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ARYL-2-HALOGENOALKYLAMINES—XXII*.

DERIVATIVES OF PHENOXYACETIC ACID: SYNTHESIS AND ANTINEOPLASTIC ACTIVITIES

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Abstract—The preparation of *o*-, *m*-, and *p*-(ethyl-2-chloroethylamino) and *o*-(di-2-chloroethylamino)-phenoxyacetic acid is described. The results of a preliminary screening of these new compounds against the transplanted Walker rat carcinoma and the mouse lymphoid leukaemia, L1210, are reported.

BAKER *et al.*[1] suggested that an irreversible inhibitor of lactic acid dehydrogenase would be of considerable interest in cancer chemotherapy. It was considered that an effective exo-alkylating irreversible inhibitor of this enzyme might result from "the placement of a suitable alkylating group on an inhibitor, such as phenoxyacetic acid, that has sufficient chemical reactivity and the proper dimensions to be able to alkylate a nucleophilic group of the enzyme near the active site". Amongst the alkylating groups which they incorporated into known lactic acid dehydrogenase inhibitors were haloacyl, haloacetamido and nitrogen mustard groups.[2]

Since it was felt that aromatic 2-chloroethylamino groups, by virtue of their S_N1 reaction mechanism were more likely to survive to reach and react with target enzyme sites the present work has concentrated on this type of alkylating group. In the absence of information concerning the location of possible nucleophilic centers adjacent to the active site of the enzyme it was considered desirable to prepare and test compounds carrying an alkylating group on each of the available positions on the aromatic ring of the inhibitor molecule. As monofunctional alkylating capacity should be sufficient to produce an irreversible antagonist the preparation of *o*-, *m*-, and *p*-(N-2-chloroethyl-*N*-ethylamino)phenoxyacetic acids has been undertaken.

Although most of the earlier work[3] suggested that only compounds having at least two alkylating groups in the molecule showed appreciable antitumour activity more recent studies have indicated that, when attached to suitable carrying structures, monofunctional alkylating groups confer significant activity and, moreover, there are suggestions that the antitumour effect may be more specific than that observed for difunctional agents. For example, Reist *et al.*[4] showed that a monofunctional nitrogen mustard derivative derived from glucose was active against the L1210 leukaemia; Creech[5] and Preston *et al.*[6] have demonstrated that certain 2-chloroethylamino-acridines are active anti-tumour agents and more recently the nitrogen mustard group has been effectively replaced by a 2-chloroethylthio group[7]; Hebborn *et al.*[8] found that *p*-(*N*-2-bromoethyl-*N*-ethylamino)benzenesulphonamide showed

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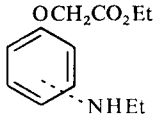
tumour inhibitory activity at a dose which did not cause leucopenia; and finally 1-ethyleneimino-2,4-dinitrobenzene has a high chemotherapeutic index in the Walker tumour assay[9] and produces little damage to blood forming elements.[10] Thus, despite the earlier indications, there has been a revival of interest in monofunctional agents.

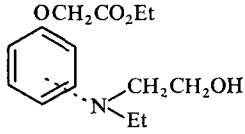
It was of interest to have the corresponding difunctional analogues for comparison with the monofunctional derivatives of phenoxyacetic acid mentioned above. *m*-(Di-2-chloroethylamino)phenoxyacetic acid has been prepared by Skinner *et al.*[11] and the *p*-isomer was described by Davis *et al.*[12] Although *o*-(di-2-chloroethylamino)-phenoxyacetic acid is listed in a survey of nitrogen mustard gas derivatives[13] it would appear from the reference cited[11] therein that this compound had not then been prepared. It has now been obtained by a method similar to that employed to prepare the monofunctional analogue.

MATERIALS

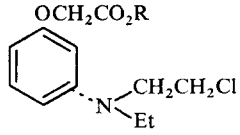
Ethylation of ethyl *m*- and *p*-aminophenoxyacetates by the method of Rice and Kohn[14] afforded the *N*-ethyl derivatives (I) which on treatment with ethylene oxide in aqueous acetic acid gave the corresponding ethyl [ethyl-(2-hydroxyethyl)amino] phenoxyacetates (II). These were converted into the chloroethyl derivatives (III, R = Et) by the action of phosphoryl chloride and subsequently hydrolysed with concentrated hydrochloric acid giving the required *m*- and *p*-[ethyl-(2-chloroethyl) amino] phenoxyacetic acids (III, R = H).



(*m*-or *p*-)
(I)



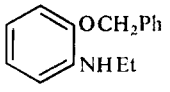
(*m*-or *p*-)
(II)



(*m*-or *p*-)
(III)

Lactam formation from derivatives of *o*-aminophenoxyacetic acid[11,15] precluded the use of a similar method for the preparation of the ortho-analogue. It was necessary to substitute fully the nitrogen atom before introducing the acidic side chain. *o*-Aminophenol was recovered unchanged on heating with Raney nickel in ethanol and this method of ethylation was not applicable to *o*-benzyloxyaniline since debenzylation occurred. The *p*-toluenesulphonyl derivative of *o*-benzyloxyaniline was converted into its *N*-ethyl derivative but all attempts at selective removal of the *p*-toluene-sulphonyl group derivative failed. *N*-ethyl-*o*-benzyloxyaniline (IV) was eventually obtained by treating *o*-benzyloxyaniline with ethyl ortho-formate in the presence of sulphuric acid and hydrolysing the product with hydrochloric acid.[16]

Treatment of the base (IV) with ethylene oxide gave benzyl *o*-[ethyl-(2-hydroxy-ethyl)amino] phenyl ether (V) which on hydrogenolysis in ethanol with palladium charcoal as catalyst afforded the phenol and this condensed with ethyl bromoacetate in the presence of potassium ethoxide to give *o*-[ethyl-(2-hydroxyethyl)amino] phenoxyacetate (VI). When treated with phosphoryl chloride the ester (VI) yielded the chloroethyl derivative (VII, R = Et) which on hydrolysis gave *o*-[ethyl-(2-chloroethyl) amino] phenoxyacetic acid (VII, R = H).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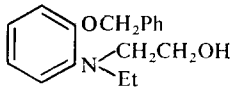
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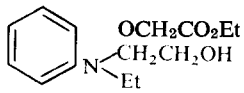
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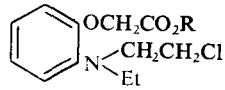
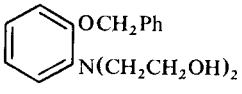
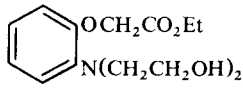

(VII)

Hydroxyethylation of *o*-benzyloxyaniline in the usual manner afforded *o*-(di-2-hydroxyethylamino)phenylbenzyl ether (VIII) which was debenzylated and converted successively into ethyl *o*-(di-2-hydroxyethylamino)phenoxyacetate (IX), ethyl *o*-(di-2-chloroethylamino)phenoxyacetate (X, R = Et) and *o*-(di-2-chloroethylamino)phenoxyacetic acid (X, R = H) by procedures described above.


(VIII)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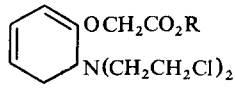

(X)

Melting points were determined with a Gallenkamp heated metal block apparatus and are corrected. Infra-red spectra were recorded on a Perkin–Elmer spectrophotometer Model 137B.

Ethyl p-ethylaminophenoxyacetate

Raney nickel (10 g) was added to a solution of ethyl *p*-aminophenoxyacetate (4·9 g) in ethanol (50 ml) and the mixture was heated under reflux with stirring for 4 hr. After cooling, the catalyst was removed by filtration and the filtrate was evaporated under reduced pressure at 50–60°. The residue was crystallized from light petroleum (b.p. 60–80°). *Ethyl p-ethylaminophenoxyacetate* (4·0 g, 69%) formed prisms, m.p. 50–51°; ν_{max}^{nujol} 3250, 1740 and 812 cm^{-1}. (Found: C, 64·8%; H, 7·3%; N, 6·4%; Calc. for $C_{12}H_{17}NO_3$: C, 64·6%; H, 7·7%; N, 6·3%.)

Ethyl p-[ethyl-(2-hydroxyethyl)amino]phenoxyacetate

Ethylene oxide (30 ml) was added to a solution of ethyl *p*-ethylaminophenoxyacetate (22·3 g) in glacial acetic acid (60 ml) and water (90 ml). The mixture was stirred at 20° for 24 hr and then treated with charcoal. Neutralization with solid $NaHCO_3$ caused the separation of an oil which was extracted with chloroform. Distillation of the dried (Na_2SO_4) extract afforded *ethyl* p-[*ethyl-(2-hydroxyethyl)amino*]*phenoxyacetate* (23 g, 86%), b.p. 184°/0·1–0·2 mm; ν_{max}^{film} 3330, 1740 and 815 cm^{-1}. (Found: 63·0%; H, 8·1%; N, 5·5%. Calc. for $C_{14}H_{21}NO_4$: C, 62·9%; H, 7·9%; N, 5·2%.)

Ethyl p-[*ethyl-(2-chloroethyl)amino*]*phenoxyacetate*

Phosphoryl chloride (30 ml) was added to a solution of ethyl *p*-[ethyl-(2-hydroxyethyl)amino]phenoxyacetate (26·7 g) in benzene (150 ml) and the mixture was heated for 2 hr on a steam bath. The cooled mixture was shaken with water (2 × 100 ml) and then with aqueous HCl (2 × 50 ml, 6N). The combined aqueous extracts were neutralized with solid $NaHCO_3$ and extracted with ether. The dried (Na_2SO_4) extract yielded an oil (25 g) which decomposed on attempted distillation. This oil, which showed ν_{max}^{film} 1750, 816, 740, and no (O-H) absorption at 3330 cm^{-1}, was proved to be the required ester by the preparation of its *picrate*, which formed prisms, m.p. 122–123°,

from benzene-light petroleum (b.p. 30–40°). (Found: C, 46·6%; H, 4·7%; Cl, 7·0%; N, 11·0%. Calc. for $C_{20}H_{23}ClN_4O_{10}$: C, 46·7%; H, 4·5%; Cl, 6·9%; N, 10·9%.)

p-[*Ethyl*-(2-*chloroethyl*)*amino*]*phenoxyacetic acid*

A solution of the above ester (4·3 g) in concentrated HCl (50 ml) was heated on a steam bath for 2 hr and then evaporated under reduced pressure at 60–70°. The residual oil was dissolved in water (20 ml) and the pH adjusted to 5 by the addition of saturated aqueous sodium acetate. The precipitated p-[*ethyl*-(2-*chloroethyl*)*amino*] *phenoxyacetic acid* (3·5 g, 90%) formed small flattened prisms, m.p. 126–127° (decomp.) from benzene-light petroleum (b.p. 30–40°); ν_{max}^{nujol} 1725, 812 and 750 cm^{-1} (Found: C, 56·6%; H, 6·3%; Cl, 13·7%; N, 5·7%. Calc. for $C_{12}H_{16}ClNO_3$: C, 55·9%; H, 6·2%; Cl, 13·8%; N, 5·4%.)

The following were prepared from ethyl *m*-aminophenoxyacetate by procedures essentially the same as described for the *p*-isomers:

Ethyl m-*ethylaminophenoxyacetate*, liquid, b.p. 145°/0·5 mm, yield 79%, ν_{max}^{film} 3320, 1760, 755 and 688 cm^{-1}. (Found: C, 64·9%; H, 7·6%; N, 6·4%). *Ethyl* m-[*ethyl*-(2-*hydroxyethyl*)*amino*]*phenoxyacetate*, liquid b.p. 180°/0·05 mm, yield 88%, ν_{max}^{film} 3230, 1740, 750 and 690 cm^{-1}. (Found: C, 62·9%; H, 8·1%; N, 5·4%). *Ethyl* m-[*ethyl*-(2-*chloroethyl*)*amino*]*phenoxyacetate*, liquid, yield 85% ν_{max}^{film} 1760, 750 and 690 cm and no O-H absorption, which gave a *picrate*, m.p. 99–100°, prisms from benzene-light petroleum (b.p. 30–40°). Found: C, 46·8%; H, 4·7%; Cl, 7·2%; N, 11·1%.)

m-[*Ethyl*-(2-*chloroethyl*)*amino*]*phenoxyacetic acid*, small needles, m.p. 123–124° (decomp.) from benzene-light petroleum (b.p. 30–40°), ν_{max}^{nujol} 1735, 753, 723, and 690 cm^{-1}. (Found: C, 55·6%; H, 6·3%, Cl, 13·9%; N, 5·6%.)

Benzyl o-(p-*toluenesulphonyl*)*aminophenyl ether*

A solution of *p*-toluenesulphonyl chloride (32 g) in pyridine (100 ml) was added during 30 min to a stirred, cooled (0°) solution of *o*-benzyloxyaniline (35 g) in pyridine (50 ml). The mixture was stirred for a further 2 hr and then kept at room temperature overnight. The solid separating on pouring the solution on to ice containing an excess of HCl was collected and crystallized from ethanol. *Benzyl* o-(*p-toluenesulphonyl*) *aminophenyl ether* formed short needles, m.p. 100–101°. (Found: C, 67·8%; H, 5·5%; N, 4·2%; S, 9·3%. Calc. for $C_{20}H_{19}NO_3S$: C, 68·0; H, 5·4%; N, 4·0%; S, 9·1%.)

Benzyl o-[*ethyl*-(p-*toluenesulphonyl*)*amino*]*phenyl ether*

The potassium salt of benzyl *o*-(*p*-toluenesulphonyl)aminophenyl ether (20 g, prepared by adding an excess of concentrated aqueous KOH to the sulphonamide, collecting the precipitate and washing with dry ether) and ethyl iodide (10 ml) in ethanol (250 ml) were heated on a steam bath for 2½ hr. The solid separating on pouring the mixture on to ice was collected and crystallized from benzene-light petroleum (b.p. 30–40°). *Benzyl* o-[*ethyl*-(p-*toluenesulphonyl*)*amino*]*phenyl ether* formed rhombs, m.p. 92–93° (Found: C, 69·3%; H, 6·0%; N, 3·7%; S, 8·6%; Calc. for $C_{22}H_{23}NO_3S$: C, 69·3%; H, 6·1%; N, 3·7%; S, 8·4%.)

N-*Ethyl*-o-*benzyloxyaniline*

o-Benzyloxyaniline (26 g), triethyl orthoformate (35 g) and concentrated H_2SO_4 (0·25 ml) were heated at 185° for 20 min, then at 200° for 10 min more; during this time 15 ml of ethanol distilled off. The product was heated under reflux with aqueous

HCl (100 ml, 3N) for 1 hr. The oil which separated on adding an excess of NaOH to the cooled mixture was extracted with ether. On distillation of the dried (Na_2SO_4) extract N-*ethyl*-o-*benzyloxyaniline* (18 g) b.p. 127°/0·01 mm, was obtained; ν_{max}^{film} 3320, 740 and 700 cm^{-1}. (Found: C, 79·3%; H, 7·1%; N, 6·3%. Calc. for $C_{15}H_{17}NO$: C, 79·3%; H, 7·5%; N, 6·2%.)

Benzyl o-[*ethyl*-(2-*hydroxyethyl*)*amino*]*phenyl ether*

Ethylene oxide (30 ml) was added to a solution of *N*-ethyl-*o*-benzyloxy aniline (18 g) in glacial acetic acid (100 ml) and water (100 ml). After 24 hr at room temperature the solution was concentrated to low bulk under reduced pressure; water was then added (250 ml) and the product was extracted with chloroform. Distillation of the dried (Na_2SO_4) extract afforded *benzyl* o-[*ethyl*-(2-*hydroxyethyl*)*amino*]*phenyl ether*, b.p. 154–156°/ 0·01–0·05 mm (25 g); ν_{max}^{film} 3250, 745 and 695 cm^{-1}. (Found: C, 75·5%; H, 8·0%; N, 4·9%. Calc. for $C_{17}H_{21}NO_2$: C, 75·2%; H, 7·8%; N, 5·2%.)

Ethyl o-[*ethyl*-(2-*hydroxyethyl*)*amino*]*phenoxyacetate*

A solution of benzyl *o*-[ethyl-(2-hydroxyethyl)amino]phenyl ether (16 g) in ethanol (50 ml) containing palladium-charcoal catalyst (1 g, 5% Pd) was shaken in an atmosphere of hydrogen until the theoretical amount of gas had been taken up (40 min). The oil (11 g), obtained on evaporating the filtered solution, was dissolved in ethanol (50 ml) and added to a solution of potassium (1·96 g) in ethanol (50 ml). After adding ethyl bromoacetate (10 ml) the mixture was heated on a steam bath for 1 hr. The oil, obtained after adding water (100 ml) to the cooled solution and concentrating under reduced pressure, was extracted with ether. Distillation of the dried (Na_2SO_4) extract yielded *ethyl* o-[*ethyl*-(2-*hydroxyethyl*)*amino*]*phenoxyacetate* (14 g), b.p. 144–148°/ 0·01–0·05 mm; ν_{max}^{film} 3200, 1745 and 750 cm^{-1}. (Found: C, 63·2%; H, 8·1%; N, 5·4%. Calc. for $C_{14}H_{21}NO_4$: C, 62·9%; H, 7·9%; N, 5·2%.)

Ethyl o-[*ethyl*-(2-*chloroethyl*)*amino*]*phenoxyacetate*

On treating the above hydroxyethylamine (10 g) with phosphoryl chloride as described above the chloroethyl derivative (10 g) was obtained as an oil, ν_{max}^{film} 1745 and 750 cm^{-1} and no hydroxyl group absorption. It formed a *picrate*, needles, m.p. 102–104°, from aqueous methanol. (Found: C, 46·8%; H, 4·6%; N, 11·1%. Calc. for $C_{20}H_{23}ClN_4O_{10}$: C, 46·7%; H, 4·5%; N, 10·9%.

o-[*Ethyl*-(2-*chloroethyl*)*amino*]*phenoxyacetic acid hydrochloride*

The above ester (2 g) was hydrolysed as described for the *p*-isomer. As no material separated on adjusting the pH to 5 the solution was saturated with NaCl and extracted with chloroform (4 × 50 ml). Evaporation of the dried (Na_2SO_4) extract gave an oil which could not be induced to crystallize. A solution of the product in ether-dichloromethane (1:1) was saturated with HCl gas and evaporated under reduced pressure. The residue (1·2 g) solidified after keeping for several days under light petroleum (b.p. 30–40°). The hydrochloride of o-[*ethyl*-(2-*chloroethyl*)*amino*]*phenoxyacetic acid* formed prisms, m.p. 189–190° (decomp.), from ether-methanol; ν_{max}^{nujol} 2600–2400, 1745, 768 and 732 cm^{-1}. (Found: C, 49·2%; H, 5·9%; Cl, 23·6%; N, 4·7%. Calc. for $C_{12}H_{17}Cl_2NO_3$ C, 49·0%; H, 5·8%; Cl, 24·1%; N, 4·8%.)

By methods essentially similar to those already described the following compounds were prepared from *o*-benzyloxyaniline:

Benzyl o-[*di*-(2-*hydroxyethyl*)*amino*]*phenyl ether*. This is obtained as an oil which showed a broad absorption band near 3300 cm^{-1} (O-H). It formed a *picrate*, needles, m.p. 132–134°, from benzene-ethanol. (Found: C, 53·3%; H, 4·6%; N, 11·0%. Calc. for $C_{23}H_{24}N_4O_{10}$: C, 53·5%; H, 4·7%; N, 10·9%.)

o-[*Di*-(2-*hydroxyethyl*)*amino*]*phenol*. Prisms, m.p. 49–50°, from benzene-light petroleum (b.p. 30–40°); ν_{max}^{nujol} 3500–2500, and 753 cm^{-1}. (Found: C, 61·0%; H, 7·5%; N, 7·0%. Calc. for $C_{10}H_{15}NO_3$: C, 60·9%; H, 7·7%; N, 7·1%.)

Ethyl o-[*di*-(2-*chloroethyl*)*amino*]*phenoxyacetate*. This is obtained as a yellow oil after chromatographic purification of the product formed by the action of phosphoryl chloride on crude ethyl *o*-[di-2-hydroxyethyl)amino]phenoxyacetate. This oil showed ν_{max}^{film} 1745 and 750 cm^{-1}. (Found: C, 52·3%; H, 6·2%; Cl, 22·2%; N, 4·4%. Calc. for $C_{14}H_{19}Cl_2NO_3$: C, 52·5%; H, 5·9%; Cl, 22·2%; N, 4·4%).

o-[*Di*-(2-*chloroethyl*)*amino*]*phenoxyacetic acid hydrochloride*. Prisms, m.p. 174–175° (decomp.), from ethanol; ν_{max}^{nujol} 2500–2300, 1735, 763 and 732 cm^{-1} (C-Cl). (Found: C, 43·9%; H, 5·0%; Cl, 31·9%; N, 4·3%. Calc. for $C_{12}H_{15}Cl_3NO_3$: C, 43·9%; H, 4·9%; Cl, 32·4%; N, 4·3%.). In this case the hydrochloride was obtained directly in 71 per cent yield on evaporating the original hydrochloric acid hydrolysis solution to dryness under reduced pressure.

METHODS

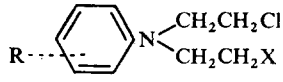
The protocols for testing the compounds as inhibitors of the growth of the transplanted Walker rat carcinoma 256 and the ADJ/PC6A mouse plasma cell tumour are as described by Connors *et al.*[17] and Wade *et al.*[18] respectively. The method of assay against the mouse lymphoid leukaemia, L1210, is essentially that already described[19] except that the C_{57}/DBA2 hybrid strain of mouse was used as host. Compounds were administered by a single intraperitoneal injection in arachis oil starting on the day following implantation of the Walker tumour or 10 days after implantation of the ADJ/PC6A tumour. In the case of the L1210 leukaemia similar injections were given for five consecutive days after the initial inoculation except as indicated in Table 2. In Table 1 the results of the Walker tumour test are expressed at C/T ratios, that is, the weight of the tumours in control animals/the weight of the tumours in treated animals. The chemotherapeutic index (C.I.) is LD_{50}/ED_{90}, where ED_{90} is the dose calculated to produce 90 per cent inhibition of tumour growth. The T/C ratios shown for the L1210 assay (Table 2) equals [the average survival time of treated animals/the average survival time of controls] × 100.

RESULTS AND DISCUSSION

Chemical reactivity

The chemical reactivity of the various derivatives has been determined by measuring the rate of hydrolysis in boiling aqueous acetone as in earlier papers of this series. This simple method, which was developed to place compounds in an order of reactivity, has been criticised[20] although the correlation with biological activity is generally better than with reaction rates obtained by more sophisticated methods.[21] The limitations of the present method and of any other method of assessing chemical reactivity *in vitro* are fully appreciated (see ref 3 p. 100) and lack of correlation with biological activity is only to be expected if metabolic and transport differences also have to be taken into account.

TABLE 1. SCREENING AGAINST WALKER 256 (S.C.) TUMOUR



Compound R	X	% Reaction* acid H	acid Cl	Na salt H	Na salt Cl	Dose mg/kg†	Survivors	C/T† ratio	Approx LD_{50} mg/kg‡	C.I.
						64	0/3	—		
						32	3/3	1		
o-OCH_2CO_2H hydrochloride	H	100	—	100	—	16	3/3	1	40	—
						8	3/3	1·2		
						4	3/3	0·8		
						160	0/3	—		
m-OCH_2CO_2H	H	38	40	60	89	80	3/3	1	113	—
						40	3/3	1		
						20	3/3	0·9		
						40	1/3	2		
p-OCH_2CO_2H	H	70	90	61	98	20	3/3	1	35	—
						10	3/3	1·7		
						5	3/3	1·3		
						32	1/3	1		
o-OCH_2CO_2H hydrochloride	Cl	100	—	100	—	16	2/3	1	20	—
						8	3/3	1		
						4	3/3	1		
						160	0/3	—		
m-OCH_2CO_2H	Cl	10	14	15	23	80	3/3	92	113	1·5
						40	3/3	2		
						20	3/3	1		
						40	1/3	—		
						20	3/3	∞		
p-OCH_2CO_2H	Cl	—	—	—	60	10	3/3	11	35	3·5
						5	3/3	1		
						2·5	3/3	1		
						1·25	3/3	0·9		
						30	0/3	—		
						10	3/3	∞		
o-$CH_2CH_2CO_2H$	Cl	—	—	—	73	5	3/3	∞	20	4·5
						2	3/3	2·1		
						1	3/3	2·5		
						160	0/3	—		
o-OCH_2CO_2Et	H	H = 93%		Cl = 94%		80	0/3	—	70	—
						40	3/3	1·1		
						20	3/3	0·9		
						40	0/3	—		
						35	1/3	3		
o-OCH_2CO_2Et	Cl	H = 75%		Cl = 74%		20	3/3	3	28	—
						15	3/3	1·2		
						10	3/3	2		
						5	3/3	1·2		

* Release of hydrogen or chloride ions on refluxing for ½ hr in 1:1 acetone-water, $c = 0{\cdot}02$ M for chlorethylamines or 0·01 M for di(chloroethyl)amines (see ref. 22).

† See text.

‡ For tumour bearing animals.

TABLE 2. SCREENING AGAINST MOUSE LEUKAEMIA, L1210

Compound R	X	Dose mg/kg	No. of daily doses	T/C Ratio	LD_{50} for host mouse mg/kg
o-OCH_2CO_2H hydrochloride	H	60	5	59	60
		30	5	55	
		15	5	111	
m-OCH_2CO_2H	H	56	5	50	56
		28	5	96	
		14	5	93	
p-OCH_2CO_2H	H	14	5	54	14
		7	5	96	
		3·5	5	91	
o-OCH_2CO_2Et	H	100	1	28	100
		50	1	70	
		25	1	112	
o-OCH_2CO_2Et	Cl	100	1	26	100
		50	1	70	
		25	1	110	

In the present series of compounds the effects of substituents on the chemical reactivity of the aromatic 2-chloroethylamino groups follows the pattern observed in earlier papers. In the mono-2-chloroethylamino compounds highest reactivity is shown by the *o*-phenoxyacetic acid derivative, followed by the *p*-derivative and the *m*-derivative has the lowest reactivity. *o*-Substituted chloroethylarylamines generally have high reactivity due to the effect of the bulky substituent on the coplanarity of the of the nitrogen valency bonds with the aromatic ring system. Higher reactivity is shown by the sodium salts owing to the effect of the negative charge on the carboxylate group in the side chain.

The difunctional analogues have lower reactivity than the monofunctional compounds due to the mutual deactivating effect of the chlorine atoms. This effect has been observed before, for example, *NN*-di-2-chloroethylaniline undergoes 20 per cent reaction whereas *N*-2-chloroethylaniline undergoes 55 per cent reaction under the standard conditions. In the case of the *o*-phenoxyacetic acid derivatives the effect on valency bond coplanarity over-rides, to some extent, this mutual deactivating effect. The reactivity of the *o*-phenoxyacetic acid esters is lower than that of the corresponding acids; this is not unexpected since even in the case of the hydrolysis of the free acids there will be carboxylate ion present during part of the reaction. The higher reactivity of *o*-(di-2-chloroethylamino)phenoxyacetic acid as compared with *o*-(di-2-chloroethylamino)phenylpropionic acid reflects the greater electron donating capacity of an oxygen atom as compared with a methylene group.

Toxicities

There is no clear cut correlation of toxicity with chemical reactivity though there is a tendency for the more reactive chloroethylamines to be more toxic. *m*-Derivatives of lowest reactivity are less toxic than the corresponding *o*- and *p*-isomers. The esters of *o*-[ethyl-(2-chloroethyl)amino] and *o*-(di-2-chloroethylamino)phenoxyacetic acids are less toxic than the corresponding acids.

Antineoplastic activities

No activity against the Walker tumour is shown by the monofunctional *o*- or *m*-phenoxyacetic acid derivatives and only marginal activity by the *p*-isomer (Table 1). This is in accord with most of the earlier work on alkylating agents[3]—however, if irreversible lactic acid dehydrogenase inhibitory activity could produce tumour growth inhibition difunctionality should not be necessary. The inactivity of *o*-(di-2-chloroethylamino) phenoxyacetic acid against the Walker tumour is unexpected especially as the isosteric phenylpropionic acid derivative and the isomeric *m*- and *p*-phenoxyacetic acid derivatives show appreciable activity (Table 1). However, *o*-(di-2-chloroethylamino)phenoxyacetic acid shows significant activity against the ADJ/PC6A mouse plasma cell tumour (LD_{50} 47 mg/kg; ED_{90} 2·1 mg/kg; C.I. 22). The ester of *o*-(di-2-chloroethylamino)phenoxyacetic acid exhibits moderate activity against the Walker tumour but high activity against the ADJ/PC6A tumour (LD_{50} 57 mg/kg; ED_{90} 0·74 mg/kg; C.I. 77).

The four monofunctional derivatives and the ester of *o*-(di-2-chloroethylamino) phenoxyacetic acid showed no activity against the L1210 mouse leukaemia (Table 2). This system is not very sensitive to alkylating agents and even compounds that have shown good activity against the Walker tumour have not been particularly effective against the leukaemia.

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