thought to be a major factor in explaining the increased selectivity of chlorambucil for proteins. It was noted (11, 12) that with aralkyl carboxylic acids the diffusion through cellular membranes seems to be promoted by unionized carboxyl groups. Several instances noted in the recent literature suggest that various derivatives of cytotoxic aralkyl acids, notably esters and amides, significantly decrease the toxicity of these compounds and may considerably enhance therapeutic indexes. Niculescu-Duvaz *et al.* (13), in a study of nitrogen mustards of various methylbenzoic acids, noted that the methyl ester of one isomer (unavailable as the free acid) showed an LD_{50} value of 300 mg/kg, as opposed to a range of 15–62 for the other acids, with antineoplastic activity similar in degree to the other compounds.



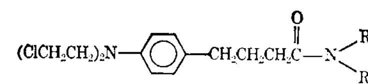
I



II

Vollmer *et al.* (14, 15) and Schaeppi *et al.* (16) reported on phenesterine, estradiol mustard, and dehydroepiandrosterone mustard, which are esters of 4-[p-[bis(2-chloroethyl)amino]phenyl]acetic acid (II); these appear to be significantly less toxic and more suitable for use than II itself. Bardos *et al.* (17) and Masnyk (18), in comparing a number of nitrogen mustard derivatives of aryl amides, phenols, and aryl acids, showed that the amide and ester derivatives generally appear to be significantly less toxic and more selective. They suggested the possibility that the active component is the original acid, formed by enzymatic hydrolysis of the appropriate ester or amide linkage. Numerous studies have been

Table I—4-[p-[Bis(2-chloroethyl)amino]phenyl]butyramides



Compound	R_1	R_2	Formula	Pure Yield, %	Melting Point
1	H	C_6H_5	$C_{20}H_{24}Cl_2N_2O$	38.2	114–115°
2	CH_3	C_6H_5	$C_{21}H_{26}Cl_2N_2O$	49.5	Oil
3	H	—C_6H_4—Br	$C_{20}H_{23}BrCl_2N_2O$	69.7	134.4–135.2°
4	H	—$CH_2CH(CH_3)_2$	$C_{18}H_{28}Cl_2N_2O$	72.7	86–87°
5	H	—C_6H_4—OCH_3	$C_{21}H_{26}Cl_2N_2O_2$	84.0	123.5–124.5°
6	H	—C_6H_4—SCH_3	$C_{21}H_{26}Cl_2N_2OS$	58.5	137.5–138.5°

Table II—Analytical Data for 4-[p-[Bis(2-chloroethyl)amino]phenyl]butyramides

Compound	Nitrogen Analysis, %		IR Bands, cm^{-1}	
	Calc.	Found	N—H	C=O
1	7.39	7.56	3290	1655
2	7.12	7.17	—	1655
3	6.12	5.84	3260	1650
4	7.80	7.86	3260	1650
5	6.85	6.67	3270	1655
6	6.56	6.50	3270	1655

Table III—Antitumor Data of 4-[p-[Bis(2-chloroethyl)amino]phenyl]butyramides

Compound	NSC Number	L-1210[a] Dose Level[e]	L-1210[a] % T/C[f]	WA[b] Dose Level[e]	WA[b] % T/C[f]	KB[c] ED_{50}[g]	PS[d] Dose Level[e]	PS[d] % T/C[f]
1	136479	8	109	—	—	—	25	120
2	136480	8	110	—	—	—	—	—
3	145697	20	111	—	—	100	—	—
4	128885	8	112	5.3	606[h]	—	—	—
5	128827	8	110	6.1	571	—	—	—
6	128886	8	115	3.2	606[h]	—	—	—

[a] L-1210 is an ascites leukemia. [b] WA is Walker carcinosarcoma 256, intramuscular. [c] KB is a cell culture screen. [d] PS is P-388 lymphocytic leukemia. [e] Milligrams per kilogram body weight. [f] Measured as percentage increase in lifespan. [g] Micrograms per milliliter. [h] Maximum value allowed in test system prior to termination; comparable to chlorambucil.

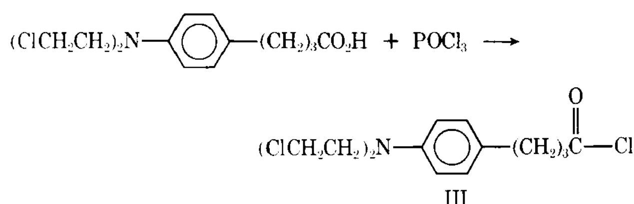
conducted on the premise that a lipophilic compound can be transported across cell walls considerably faster than the corresponding ionic forms, presumably by an appropriate carrier (19–23).

This information led the authors to prepare compounds similar in structure to chlorambucil but in which the carboxylic acid group was converted to a relatively nonpolar amide group. Hopefully, the amides would be transported to the neoplastic cells, perhaps more rapidly than the corresponding acid, after which they would be hydrolyzed to the acid form. Several of the prepared compounds exhibit encouraging antitumor activity in initial stages of testing.

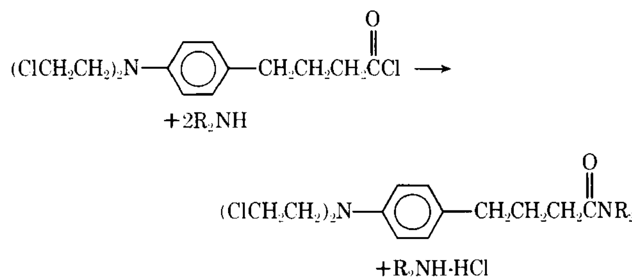
The starting point for the preparation of the amides was the corresponding acid chloride (III), prepared according to the reaction shown in Scheme I. Formation of III was confirmed by appropriate IR spectral bands in the 1800-cm^{-1} region. Once the acid chloride was prepared, it was used immediately for the preparation of the appropriate amides (Scheme II). Tables I–III present analytical and antitumor screening data for the compounds reported.

EXPERIMENTAL[1]

4-[p-[Bis(2-chloroethyl)amino]phenyl]butyryl Chloride (I) (Scheme I)—Phosphorus oxychloride (35.0 g, 0.229 mole) was added dropwise to a solution of chlorambucil (3.0 g, 9.86 mmoles) in 25 ml of dry chloroform in a 100-ml round-bottom flask fitted with a reflux condenser and drying tube. The reaction mixture was stirred with a magnetic stirrer and was heated with stirring to 60° for 4 hr. After cooling to room temperature, excess phosphorus oxychloride was removed *in vacuo*. The resulting oil was washed several times with petroleum ether (bp 30–60°) to remove any traces of phosphorus oxychloride, dissolved in 10 ml of dry chloroform or acetonitrile, and used immediately for preparation of the amide of choice. The formation of I was confirmed by the presence of the appropriate IR C=O band at 1800 cm^{-1}.



Scheme I



Scheme II

Synthesis of the Amides (Scheme II)—The appropriate amine (8.64 mmoles), dissolved in 25 ml of acetonitrile, was added dropwise to a stirring solution of the acid chloride previously obtained in 10 ml of acetonitrile at 0°. The reaction mixture was allowed to warm slowly to room temperature and was washed with water to remove the amine hydrochloride. The aqueous solution was extracted with chloroform, and the extract was dried and passed through a column of neutral and basic (3:1) alumina. The eluate containing the amide was evaporated to one-fourth of its original volume *in vacuo*, and petroleum ether was added. Crystals of the amide were obtained and recrystallized from chloroform–petroleum ether. In the case of the oils, the resulting oil was chromatographed a second time, using the same procedure as before, and the chloroform was removed *in vacuo*.

[1] All melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are corrected. IR spectra were determined on Perkin-Elmer 137 and 237B spectrophotometers. Elemental analyses were performed by Midwest Microlab, Indianapolis, Ind., and Micro-tech Labs, Skokie, Ill.

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Flame-Ionization and Electron-Capture GLC Determination of 1-(2,6-Dimethylphenoxy)-2-aminopropane in Plasma

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Abstract □ A sensitive flame-ionization GLC method is described for the determination of the new anticonvulsant, 1-(2,6-dimethylphenoxy)-2-aminopropane, in plasma. The method is compared with a simultaneously developed electron-capture technique, which was found to give no advantage applicable to the analysis. Relative standard deviations of replicate samples extracted for analysis by the flame-ionization method are less than 7%. The lower limit of detection of this method is 7 ng/ml.

Keyphrases □ 1-(2,6-Dimethylphenoxy)-2-aminopropane—flame-ionization and electron-capture GLC analysis in human plasma □ GLC, flame ionization and electron capture—analysis, 1-(2,6-dimethylphenoxy)-2-aminopropane in human plasma

The new anticonvulsant drug, 1-(2,6-dimethylphenoxy)-2-aminopropane (I), was tested clinically and shown to be effective in the treatment of psychomotor seizures (1). Two urinary metabolites were characterized (2), and a method for the quantitation of I in urine was published (3). To determine the half-life of this drug and to establish an easily measurable parameter as a basis for dosage adjustment, a method for quantitation of I in plasma was developed[1].

EXPERIMENTAL

Reagents—1-(2,6-Dimethylphenoxy)-2-aminopropane[2] (I) and the internal standard, 1-(2,3-dimethylphenoxy)-2-aminopropane[2] (II), were supplied as the hydrochloride salts and were used without further purification. All extracting solvents were reagent grade for flame work and specially purified[3] for electron-capture work. Water for standards and buffers used in the electron-capture procedure was made by extracting 0.1 *N* HCl (distilled water) four times with 0.1 volume of chloroform, adjusting the pH to 12 with sodium hydroxide pellets, and repeating the extractions. The resulting aqueous phase was redistilled in an all-glass system and stored in a ground-glass stoppered bottle. Trifluoroacetic anhydride[4] and pentafluoropropionic anhydride[5] were used for derivatization.

Apparatus—A gas chromatograph[6] equipped with flame-ionization and scandium tritide (4) electron-capture detectors was used. The columns were 91.5-cm × 2-mm (i.d.) glass U-tubes packed with 2.8% OV-210–3.2% OV-1 on 80–100-mesh Chromosorb W-HP for flame-ionization analysis and 3% OV-17 on 100–120-mesh Gas Chrom Q for electron-capture analysis. Instrument temperatures were 270, 250, and 120° at the detector, injector, and column oven, respectively. The electron-capture detector was operated in the dc mode at a potential of 90 v. The flow of nitrogen through the OV-210–OV-1 column was set at 25.5 ml/min, giving retention times of 4.58 and 6.00 min for the trifluoroacetamide derivatives of I and II, respectively. Nitrogen was also used as carrier gas with the electron-capture detector at a flow rate of 12.0 ml/min, with a detector purge of 35.0 ml/min giving retention times of 3.00 and 4.37 min for the pentafluoropropionamide derivatives of I and II, respectively.

Special centrifuge tubes[7], tapered to a 1.0–1.5-mm diameter capillary at the bottom, were used to obtain almost total recovery of the final chloroform extract.

Procedure—In a 16 × 150-ml culture tube (Teflon-lined screw cap), 2.00 ml of plasma, 0.50 ml of the internal standard (800 ng II/ml of distilled water), 0.5 ml of 2.0 *N* NaOH, and 5 ml of ethyl acetate are combined and shaken for 10 min. After centrifugation, the ethyl acetate is transferred to a tapered centrifuge tube, 1.0 ml of 0.1 *N* HCl is added, and the mixture is shaken for 5 min. The ethyl acetate is aspirated completely after centrifugation, 0.5 ml of 2.0 *N* NaOH and 0.1 ml of chloroform are added to the aqueous phase, and the tube is shaken by hand for 1 min. The tube is centrifuged and the lower phase is transferred to a conical vial[8] with a microsyringe. Trifluoroacetic anhydride (50 μl) is added, and the vial is capped and allowed to stand at room temperature for 30 min. The chloroform and excess reagent are evaporated at room temperature under a stream of nitrogen, and the residue is dissolved in 25 μl of redistilled carbon disulfide. Approximately 1 μl is injected into the chromatograph. Quantitation is by peak height ratio (I/II).

For analysis by electron-capture detection, 1 ml of plasma is extracted and the internal standard concentration is lowered to 80 ng II/ml. All extractions prior to derivatization are performed as already described. Pentafluoropropionic anhydride is substituted for trifluoroacetic anhydride as the derivatizing reagent. After the 30-min reaction time, the chloroform and excess reagent are evaporated at room temperature under a stream of nitrogen and the residue is taken up in 100 μl of chloroform. The chloroform is transferred to a tapered centrifuge tube containing 0.5 ml of 2.0 *N* NaOH and the final extraction is repeated as already described. The organic phase is transferred and evaporated under nitrogen at

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