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ARYL-2-HALOGENOALKYLAMINES—XXIII.

DERIVATIVES OF OXANILIC ACID AND SALICYLIC ACID: SYNTHESIS AND ANTINEOPLASTIC ACTIVITIES

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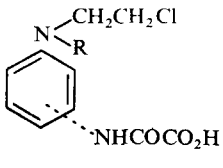
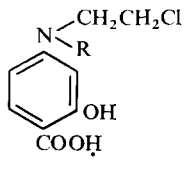
Abstract—The preparation of *p*-[ethyl-(2-chloroethyl)amino]oxanilic acid, 5-[ethyl-(2-chloroethyl)amino]salicylic acid and 5-(di-2-chloroethylamino)salicylic acid is described. The results of a preliminary screening of these new compounds against the transplanted Walker rat carcinoma are reported.

THE POSSIBLE value of irreversible inhibitors of lactic acid dehydrogenase, formed by placing alkylating groups on known inhibitors of the enzyme, has already been discussed.[1,2] In Part XXII of this series various chloroethylamino derivatives of phenoxyacetic acid were described. The present paper deals with similar derivatives of the lactic acid dehydrogenase inhibitors, oxanilic acid and salicylic acid.

Some chloroethylamino derivatives of these acids have been described. Thus Benn *et al.*[3] prepared *p*-(di-2-chloroethylamino)oxanilic acid (Ia, R = CH_2CH_2Cl) and Baker *et al.*[4] have obtained *m*-(di-2-chloroethylamino)oxanilic acid (Ib, R = CH_2CH_2Cl) and also 4-(2-chloroethylamino)salicylic acid (II, R = H), 4-[methyl-(2-chloroethyl)amino] salicylic acid (II, R = Me), and 4-(di-2-chloroethylamino) salicylic acid (II, R = CH_2CH_2Cl). The preparation of *p*-[ethyl-(2-chloroethyl)amino] oxanilic acid (Ia, R = Et), 5-[ethyl-(2-chloroethyl)amino]salicylic acid (III, R = Et) and 5-(di-2-chloroethylamino)salicylic acid (III, R = CH_2CH_2Cl) is now described.



(I) a, *p*-isomer
b, *m*-isomer



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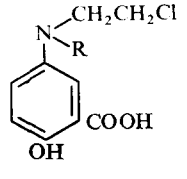

(III)

MATERIALS

Ethyl *p*-nitro-oxanilate (IV, R = NO_2), required as starting material for the synthesis of *p*-[ethyl-(2-chloroethyl)amino]oxanilic acid, has been prepared by the prolonged action of ethyl oxalate on *p*-nitroaniline.[5,6] This compound can be obtained in improved yield and with a much shorter reaction time by the condensation of ethyl

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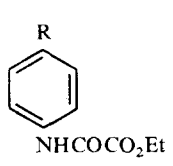
oxalate with *p*-nitroaniline in ethanol solution containing sodium ethoxide. Ethyl *p*-nitro-oxanilate may also be obtained in high yield by the action of the less accessible ethyl oxalyl chloride on *p*-nitroaniline (cf. ref. 4) but the improved method using ethyl oxalate is more convenient.



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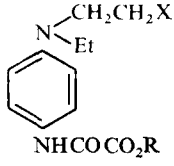

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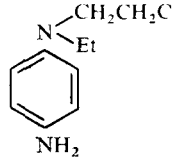
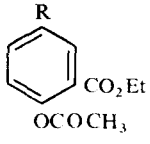


(VI)

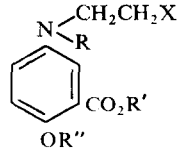
Catalytic hydrogenation of the nitro-compound (IV, R = NO_2) afforded the amino-derivative (IV, R = NH_2) which on ethylation by the method of Rice and Kohn[7] gave ethyl *p*-ethylamino-oxanilate (IV, R = NHEt). This base was hydroxyethylated by the action of ethylene oxide in aqueous acetic acid and then on treating the product (V, X = OH, R = Et) with phosphoryl chloride ethyl *p*-[ethyl-(2-chloro-ethyl)amino]oxanilate (V, X = Cl, R = Et) was obtained.

On heating the ester (V, X = Cl, R = Et) under reflux with concentrated hydrochloric acid decomposition of the oxanilic acid initially formed occurs with the production of *p*-[ethyl-(2-chloroethyl)amino]aniline (VI). When the ester is allowed to stand at room temperature in concentrated hydrochloric acid a mixture of the required acid (V, X = Cl, R = H) and the amine (VI) is formed. The precipitate obtained when an aqueous solution of the mixture is adjusted to pH 5 was shown to be the salt formed from the acid (V, X = Cl, R = H) and the amine (VI). The free acid, generated from this salt, proved to have limited stability.

Ethyl 5-nitrosalicylate was converted into its *O*-acetyl derivative (VII, R = NO_2) and this on catalytic hydrogenation afforded ethyl *O*-acetyl-5-aminosalicylate (VII, R = NH_2). The *N*-ethyl derivative (VIII, R = NHEt), prepared by heating the amino-ester with Raney nickel in ethanol, was converted into ethyl *O*-acetyl-5-[ethyl-(2-hydroxyethyl)amino]salicylate (VIII, R = R′ = Et, R″ = CH_3CO, X = OH) on treatment with ethylene oxide in glacial acetic acid. On heating the product obtained by the action of phosphoryl chloride on the hydroxyethylamino-ester with concentrated hydrochloric acid 5-[ethyl-(2-chloroethyl)amino]salicylic acid (VIII, R = Et, R′ = R″ = H, X = Cl) was obtained.



(VII)



(VIII)

When ethyl *O*-acetyl-5-aminosalicylate (VII, R = NH_2) was treated with ethylene oxide in 6N aqueous acetic acid a mixture of mono- and di-hydroxyethylated products was formed. Chromatographic resolution gave crystalline ethyl *O*-acetyl-5-(2-hydroxy-ethylamino)salicylate (VIII, R = H, R′ = Et, R″ = CH_3CO, X = OH); the more

absorbed fraction contained the dihydroxyethylamino-derivative (VIII, R = CH_2CH_2-OH, R′ = Et, R″ = CH_3CO, X = OH) which became the main product when the reaction was carried out in glacial acetic acid. The liquid dihydroxyethylamino-ester was converted into 5-(di-2-chloroethylamino)salicylic acid (VIII, R = CH_2CH_2Cl, R′ = R″ = H, X = Cl) in the usual manner.

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-*nitro-oxanilate*

Method a. A solution of *p*-nitroaniline (6·9 g) and diethyl oxalate (14·6 g) in ethanol (100 ml) was rapidly added to a vigorously stirred solution of sodium (1·15 g) in ethanol (50 ml). Stirring was continued for 4 min after the addition was complete and then the mixture was rapidly poured on to crushed ice. The precipitated ethyl *p*-nitro-oxanilate was crystallized from ethanol; yield 7·7 g (64·7%), m.p. 175–176° (lit.[5,6] m.p. 171°); ν_{max}^{nujol} 3200, 1710, 1690, 1490, 1550, 1338 and 858 cm^{-1}.

Method b. Ethyl oxalyl chloride (13·7 g) in methylene chloride (100 ml) was added to *p*-nitroaniline (27·6 g) in methylene chloride (500 ml) and the solution was heated under reflux on a steam bath for 30 min. The cooled solution was filtered and concentrated to 150 ml. On adding light petroleum (200 ml, b.p. 30–40°) ethyl *p*-nitro-oxanilate, m.p. 175–176°, separated; yield 22·3 g (94%).

Ethyl p-*amino-oxanilate*

A solution of ethyl *p*-nitro-oxanilate (10 g) in ethanol (200 ml) and ethyl acetate (200 ml) containing palladium charcoal (350 mg, 5% Pd) was shaken in an atmosphere of hydrogen for 1 hr. The filtered solution was concentrated to 50 ml and then poured on to crushed ice when *ethyl* p-*amino-oxanilate* (8·5 g, 97%), m.p. 104–106°, separated. On crystallization from benzene, prisms, m.p. 107–109°, were obtained; ν_{max}^{nujol} 3300, 3200, 1710, 1675, 1510 and 834 cm^{-1}. (Found: C, 57·6%; H, 5·6%; N, 13·7%; Calc. for $C_{10}H_{12}N_2O_3$: C, 57·7%; H, 5·8%; N, 13·5%.)

Ethyl p-*ethylamino-oxanilate*

A solution of ethyl *p*-amino-oxanilate (2·7 g) in ethanol (50 ml) containing Raney nickel catalyst (5 g) was heated under reflux for 4 hr. Evaporation of the filtered solution under reduced pressure gave an oil (2·3 g) which solidified in contact with light petroleum (b.p. 30–40°). *Ethyl* p-*ethylamino-oxanilate* formed pale yellow needles, m.p. 69–70°, from benzene-light petroleum (b.p. 60–80°); ν_{max}^{nujol} 3200, 1710, 1680, 1510 and 834 cm^{-1}. (Found: C, 60·8%; H, 6·9%; N, 12·1%. Calc. for $C_{12}H_{16}N_2O_3$: C, 61·0%; H, 6·8%; N, 11·9%.)

Ethyl p-[*ethyl-(2-hydroxyethyl)amino*]*oxanilate*

A solution of ethyl *p*-ethylamino-oxanilate (20 g) and ethylene oxide (25 ml) in acetic acid (75 ml) and water (75 ml) was kept at room temperature for 24 hr. The filtrate obtained after treatment with charcoal was neutralized with solid $NaHCO_3$ when the product separated. *Ethyl* p-[*ethyl-(2-hydroxyethyl)amino*]*oxanilate* formed canary yellow needles, m.p. 93–94°, from benzene-light petroleum (b.p. 30–40°); yield 15·5 g (65%); ν_{max}^{nujoi} 3320, 3200, 1705, 1670, 1510 and 822 cm^{-1}. (Found: C, 59·8%, H, 6·9%; N, 10·1%. Calc. for $C_{11}H_{20}N_2O_4$: C, 60·0%; H, 7·2%; N, 10·0%.)

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

Ethyl *p*-[ethyl-(2-hydroxyethyl)amino]oxanilate (15 g) and phosphoryl chloride (50 ml) were heated under reflux for 30 min and the cooled mixture was then poured on to ice. On neutralization with solid $NaHCO_3$ *ethyl* p-[*ethyl*-(2-*chloroethyl*)*amino*] *oxanilate* (15 g, 94%) separated. It formed yellow plates, m.p. 99–100°, from benzene-light petroleum (b.p. 30–40°); ν_{max}^{nujol} 3250, 1720, 1690, 1520 and 835 cm^{-1}. (Found: C, 56·1%; H, 6·2%; Cl, 12·2%; N, 9·6%. Calc. for $C_{14}H_{19}ClNO_3$: C, 56·3%; H, 6·4%; Cl, 11·9%; N, 9·4%.)

Hydrolysis of Ethyl p-[*ethyl*-(2-*chloroethyl*)*amino*]*oxanilate*

(a) Ethyl *p*-[ethyl-(2-chloroethyl)amino]oxanilate (0·5 g) was heated under reflux for 2 hr in concentrated HCl (10 ml). The solution was then evaporated under reduced pressure and the residue was dissolved in hot ethanol. The material precipitated on adding ether to the cooled solution was recrystallized from methanol-ether giving the *dihydrochloride* of p-[*ethyl*-(2-*chloroethyl*)*amino*]*aniline* (0·4 g) as prisms, m.p. 191–193° (decomp.); ν_{max}^{nujol} 2500, 1925, 833 and 680 cm^{-1}. (Found: C, 44·6%; H, 6·4%; Cl, 38·4%; N, 10·3%. Calc. for $C_{10}H_{17}Cl_3NO_2$: C, 44·2%; H, 6·3%; Cl, 39·2%; N, 10·3%.)

(b) A solution of ethyl *p*-[ethyl-(2-chloroethyl)amino]oxanilate (0·5 g) in concentrated HCl (10 ml) was kept at room temperature for 18 hr. After evaporation under reduced pressure the residue was dissolved in water (5 ml) and the pH was adjusted to 5 with saturated aqueous sodium acetate. The precipitate was crystallized from benzene. The p-[*ethyl*-(2-*chloroethyl*)*amino*]*oxanilate* of p-[*ethyl*-(2-*chloroethyl*)*amino*] *aniline* formed needles, m.p. 129–130°; ν_{max}^{nujol} 3240, 2600, 1645, 1610 and 812 cm^{-1}. (Found: C, 57·0%; H, 6·5%; Cl, 14·8%; N, 11·6%; Calc. for $C_{22}H_{30}Cl_2N_4O_3$: C, 56·3%; H, 6·4%; Cl, 15·1%; N, 11·9%.)

The salt (6 g) was suspended in ether and shaken with aqueous sodium hydroxide (12·7 ml, N). At first the salt dissolved but later the sodium salt of the acid separated as needles. p-[*Ethyl*-(2-*chloroethyl*)*amino*]*oxanilic acid* was obtained as an amorphous solid (3 g), m.p. 190–193° (decomp.), by dissolving the sodium salt in dilute HCl and adjusting the pH to 5 with sodium acetate. The acid showed ν_{max}^{nujol} 3200, 1850, 1670–1630 and 1570 cm^{-1}. (Found: Cl, 13·0%; N, 10·3%. Calc. for $C_{12}H_{15}ClN_2O_3$: Cl, 13·1%; N, 10·4%.). It decomposed on attempted crystallization and did not form solid piperidine or aniline salts. It was not possible to determine the rate of reaction under standard conditions since it decomposed on heating in 50% aqueous acetone. The oil, obtained when the initial ether extract was dried (Na_2SO_4), saturated with HCl and evaporated, solidified in contact with ethanol. The product, m.p. 191°, was shown to be the dihydrochloride of *p*-[ethyl-(2-chloroethyl)amino]aniline—identical i.r. spectra and giving no m.p. depression on admixture with an authentic specimen.

Ethyl O-*acetyl*-5-*nitrosalicylate*

Ethyl 5-nitrosalicylate (32 g), anhydrous sodium acetate (15 g), and acetic anhydride (100 ml) were heated under reflux for 4 hr. The precipitate formed on pouring the mixture on to ice was crystallized from aqueous ethanol giving *ethyl* O-*acetyl*-5-*nitro-salicylate* (36 g, 92%) as flattened prisms, m.p. 63–65°; ν_{max}^{nujol} 1775, 1715, 1530, 1356, 910, 842, 752 and 872 cm^{-1}. (Found: C, 52·0%; H, 4·4%; N, 5·8%. Calc. for $C_{11}H_{11}NO_6$: C, 52·2%; H, 4·4%; N, 5·5%.)

Ethyl O-acetyl-5-aminosalicylate

Ethyl *O*-acetyl-5-nitrosalicylate (20 g) in ethanol (250 ml) and ethyl acetate (250 ml) containing palladium charcoal catalyst (1 g, 5% Pd) was shaken in an atmosphere of hydrogen for 1 hr. The filtered solution was evaporated under reduced pressure and the residue was crystallized from benzene. *Ethyl O-acetyl-5-aminosalicylate* (15 g, 85·2%) formed prisms, m.p. 85–87°, ν_{max}^{nujol} 3380, 3290, 1750, 1705, 900 and 850 cm^{-1}. (Found: C, 59·0%; H, 5·9%; N, 6·4%. Calc. for $C_{11}H_{13}NO_4$: C, 59·2%; H, 5·9%; N, 6·3%.)

Ethyl O-acetyl-5-ethylaminosalicylate

Ethyl *O*-acetyl-5-aminosalicylate was ethylated by heating with Raney nickel in ethanol as described above. *Ethyl O-acetyl-5-ethylaminosalicylate*, prisms, m.p. 55–57°, from benzene-light petroleum (b.p. 30–40°) was obtained in 59% yield; ν_{max}^{nujol} 3300, 1745 and 1680 cm^{-1}. (Found: C, 62·1%; H, 7·1%; N, 5·8%. Calc. for $C_{13}H_{17}NO_4$: C, 62·1%; H, 6·8%; N, 5·6%.)

Ethyl O-acetyl-5-[ethyl-(2-hydroxyethyl)amino]salicylate

Ethyl *O*-acetyl-5-ethylaminosalicylate (18 g) and ethylene oxide (30 ml) in glacial acetic acid (100 ml) were kept at room temperature for 54 hr. The oil which separated on adding water (200 ml) and neutralizing with solid $NaHCO_3$ was extracted with ether. Distillation of the dried (Na_2SO_4) extract afforded *ethyl O-acetyl-5-[ethyl-(2-hydroxyethyl)amino]salicylate* (9 g, 42·8%), b.p. 200–201°/0·005 mm; ν_{max}^{film} 3300, 1750 and 1700 cm^{-1}. (Found: C, 60·5%; H, 7·2%; N, 5·2%. Calc. for $C_{15}H_{21}NO_5$: C, 61·0%; H, 7·2%; N, 4·7%.)

5-[Ethyl-(2-chloroethyl)amino]salicylic acid

Ethyl *O*-acetyl-5-[ethyl-(2-hydroxyethyl)amino]salicylate (8 g) and phosphoryl chloride (10 ml) in benzene (100 ml) were heated under reflux for 2 hr. The benzene layer obtained when the cooled solution was poured on to ice was washed with aqueous Na_2CO_3 and water and then dried (Na_2SO_4). Evaporation under reduced pressure gave the chloroethylamino ester as an oil (6 g); ν_{max}^{film} 1745, 1700, 908 and 830 cm^{-1} and no (OH) absorption. This oil (2 g) was hydrolysed without purification by heating on a steam bath for 1 hr with concentrated HCl (10 ml). On adjusting the pH of the cooled solution to 3 by adding solid sodium acetate *5-[ethyl-(2-chloroethyl)amino]salicylic acid* separated (1·1 g). The acid formed prisms, m.p. 148–150° (decomp.), from ethanol; ν_{max}^{nujol} 3300, 2300 and 1610 cm^{-1}. (Found: C, 53·8%; H, 5·9%; Cl, 14·7%; N, 5·8%. Calc. for $C_{11}H_{14}ClNO_3$: C, 54·2%; H, 5·8%; Cl, 14·6%; N, 5·8%.)

Hydroxyethylation of ethyl O-acetyl-5-aminosalicylate

(a) Ethyl *O*-acetyl-5-aminosalicylate (23 g) and ethylene oxide (50 ml) in aqueous acetic acid (230 ml, 6N) was kept at room temperature for 60 hr. After neutralization with solid $NaHCO_3$ the mixture was extracted with ether. The dried extract was evaporated and a solution of the residue in benzene was passed through a column of partially deactivated alumina—prepared by adding dilute acetic acid (10 ml, 10% v/v) to Spence Type H alumina (100 g). On elution with benzene an early fraction (10 g) was obtained which was crystallized from benzene-light petroleum (b.p. 30–40°). *Ethyl O-acetyl-5-(2-hydroxyethylamino)salicylate* formed needles, m.p. 79–80°;

ν_{max}^{nujol} 3300, 3200, 1745, 1705, 918 and 830 cm^{-1}. (Found: C, 58·5%; H, 6·8%; N, 5·1%. Calc. for $C_{13}H_{17}NO_5$: C, 58·4%; H, 6·4%; N, 5·2%.)

Continued elution with benzene afforded an oil (20 g) which showed strong absorption at 3300 cm^{-1} (OH).

(b) Hydroxyethylation of ethyl O-acetyl-5-aminosalicylate (37 g) in glacial acetic acid, as described for the ethylamino analogue, only gave the more absorbed material (27 g) which was shown to be the di-2-hydroxyethylamino derivative by conversion into the di-2-chloroethylamino acid.

5-(di-2-chloroethylamino)salicylic acid

The more absorbed product (20 g), obtained by methods (a) or (b), was treated with phosphoryl chloride in benzene and the non-crystalline product (17 g) was hydrolysed with concentrated HCl as described above. On adjusting the pH of the acid solution to 5, 5-(*di-2-chloroethylamino*)*salicylic acid* (7·8 g) separated. The acid formed flattened prisms, m.p. 125–126°, from ethanol. This material contained ethanol of crystallization as shown by analysis and a strong absorption band at 3350 cm^{-1}. (Found: C, 48·6%; H, 5·2%; Cl, 21·8%; N, 4·6%. Calc. for $C_{11}H_{13}Cl_2NO_3.C_2H_5OH$: C, 48·2%; H, 5·9%; Cl, 21·9%; N, 4·3%.)

On vacuum drying for 4 hr at 60° the solvent of crystallization was lost; the absorption band at 3350 cm^{-1} disappeared and the m.p. rose to 127–128°. The anhydrous material showed ν_{max}^{nujol} 3200–2500, 1670, 900, 830 and 719 cm^{-1}. (Found: C, 47·5%; H, 4·8%; Cl, 25·2%; N, 5·0%. Calc. for $C_{11}H_{13}Cl_2NO_3$; C, 47·5%; H, 4·7%; Cl, 25·5%; N, 5·0%.)

METHODS

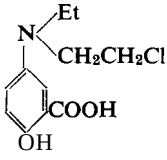
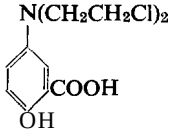
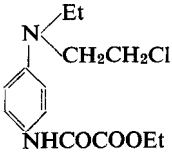
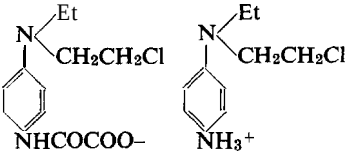
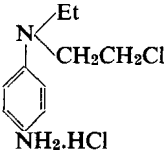
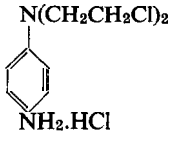
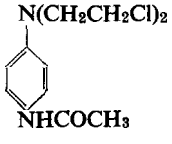
The protocol for testing the compounds as inhibitors of the growth of the transplanted Walker rat carcinoma 256 and the method of assay against the mouse lymphoid leukaemia, L1210, are described in Part XXII.[2] In both tests compounds were administered by a single i.p. injection in arachis oil on the day following implantation or inoculation. In Table 1 the results of the Walker tumour test are expressed as C/T ratios, that is, the weight of tumours in control rats/the weight of tumours in treated rats. The T/C ratio shown for the L1210 assay (Table 2) equals [the average survival time of treated mice divided by the average survival time of controls] × 100.

RESULTS AND DISCUSSION

Chemical reactivity

The rates of reaction under standard conditions[9] of 5-[ethyl-(2-chloroethyl)amino]- and 5-(di-2-chloroethylamino)-salicylic acid follow the usual pattern—the monofunctional derivative being the more reactive. The sodium salts of these acids release chloride ions at about the same rate though the extent of ester formation (measured by the difference between hydrogen ion and chloride ion release)[9] is considerably different. It was not possible to determine the rate of reaction of the oxanilic acid derivative since decomposition occurred in boiling aqueous acetone. Hydrolysis of *p*-(ethyl-2-chloroethyl)amino- and *p*-(di-2-chloroethyl)amino-aniline was complete under the standard conditions. Acetylation of the free amino group reduces the

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

Compound	% Reaction* acid H	acid Cl	Na salt H	Na salt Cl	Dose mg/kg†	Survivors	C/T† ratio	Approx. LD_{50} mg/kg‡
	54	84	16	60	32 16 8 4	0/3 3/3 3/3 3/3	— 1·2 1·2 1·2	23
	35	38	48	63	32 16 8 4	0/3 213 3/3 3/3	— 17 1·4 0·9	14
	—	—	—	—	80 40 20 10	2/3 2/3 3/3 3/3	1 1 1 1	45
	—	—	—	—	160 80 40 20	0/3 3/3 3/3 3/3	— 1·3 1 1	113
	100§	loo	—	—	40 20 10	0/3 3/3 3/3 3/3	— 1 1·2 1·2	28
	100§	loo	—	—	18 4	213 3/3 3/3	1 1·2	14
	42‖	—	—	—	32 16 8 4	1/3 3/3 3/3 3/3	∞ 51 4 1·3	28

* Release of hydrogen or chloride ions on refluxing for ½ hr in 1 :1 acetone-water, C = 0·02 M for chloroethylamines or 0·01 M for di(chloroethyl)amines (see ref. 9).

† gee text.

‡ For tumour bearing animals.

§ For hydrochloride.

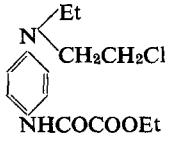
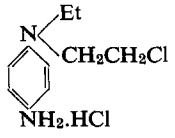
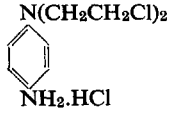
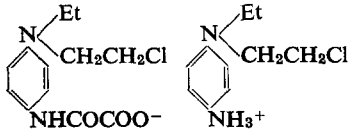
‖ For free base.

chemical reactivity of the 2-chloroethylamino group due to the lower electron releasing capacity of an acetylamino group as compared with a free amino group.

Toxicities

5-[Ethyl-(2-chloroethyl)amino]salicylic acid is somewhat less toxic than the difunctional analogue and *p*-[ethyl-(2-chloroethyl)amino]aniline is less toxic than *p*-(di-2-chloroethylamino)aniline. The *p*-[ethyl-(2-chloroethyl)amino]oxanilic acid salt of

TABLE 2. SCREENING AGAINST MOUSE LEUKAEMIA, L1210

Compound	Dose† mg/kg	T/C Ratio	LD_{50} for host mouse mg/kg
	10 5 2·5	54 91 93	10
	56 28 14	96 93 93	56
	14 7 3·5	69 94 100	14
	36 18 9	48 98 100	36

† See footnote to Table 1.

p-[ethyl-(2-chloroethyl)amino]aniline is considerably less toxic than the hydrochloride. Acetylation reduces the toxicity of *p*-(di-2-chloroethylamino)aniline.

Antineoplastic activities

None of the mono-2-chloroethylamino derivatives shows activity against the Walker tumour or the L1210 leukaemia (Tables 1 and 2). It was not possible to test free *p*-[ethyl-(2-chloroethyl)amino]oxanilic acid owing to its instability. Since its salt with the inactive *p*[ethyl-(2-chloroethyl)amino]aniline produces no growth inhibition it is reasonable to conclude that the acid is without effect on the Walker tumour. Though there is no great difference in the toxicities of the two salicylic acid derivatives only the difunctional derivative shows activity against the Walker tumour and this only at near toxic doses. *p*-(Di-2-chloroethylamino)aniline also shows activity only at doses approaching the toxic level. There are thus two more examples in this paper of the greater antineoplastic activity of a difunctional derivative as compared with a

monofunctional derivative of the same basic structure. Appreciable activity is shown by *p*-(di-2-chloroethylamino)acetanilide—the dose (ED_{90}) calculated to produce 90 per cent inhibition of the growth of the Walker tumour is 14 mg/kg and the chemotherapeutic index (LD_{50}/ED_{90}) is 2. This compares well with the index (3) reported for this compound when assayed against the Walker tumour carried in the Holtzmann rat.[8]

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