Aryl-2-halogenoalkylamines. Part XIV. Some Compounds possessing Latent Cytotoxic Activity.*

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In an attempt to obtain aryldi-2-chloroethylamines of greater specificity as tumour-growth inhibitors a series of derivatives has been prepared in which the reactivity of the chlorine atoms would be increased by a change in the molecule such as could occur *in vivo*. It was hoped that the required change would not take place equally well at all sites in the organism. In this communication are described some acyl, phosphoryl, and phenylureido-derivatives of *NN*-di-2-chloroethyl-*p*-phenylenediamine, certain *NN*-di-2-chloroethylamino-derivatives of hippuric acid, and also some acyl derivatives of *p*-di-(2-chloroethyl)aminophenol. The rates of hydrolysis and the biological activities of some of the new compounds are briefly discussed.

Many aryl-2-halogenoalkylamines are cytotoxic towards rapidly proliferating tissues but their lack of specificity towards neoplastic tissues has limited their use as chemotherapeutic agents (see Part XII, *J.*, 1953, 2386). Earlier papers of this series have described derivatives possessing groups which it was hoped would influence the ease with which the compound would diffuse into target cells. In further attempts to exploit some cell variable, as well as the state of rapid division, derivatives have now been prepared which may not themselves be active but which may be converted by enzymic action into effective compounds. As there is, as yet, no clear indication of an enzyme system which predominates in neoplastic tissue the first studies are necessarily empirical. In the present communication are described compounds which could be activated, in the sense that their chlorine atoms could become more reactive, by processes known to occur *in vivo*.

The *p*-amino-derivative of *NN*-di-2-chloroethylaniline (I; R = H) is known to be very effective (Part II, *J.*, 1949, 1972) and it has been shown (Haddow, *Ann. Rep. British Empire Cancer Campaign*, 1952, **30**, 28) that certain acylated derivatives also exhibit activity as inhibitors of the transplanted Walker rat carcinoma although the chemical reactivity of the chlorine atoms in these derivatives is lower than in the parent amine. For example, the acetyl (I; R = Ac) and the propionyl (I; R = Et·CO) derivative are effective; the benzoyl derivative (I; R = Bz) is, however, inactive. These results suggested that hydrolysis to the amine was responsible for activity, the more resistant benzoate being unaffected.

A number of new acylated derivatives of the amine (I) has now been prepared. Particular attention has been directed to including substituents in the acyl group which would result in lowered reactivity in the chloroethyl group. The incorporation of such groups leads to a greater activation on subsequent hydrolysis of the amide linkage. The compounds obtained include the mono-, di-, and tri-chloroacetyl, the iodoacetyl, the phenylacetyl, and the *p*-nitrobenzoyl derivatives. Some of the compounds prepared, together with their rates of hydrolysis under standard conditions and their biological activities (where available), are given in the Table. Especially interesting is the trifluoroacetyl

* Part XIII, *J.*, 1955, 890.

derivative* (I; R = CF_3·CO) which is hydrolysed only to the extent of 7%, compared with 42% for the acetate.

4-(Di-2-chloroethylamino)-2-methylaniline and the 2-methoxy-analogue have also been prepared mainly because they would have been reductive fission products of various azo-derivatives (see below); but the acetyl and the benzoyl derivative of the former were also prepared for biological test.

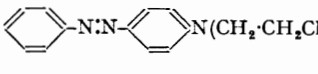
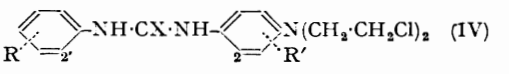
(I) *p*-NHR·C_6H_4·$N(CH_2·CH_2Cl)_2$ *p*-$(RO)_2PO·NH·C_6H_4·N(CH_2·CH_2Cl)_2$ (II)

In view of the reported higher activity of phosphoramidases in malignant tissue, compared with that on normal tissue [Ichihara, *J. Biochem.* (*Japan*), 1933, **18**, 87; Gomori, *Proc. Soc. Exp. Biol. Med.*, 1948, **69**, 407; see also Friedman and Seligman, *J. Amer. Chem. Soc.*, 1954, **76**, 655, 658] it appeared profitable to examine phosphoramidates related to the amine (I; R = H). Accordingly the diethyl (II; R = Et) and the diphenyl phosphoramidate (II; R = Ph) were prepared. Only the diethyl derivative was an active tumour-growth inhibitor.

Another type of compound which is probably activated by conversion into the amino-derivative (I; R = H) is di-2-chloroethylaminoazobenzene (III), some derivatives of which were described in Parts II and XII (*J.*, 1949, 1972; 1953, 2386). An extended series of azo-derivatives will be reported later. It is known that the azo-linkage may be replaced by a ureido-group in certain trypanocidal dyes without loss of biological activity (Burger, "Medicinal Chemistry," Interscience, New York, 1951, p. 842), so several urea derivatives (IV; X = O) have been prepared by the action of the appropriate *iso*cyanate on the amine (I; R = H).

In the *p*-(di-2-chloroethylamino)azobenzene series early results indicated that the insertion of electron-releasing substituents *ortho* to the azo-linkage favourably influenced cytotoxic activity. For this reason the 2-methyl- (IV; R = H, R′ = *o*-Me, X = O), 2′-methyl- (IV; R = *o*-Me, R′ = H, X = O), and 4′-methyl-diphenylurea derivative (IV; R = *p*-Me, R′ = H, X = O) were also prepared. Two thiourea derivatives (IV; R = R′ = H, X = S; and R = H, R′ = *o*-Me, X = S) have also been obtained.

The chlorine atoms in the azo-compounds have less chemical reactivity than those in the ureido-derivatives (see Table). The latter would, however, give rise to the even more reactive amino-compounds on hydrolysis. It was established that the ureido-linkage was stable under the standard conditions used to determine hydrolysis rates and hence the relatively high values found for the ureido-derivatives could not have been due to the partial formation of the very reactive *p*-phenylenediamine derivative (I; R = H). Prolonged heating of the *p*-tolylureido-derivative (IV; R = *p*-Me, R′ = H, X = O) in 50% aqueous acetone gave the corresponding di-2-hydroxyethylaminophenylureido-compound.

(III)  (IV) 

(IV*a*) Ph·N:CH·C_6H_4·$N(CH_2·CH_2Cl)_2$-*p* Ph·CH:CH·$C_6H_4(N·CH_2·CH_2Cl)_2$-*p* (IV*b*)

(IV*c*) *p*-$(Cl·CH_2·CH_2)_2N·C_6H_4·CH:N·C_6H_4·N(CH_2·CH_2Cl)_2$-*p*

The azomethine (IV*a*) was of considerable interest since it could be regarded as an isostere of two cytotoxic agents, namely, the azo-compound (III) and the stilbene derivative (IV*b*) (Ross, *J.*, 1949, 183), and for this reason the parent substance and its 4′-methoxy- and 3′-nitro-derivatives were prepared. In the organism the azomethine could yield the weakly growth-inhibitory *p*-(di-2-chloroethylamino)benzaldehyde by hydrolytic fission or *N*-4-(di-2-chloroethylamino)benzylaniline by enzymic reduction. The last compound, which would be expected to be active on account of the reactivity of its chlorine atoms, was prepared, for test, by catalytic hydrogenation of the azomethine (IV*a*). A tetra-2-chloroethylaminoazomethine (IV*c*) was obtained by condensing *p*-(di-2-chloroethylamino)-benzaldehyde with *p*-*NN*-di-2-chloroethyl-*p*-phenylenediamine. The closely related phenylhydrazone of *p*-(di-2-chloroethylamino)benzaldehyde was also prepared for test.

* The preparation of this compound was suggested in discussion with Dr. L. N. Owen.

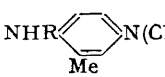
The hydrolysis rate of the azomethines could not be determined since decomposition into the constituent aldehyde and amine occurred under the standardised conditions for this test.

p-(Di-2-chloroethylamino)benzoic acid (V) is a moderately effective cytotoxic agent, for although the reactivity of the chlorine atoms in the free acid is low it is much higher in the ionised form which will predominate at physiological pH. In its esters activation of the chlorine atoms will be manifest only after hydrolysis of the ester linkage. The methyl ester has low biological activity whereas the ethyl ester is inactive; the methyl ester would be expected to be the more easily hydrolysed (cf. Hammett, " Physical Organic Chemistry," New York, 1940, p. 211).

Two other compounds of lowered chemical reactivity which could give rise to the aminobenzoic acid derivative (V) have now been prepared : *p*-(di-2-chloroethylamino)benzamide and ethyl *p*-di-(2-chloroethylamino)hippurate (VI) were obtained by the action of the acid

(V) p-$HO_2C{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2Cl)_2$ $HO_2C{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2Cl)_2$-$p$ (VI)

chloride on ammonia and glycine ester respectively. During this work the preparation of ethyl *p*-(di-2-chloroethylamino)benzoate was improved. The corresponding *m*-substituted amide and hippuric acid were similarly prepared. These amides and hippuric acid derivatives are not effective compounds and this suggests that the amide linkage is not readily hydrolysed *in vivo*.

Substituent R	Hydrolysis (%) *	Biological activity †
Diamine (I)		
Ac	42	+ve
Et·CO	40	+ve
Pr·CO	38	—
$CH_2F{\cdot}CO$	19	+ve
$CF_3{\cdot}CO$	7	—
$CH_2Cl{\cdot}CO$	23	—
$CHCl_2{\cdot}CO$	17	—
$CCl_3{\cdot}CO$	13	+ve
$CH_2I{\cdot}CO$	19	—
Bz	23	−ve
p-$Me{\cdot}C_6H_4{\cdot}CO$	29	—
p-$O_2N{\cdot}C_6H_4{\cdot}CO$	18	—
$Ph{\cdot}CH_2{\cdot}CO$	53	—
		
Ac	47	—
Bz	23	—
Phosphoramidates (II)		
Et	41	+ve
Ph	28	−ve
Azobenzene derivative (III)		
—	<1	+ve

Substituent R	Hydrolysis (%) *	Biological activity †
Urea (IV; R′ = H; X = O)		
H	34	+ve
H (R′ = 2-Me)	38	—
2′-Me	52	—
4′-Me	47	—
Thiourea (IV; R = H, X = S)		
H	14	—
H (R′ = 2-Me)	13	—
p-$R{\cdot}CO{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2Cl)_2$		
Cl	—	−ve
NH_2	2	—
$NH{\cdot}CH_2{\cdot}CO_2Et$	3	−ve
m-$R{\cdot}CO{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2Cl)_2$		
Cl	—	−ve
NH_2	7	−ve
$NH{\cdot}CH_2{\cdot}CO_2Et$	7	−ve
p-$RO{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2Cl)_2$		
H ‡	56	+ve
Ac ‡	15	+ve
Bz ‡	Bz ‡	+ve

* In 50% aqueous acetone at 66° during ½ hr. † As inhibitors of the growth of the transplanted Walker rat carcinoma. ‡ Measured by Dr. J. J. Roberts.

The chlorine atoms in the *O*-acetate and *O*-benzoate of *p*-(di-2-chloroethylamino)-phenol were shown to be less reactive than those in the free phenol (Table). In tumour-growth inhibition tests, however, the acetate and the benzoate are more effective than the phenol. This may be due to the liberation of the phenol within cells into which the free phenol could not so easily diffuse. An attempt to obtain *p*-(di-2-chloroethylamino)phenol by heating *NN*-di-2-chloroethyl-*p*-anisidine with hydriodic acid resulted in the formation of *N*-2-chloroethyl-*N*-2-iodoethyl-*p*-anisidine, previously obtained from *NN*-di-2-chloroethyl-*p*-anisidine and sodium iodide in aqueous acetone (Ross, *J.*, 1949, 2589). The required phenol was eventually obtained in low yield by the action of phosphorus

oxychloride on *p*-(di-2-hydroxyethylamino)phenol (I.G. Farbenind A.-G., Fr. P. 648,761). Although the *p*-(di-2-chloroethylamino)phenyl benzoate was readily obtained by the action of benzoyl chloride in alkaline solution the acetate could not thus be conveniently prepared. Hydroxyethylation of *p*-aminophenyl acetate (Gatalis, *Ber.*, 1926, **59**, 850), followed by treatment with phosphorus oxychloride, gave the required *p*-(di-2-chloroethylamino)-phenyl acetate.

Experimental

4-(*Di*-2-*chlorothylamino*)-2-*methyl*- *and* -2-*methoxy*-*aniline*.—*NN*-Di-2-chloroethyl-*m*-toluidine (Ross, *J.*, 1949, 183) was nitrosated by Everett and Ross's method (*J.*, 1949, 1972), giving the 4-*nitroso*-derivative (NR_2 = 1), m. p. 103°, dark green needles from benzene–light petroleum (b. p. 60—80°) (Found: C, 50·8; H, 5·5; N, 11·0. $C_{11}H_{14}ON_2Cl_2$ requires C, 50·6; H, 5·4; N, 10·7%). Reduction of the nitroso-compound with stannous chloride as described in Part II, or more conveniently by hydrogenation over a palladium catalyst in methanol, gave the diamine which formed a *hydrochloride*, m. p. 200°, needles from ethanol–light petroleum (b. p. 60—80°) (Found: C, 46·8; H, 6·1; N, 9·9. $C_{11}H_{16}N_2Cl_2$,HCl requires C, 46·6; H, 6·0: N, 9·9%), and an *acetyl*, m. p. 125°, fine needles from benzene–light petroleum (b. p. 60—80°) (Found: C, 54·0; H, 6·4; N, 9·7. $C_{13}H_{18}ON_2Cl_2$ requires C, 54·0; H, 6·3; N, 9·7%), and a *benzoyl derivative*, m. p. 156—157°, short thick needles from ethanol (Found: C, 62·0; H. 6·0; N, 8·2. $C_{18}H_{20}ON_2Cl_2$ requires C, 61·5; H, 5·7; N, 8·0%).

NN-*Di*-2-*chloroethyl*-m-*anisidine* {obtained from *m*-anisidine as a colourless oil, n_D^{23} 1·5708, by the methods described in Part I: Found: Cl, 28·6, 28·4. $C_{11}H_{15}ONCl_2$ requires Cl, 28·6% [*styphnate*, prisms, m. p. 124—125° (decomp.) (from methanol) (Found: C, 41·3; H, 3·9; N, 11·1. $C_{17}H_{18}O_9N_4Cl_2$ requires C, 41·4; H, 3·7; N, 11·3%)]} was similarly converted into the 4-*nitroso*-derivative, m. p. 122°, stout dark green needles from benzene–light petroleum (b. p. 60—80°) (Found: C, 47·8; H, 5·2; N, 10·5. $C_{11}H_{14}O_2N_2Cl_2$ requires C, 47·7; H, 5·1; N, 10·1%), and into the 4-amino-derivative which gave a *monohydrochloride*, m. p. 194° (decomp.), short needles from ethanol–light petroleum (b. p. 60—80°) (Found: C, 43·6; H, 5·8; N, 9·6. $C_{11}H_{16}ON_2Cl_2$,HCl requires C, 44·1; H, 5·7; N, 9·4%).

Acyl Derivatives of NN-*Di*-2-*chloroethyl*-p-*phenylenediamine*.—These derivatives were prepared by the following general method. *NN*-Di-2-chloroethyl-*p*-phenylenediamine hydrochloride (Everett and Ross, *loc. cit.*) (1 mol.) was suspended in ether and shaken with 2N-sodium hydroxide (2·5 equiv.). When all the solid had dissolved the mixture was cooled in ice, and the acid chloride or anhydride was added with vigorous shaking. After 10 min. the ether layer was washed with water and dried (Na_2SO_4). In some instances it was necessary to submit the product to chromatography (benzene and activated alumina). The acyl derivatives were crystallised from benzene–light petroleum (b. p. 60—80°) unless otherwise stated. The following derivatives of *NN*-di-2-chloroethyl-*p*-phenylenediamine were obtained: N′-*butyryl*, plates, m. p. 103° (Found: C, 55·4; H, 6·7; N, 9·1. $C_{14}H_{20}ON_2Cl_2$ requires C, 55·5; H, 6·7; N, 9·2%; N′-*fluoroacetyl*, plates, m. p. 123—124° (Found: C, 49·4; H, 5·3; N, 9·7. $C_{12}H_{15}ON_2Cl_2F$ requires C, 49·2; H, 5·2; N, 9·6%); N′-*trifluoroacetyl*, prismatic needles, m. p. 109—110°, from light petroleum (b. p. 60—80°) (Found: C, 43·9; H, 4·2; N, 8·9. $C_{12}H_{13}ON_2Cl_2F_3$ requires C, 43·8; H, 4·0; N, 8·6%); N′-*chloroacetyl*, needles, m. p. 137—138° (Found: C, 46·7; H, 5·0; N, 9·3. $C_{12}H_{15}ON_2Cl_3$ requires C, 46·5; H, 4·9; N, 9·1%); N′-*dichloroacetyl*, needles, m. p. 135° (Found: C, 42·3; H, 4·3; N, 8·2. $C_{12}H_{14}ON_2Cl_4$ requires C, 41·9; H, 4·1; N, 8·1%); N′-*trichloroacetyl*, plates, m. p. 113—114·5° (Found: C, 38·9; H, 3·7; N, 7·6; Cl, 46·0. $C_{12}H_{13}ON_2Cl_5$ requires C, 38·1; H, 3·5; N, 7·4; Cl, 46·8%); N′-*iodoacetyl*, pale yellow needles, m. p. 149—150° (Found: C, 35·8; H, 4·0; N, 7·0. $C_{12}H_{15}ON_2Cl_2I$ requires C, 35·9; H, 3·8; N, 7·0%); N′-*phenylacetyl*, needles, m. p. 132·5° (Found: C, 61·4; H, 4·7; N, 8·7. $C_{18}H_{20}ON_2Cl_2$ requires C, 61·5; H, 5·7; N, 8·0%); N′-p-*nitrobenzoyl*, copper-coloured plates, m. p. 176°, from ethanol–benzene (Found: C, 53·1; H, 4·6; N, 11·7. $C_{18}H_{17}O_3N_2Cl_2$ requires C, 53·2; H, 4·5; N, 11·0%).

Phosphoramidates from NN-*Di*-2-*chloroethyl*-p-*phenylenediamine*.—*NN*-Di-2-chloroethyl-*p*-phenylenediamine hydrochloride (0·5 g.) was shaken with an excess of 2N-sodium carbonate, and the free base was extracted with ether. Triethylamine (1 ml.) was added to the dried ether solution, followed by diphenyl phosphorochloridate (0·5 ml.). After 10 min. at room temperature the solution was washed with water, dried, and evaporated. A benzene solution of the residue was passed through a column of activated alumina; further washing with benzene

slowly eluted the *diphenyl phosphoramidate* (0·55 g.) which formed prismatic needles, m. p. 128—130°, from benzene–light petroleum (b. p. 60—80°) (Found : C, 56·7; H, 5·6; N, 5·9. $C_{22}H_{23}O_3N_3Cl_2P$ requires C, 56·8; H, 5·0; N, 6·0%). The *diethyl phosphoramidate*, m. p. 113—114°, forming prismatic needles from light petroleum (b. p. 60—80°), was similarly prepared (Found : C, 46·1; H, 6·5; N, 7·7. $C_{14}H_{23}O_3N_2Cl_2P$ requires C, 45·5; H, 6·3; N, 7·6%).

4-Di-2′-chloroethylaminodiphenylurea.—A suspension of *NN*-di-2-chloroethyl-*p*-phenylenediamine hydrochloride (2·69 g.) in ether (100 ml.) was shaken with 2N-sodium carbonate (20 ml.). To the dried ethereal layer was added phenyl *iso*cyanate (1·09 ml.) and when the mixture was heated the *diphenylurea* separated. It formed flattened needles, m. p. 150—152°, from benzene (Found : C, 58·2; H, 5·5; N, 11·9. $C_{17}H_{19}ON_3Cl_2$ requires C, 58·0; H, 5·5; N, 11·9%). *4-Di-2″-chloroethylamino-2-methyl-*, m. p. 170—171°, from ethyl acetate (Found : C, 58·3; H, 6·0; N, 11·3. $C_{18}H_{21}ON_3Cl_2$ requires C, 59·0; H, 5·8; N, 11·5%), *4-di-2″-chloroethylamino-2′-methyl-*, m. p. 159—161°, from benzene–light petroleum (b. p. 60—80°) (Found : C, 59·4; H, 5·7; N, 11·2%), and *4-di-2″-chloroethylamino-4′-methyl-diphenylurea*, m. p. 162°, from benzene (Found : C, 59·5; H, 5·8; N, 11·2%), and *4-di-2″-chloroethylamino-*, m. p. 147°, from benzene–light petroleum (b. p. 60—80°) (Found : C, 55·7; H, 5·3; N, 11·6. $C_{17}H_{19}N_3Cl_2S$ requires C, 55·4; H, 5·2; N, 11·4%), and *4-di-2″-chloroethylamino-2-methyl-diphenylthiourea*, m. p. 169°, from ethyl acetate (Found : C, 56·3; H, 5·7; N, 11·3. $C_{18}H_{21}N_3Cl_2S$ requires C, 56·6; H, 5·5; N, 11·0%) were similarly prepared (all needles) from the appropriate reactants.

Hydrolysis of 4-Di-2″-chloroethylamino-4′-methyldiphenylurea.—A preliminary experiment showed that *s*-diphenylurea was unchanged when heated in 50% aqueous acetone for 10 hr. When 4-di-2″-chloroethylamino-4′-methyldiphenylurea was heated under reflux in 50% aqueous acetone for 3 hr. the corresponding *(dihydroxyethyl)amino*-derivative, m. p. 168°, needles, from ethyl acetate, was formed (Found : C, 65·3; H, 7·3; N, 13·0. $C_{17}H_{21}O_3N_3$ requires C, 65·6; H, 7·0; N, 12·8%).

N-4-*(Di-2′-chloroethylamino)benzylideneaniline.*—A solution of *p*-(di-2-chloroethylamino)-benzaldehyde (2·46 g.) and aniline (1 ml.) in dry ether (100 ml.) was concentrated to 25 ml. and then heated under reflux for 1 hr. On evaporation the solution gave a yellow oil which slowly solidified. The *azomethine* (2·35 g.) formed plates, m. p. 62—64°, from ethanol–light petroleum (b. p. 60—80°) (Found : C, 63·6; H, 5·8; N, 8·9. $C_{17}H_{18}N_2Cl_2$ requires C, 63·6; H, 5·7; N, 8·7%). When the azomethine (1 g.), dissolved in ethanol (20 ml.), was hydrogenated at room temperature over Raney nickel the theoretical volume of gas was taken up during 2½ hr. On saturation of the filtered solution with hydrogen chloride and addition of ether the *hydrochloride* of N-4-*(di-2′-chloroethylamino)benzylaniline* (0·65 g.) separated as a pale cream-coloured powder, m. p. 145° (decomp.) (Found : equiv. by potentiometric titration, 199. $C_{17}H_{20}N_2Cl_2,2HCl$ requires equiv., 198). N-4-*(Di-2′-chloroethylamino)-benzylidene-p-anisidine*, plates, m. p. 93, from ethanol (Found : C, 61·3; H, 5·8; N, 8·1. $C_{17}H_{20}ON_2Cl_2$ requires C, 61·5; H, 5·7; N, 8·0%), N-4′-*(di-2-chloroethylamino)benzylidene-3-nitroaniline*, orange needles, m. p. 126—128·5°, from benzene–light petroleum (b. p. 60—80°) (Found : C, 55·5; H, 4·8; N, 11·2. $C_{17}H_{17}O_2N_3Cl_2$ requires C, 55·7; H, 4·7; N, 11·5%), and 4 : 4′-*bis(di-2-chloroethylamino)benzylideneaniline*, straw-coloured needles, m p. 134—135°, from benzene–light petroleum (b. p. 60—80°) (Found : C, 55·0; H, 5·6; N, 9·0. $C_{21}H_{25}N_3Cl_4$ requires C, 54·7; H, 5·5; N, 9·1%), were prepared by similar condensation of the appropriate aldehyde and amine. The last compound rapidly darkened unless stored in the dark.

p-*(Di-2-chloroethylamino)benzaldehyde Phenylhydrazone.*—Heating the aldehyde (5 g.) and phenylhydrazine (2·2 g.) in ether (160 ml.) under reflux for 1 hr. gave the *phenylhydrazone* (5·9 g.), plates, m. p. 95—96° (from ethanol) (Found : C, 60·4; H, 5·8; N, 12·0. $C_{17}H_{19}N_3Cl_2$ requires C, 60·7; H, 5·7; N, 12·5%). This is converted into a dark resin in 2 weeks in a sealed container.

Ethyl p-*(Di-2-hydroxyethylamino)benzoate.*—(*a*) Ethylene oxide (25 ml.) in acetone (20 ml.) was added to a solution of ethyl *p*-aminobenzoate (20 g.) in water (40 ml.), acetone (40 ml.), and acetic acid (5 ml.). The mixture was heated at 50° and stirred for 8 hr.; then ethylene oxide (20 ml.) was added and stirring continued for a further 8 hr. The product was passed in benzene through activated alumina; the column was washed successively with benzene, benzene–ether (1 : 1), and ether. Early eluates contained *ethyl* p-*2-hydroxyethylaminobenzoate*, m. p. 61—61·5°, which formed flattened needles from benzene (Found : C, 63·0; H, 7·0; N, 6·7. $C_{11}H_{15}O_3N$ requires C, 63·1; H, 7·2; N, 6·7%). Later eluates contained the di-(hydroxyethyl)amino-ester, m. p. 92—93·5°, which formed plates from benzene (Found : C, 61·5; H, 7·5; N, 5·6. Calc. for $C_{13}H_{19}O_4N$: C, 61·6; H, 7·6; N, 5·5%). Everett and Ross (*loc. cit.*) gave m. p. 70° for the latter compound which they obtained as prisms from benzene.

All preparations now form the plates of higher m. p. and it appears that the product previously obtained was a different crystalline modification. In B.P. 128,912/1919 the di(hydroxyethyl)-amino-compound is described as having m. p. 94°.

(*b*) When ethyl *p*-aminobenzoate (16·5 g.), ethylene oxide (20 ml.), and 2N-acetic acid (20 ml.) were heated in a sealed tube at 130° for 5 hr. the di-2-hydroxyethylamino-ester, m. p. 90°, was the only product.

p-(*Di-2-chloroethylamino*)*benzoyl Chloride*.—Ethyl *p*-(di-2-hydroxyethylamino)benzoate (10 g.) was heated under reflux for 15 min. in benzene (100 ml.) containing thionyl chloride (10 ml.). Concentrated hydrochloric acid (70 ml.) was added to the cooled solution, and the mixture was distilled until all the benzene and 20 ml. of water had passed over. The remaining solution was then heated under reflux for a further 1½ hr. during which the *p*-(di-2-chloroethyl-amino)benzoic acid separated. It formed prisms, m. p. 168, from benzene–*cyclo*hexane (1 : 2) (yield 7·5 g.). The acid (2 g.) was heated for 15 min. with thionyl chloride (4 ml.) in benzene (40 ml.). The residue obtained after evaporation under reduced pressure crystallised from ether–light petroleum (b. p. 60—80°). p-(*Di-2-chloroethylamino*)*benzoyl chloride* formed plates, m. p. 83—84° (Found: C, 47·7; H, 4·5; N, 5·0. $C_{11}H_{12}ONCl_3$ requires C, 47·1; H, 4·3; N, 5·0%). The acid chloride was extremely sensitive to hydrolysis and was used immediately after purification. The *amide* was prepared by shaking an ethereal solution of the acid chloride with concentrated aqueous ammonia. It formed slender needles, m. p. 125—126°, from benzene–light petroleum (b. p. 60—80°) (Found: C, 50·5; H, 5·5; N, 10·9. $C_{11}H_{14}ON_2Cl_2$ requires C, 50·6; H, 5·4; N, 10·7%).

Ethyl p-(*Di-2-chloroethylamino*)*hippurate*.—When the Schotten–Baumann method described below for the *m*-isomer was used, only *p*-(di-2-chloroethylamino)benzoic acid could be isolated. Because of the lability of the acid chloride the reaction with glycine had to be carried out in dry acetone. Glycine ester hydrochloride (10 g.) was mixed with an excess of powdered sodium hydroxide and moistened with water. The paste was extracted with acetone (50 ml.) and the extract was dried (K_2CO_3). Finely powdered acid chloride (2 g.) was then added to the filtered solution and this was left at room temperature for ½ hr., then diluted with water and extracted with ether. *Ethyl* p-(*di-2-chloroethylamino*)*hippurate* formed thin needles, m. p. 73·5—74°, from ether–pentane (Found: C, 52·2; H, 5·8; N, 7·8. $C_{15}H_{20}O_3N_2Cl_2$ requires C, 51·9; H, 6·0; N, 8·1%).

Ethyl m-(*Di-2-chloroethylamino*)*hippurate*.—*m*-(Di-2-chloroethylamino)benzoic acid (Everett, Roberts, and Ross, *J.*, 1953, 2386), its *acid chloride*, m. p. 79—81°, prisms from ether–light petroleum (b. p. 60—80°) (Found: C, 47·2; H, 4·3; N, 5·0. $C_{11}H_{12}ONCl_3$ requires C, 47·1; H, 4·3; N, 4·9%), and its *amide*, m. p. 110—112°, flattened needles from benzene–pentane (Found: C, 50·3; H, 5·6; N, 10·5. $C_{11}H_{14}ON_2Cl_2$ requires C, 50·6; H, 5·4; N, 10·7%), were similarly prepared. A solution of the acid chloride (1 g.) in ether (25 ml.) was shaken vigorously with a solution of glycine ester hydrochloride (0·625 g.) in N-sodium hydroxide (7 ml.). Sufficient N-sodium hydroxide (4 ml.) was then added to render the solution alkaline to phenol-phthalein. The ether layer was then dried and evaporated, giving a yellow oil which was passed in benzene–light petroleum (b. p. 60—80°) (1 : 1) through activated alumina. After the early elution of amorphous material *ethyl* m-(*di-2-chloroethylamino*)*hippurate* (300 mg.) was obtained. It formed fine needles, m. p. 100°, from light petroleum (b. p. 60—80°) (Found: C, 51·9; H, 5·8; N, 7·9. $C_{15}H_{20}O_3N_2Cl_2$ requires C, 51·9; H, 6·0; N, 8·1%).

p-(*Di-2-chloroethylamino*)*phenol* (with J. J. Roberts).—*p*-(Di-2-hydroxyethylamino)phenol, prepared by the action of ethylene oxide on *p*-aminophenol in dilute acetic acid, formed prisms, m. p. 140°, from acetone (Found: C, 60·9; H, 7·8; N, 7·2. Calc. for $C_{10}H_{15}O_3N$: C, 60·9; H, 7·7; N, 7·1%). The *triacetate* formed prisms, m. p. 78°, from aqueous ethanol (Found: C, 59·1; H, 6·7. $C_{16}H_{21}O_6N$ requires C, 59·4; H, 6·5%), and the *tribenzoate* formed prisms, m. p. 84°, from ethanol (Found: C, 72·8; H, 5·4. $C_{31}H_{27}O_6N$ requires C, 73·1; H, 5·3%). The dihydroxyethylaminophenol (10 g.) and phosphorus oxychloride (10 ml.) were heated under reflux for 1 hr., after which the mixture was evaporated under reduced pressure and concentrated hydrochloric acid (20 ml.) was added. After 1 hour's heating the solution was diluted with water and extracted with chloroform. The dark extract was passed in benzene through activated alumina which was further washed with benzene. Finally elution with chloroform gave the chloroethylaminophenol as an oil which formed a *hydrochloride*, m. p. 168° (Found: C, 44·8; H, 5·5; N, 5·1. $C_{10}H_{13}ONCl_2,HCl$ requires C, 44·3; H, 5·2; N, 5·2%), and a *picrate*, m. p. 146°, small prisms from benzene (Found: C, 42·0; H, 3·8; N, 11·8. $C_{16}H_{16}O_8N_4Cl_2$ requires C, 41·5; H, 3·5; N, 12·1%). p-(*Di-2-chloroethylamino*)*phenyl benzoate*, prepared by shaking the free phenol (1 g.) in 10% aqueous sodium hydroxide with benzoyl chloride (1 g.)

for 15 min., formed prisms, m. p. 84—86°, from light petroleum (b. p. 60—80°) (Found: C, 60·1; H, 5·2; N, 4·1; Cl, 20·5. $C_{17}H_{17}O_2NCl_2$ requires C, 60·4; H, 5·1; N, 4·1; Cl, 20·9%).

p-(*Di*-2-*chloroethylamino*)*phenyl Acetate.*—*p*-Aminophenyl acetate was treated with ethylene oxide in 2N-acetic acid in the usual way, and the resultant NN-*di*-2-*hydroxyethyl* derivative formed plates, m. p. 82·5°, from benzene–light petroleum (b. p. 60—80°) (Found: C, 60·2; H, 7·2. $C_{12}H_{17}O_4N$ requires C, 60·2; H, 7·2%). Heating the diol (10 g.) with phosphorus oxychloride (10 ml.) in benzene (30 ml.) for 45 min. gave p-(*di*-2-*chloroethylamino*)*phenyl acetate* (2 g.) as an oil (Found: Cl, 25·5. $C_{12}H_{15}O_2NCl_2$ requires Cl, 25·7%).

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