486. *Aryl-2-halogenoalkylamines. Part XII.* Some Carboxylic Derivatives of* NN-*Di*-2-*chloroethylaniline.*

By J. L. Everett, J. J. Roberts, and W. C. J. Ross.

The preparation is described of a number of derivatives of *NN*-di-2-chloroethylaniline containing carboxyl groups, and the reactivities of their chlorine atoms have been assessed by determining the rates of hydrolysis in aqueous acetone. It is shown that the effect of substituents on the reactivity of the chlorine atom in a series of *NN*-di-2-chloroethylanilines can be expressed by Hammett's equation (*J. Amer. Chem. Soc.*, 1937, **59**, 96), ρ having the value $-2{\cdot}10$. The activity of a number of the derivatives as tumour-growth inhibitors is briefly discussed.

The study of aryl-2-halogenoalkylamines (" aromatic nitrogen mustards ") has made it clear that most substances which contain a di-2-chloroethylamino-group possessing a certain level of chemical reactivity are cytotoxic to proliferating tissues (Haddow, Kon, and Ross, *Nature*, 1948, **162**, 824; Ross, *J.*, 1949, 183, and subsequent papers; Ross, *Adv. Cancer Res.*, 1953, **1**, 397). However, their use as chemotherapeutic agents has been limited by their lack of specific action on malignant growths—many normal proliferating tissues are equally affected by most of the derivatives. Danielli (*Nature*, 1952, **170**, 863) has pointed out that cells may be extremely selective in the types of molecule which they concentrate within their plasma membranes. It is hoped to obtain compounds of more selective action on neoplasms by incorporating the di-2-chloroethylamino-group into molecules which have anionic, cationic, lipophilic, of hydrophilic character. The present paper describes the preparation and properties of some carboxyl-substituted derivatives of *NN*-di-2-chloroethylaniline.

A series of acids (I), where $n = 0$—4, has been prepared. The first member, *NN*-di-2-chloroethyl-*p*-aminobenzoic acid (I; $n = 0$), and its methyl and ethyl esters, have already

(I) $(Cl{\cdot}CH_2{\cdot}CH_2)_2N$—C$_6H_4$—$[CH_2]_n{\cdot}CO_2H$

been described (Ross, *J.*, 1949, 183; Everett and Ross, *J.*, 1949, 1972). The methyl or ethyl esters of *p*-aminophenyl-acetic, -propionic, -butyric, and -valeric acids were converted successively into the *NN*-di-2-hydroxyethyl esters, the *NN*-di-2-chloroethyl esters, and the *NN*-di-2-chloroethyl acids by the methods used for preparation of the benzoic acid derivatives (Everett and Ross, *loc. cit.*).

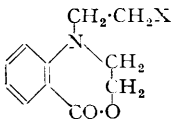
Whilst hydrolysis of methyl *NN*-di-2-chloroethyl-*m*-aminobenzoate yielded the required acid, diethyl *NN*-di-2′-chloroethyl-4-aminophthalate could not be converted into the corresponding dicarboxylic acid since conditions of reaction sufficiently drastic to effect hydrolysis also resulted in decarboxylation to *NN*-di-2-chloroethyl-*m*-aminobenzoic acid.

When methyl anthranilate is heated with two equivalents of ethylene oxide in benzene solution at 160° the main reaction product is 2 : 3 : 4 : 7-tetrahydro-4-2′-hydroxyethyl-7-keto-5 : 6-benz-1 : 4-oxazepine (II; X = OH). Methyl *N*-2-hydroxyethylanthranilate (III; X = OH, R = Me) is also formed but none of the required bishydroxyethyl compound (IV; X = OH, R = Me) could be isolated. The monohydroxyethyl compound becomes the sole product when methyl anthranilate is treated with an excess of ethylene oxide in aqueous acetic acid solution. Kiprianov (*Ukrainskii Khem. Zhurnal*, 1925, **1**, 644) claimed to have obtained both the mono- and the bis-2-hydroxyethyl derivatives when methyl anthranilate was heated with one equivalent of ethylene oxide. The formation of an oxazepine derivative by the action of ethylene oxide on methyl *N*-methylanthranilate was also described by Kiprianov.

The structure of the hydroxyethyloxazepine derivative (II; X = OH) is confirmed by molecular-weight determination, the consumption of one equivalent of alkali on hydrolysis,

* Part XI, *J.*, 1952, 4296.

the formation of a monochloro-derivative (II; X = Cl) on treatment with phosphorus oxychloride, and the production of methyl *NN*-di-2-hydroxyethylanthranilate (IV; X = OH, R = Me) by the action of methanolic hydrogen chloride. The bishydroxyethyl ester (IV; X = OH, R = Me), when treated with phosphorus oxychloride, affords methyl *NN*-di-2-chloroethylanthranilate (IV; X = Cl, R = Me) which may be hydrolysed to the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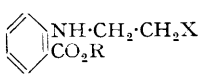
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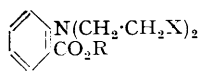
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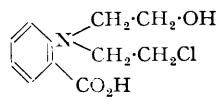
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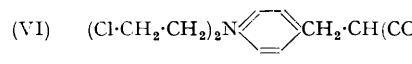
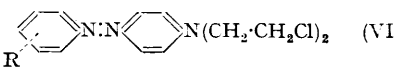
corresponding acid (IV; X = Cl, R = H). The monohydroxy-ethyl compound (III; X = OH, R = Me) has similarly been converted into the chloro-ester (III; X = Cl, R = Me) and the chloro-acid (III; X = Cl, R = H). Hydrolysis of the chloro-oxazepine derivative (II; X = Cl) with concentrated hydrochloric acid yields *N*-2-chloroethyl-*N*-2-hydroxyethylanthranilic acid (V).

Vigorous acid hydrolysis of the ester (VI; R = Et) resulted in some decarboxylation but under milder conditions it was possible to obtain the hydrochloride of *NN*-di-2-chloroethyl-*p*-aminobenzylmalonic acid (VI; R = H).

(VI) 


(VII)

NN-Di-2-chloroethyl-*p*-aminoazobenzene (VII; R = H), which is an effective inhibitor of the growth of the transplanted Walker rat carcinoma, has been prepared by coupling benzenediazonium chloride with *NN*-di-2-chloroethylaniline in acid solution (Everett and Ross, *loc. cit.*). 4-(*NN*-Di-2″-chloroethylamino)azobenzene-2′-, -3′-, and -4′-carboxylic acids (VII; R = CO_2H) have now been prepared by similarly coupling the appropriate diazotised amino-acid.

A correlation between the chemical reactivity of the aryl-2-halogenoalkylamines and their biological activity has been established (Ross, *loc. cit.*, 1953) : in view of this it was of interest to determine the rates of hydrolysis of the new compounds. Table 1 shows the rates for the esters, the free acids, and their sodium salts : the values for the salts were obtained by neutralising the acids with sodium hydroxide, adjusting the acetone concentration to 50%, and then heating in the usual way. In the case of the free acids and the

TABLE 1. *The extent of hydrolysis of 2-chloroethylamino-acids in* 50% *acetone at* 66°. *Concn. of amino-acid,* 0·02M. *Time,* 30 *min.*

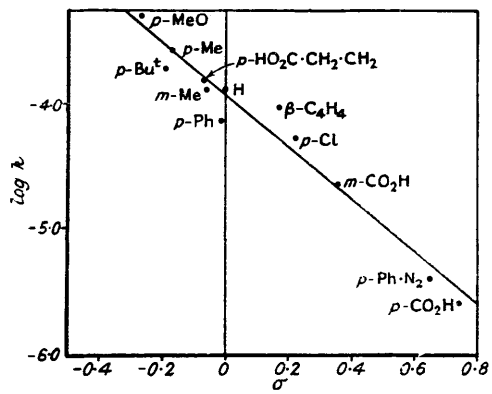
Acid	Free acid, H or Cl (%)	Ester H or Cl (%)	Sodium salt H (%)	Sodium salt Cl (%) (obs.)	Sodium salt Cl (%) (calc.)	pK_a of acid *	Solubility of acid in 4% $COMe_2$ (mg./100 ml.) †
o-$HO_2C{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2Cl)_2$	10	48	41	70	(111)	7·1	—
m-$HO_2C{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2Cl)_2$	4	4	16	26	30	6·0	9
$HO_2C{\cdot}[CH_2]_n$—C$_6$H$_4$—$N(CH_2{\cdot}CH_2Cl)_2$ *n* = 0	1	1	5	12	13	6·6	9
1	17	15	22	39	45	6·1	43
2	24	21	20	41	45	6·4	12
3	32	23	22	42	45	6·5	12
4	27	24	18	38	41	—	5

* Determined in 50% acetone. † Determined by Dr. W. Davis.

esters the amounts of hydrogen and chloride ion liberated are practically identical; the differences in the case of the sodium salts are due to ester formation by reaction of the intermediate carbonium ions with the ionised carboxyl groups (compare Ross, *J.*, 1949, 2589). The experimental values given in Table 1 for the extent of the liberation of chloride ions from the anionic form of the acid are low owing to the production of acid during the

hydrolysis, which reduces the number of carboxyl groups in the ionised form. Since the rate of hydrolysis of the free acid is known it is possible to calculate the true values for the chloride ion liberation—these values represent the actual reactivity of the halogen atom. The validity of the calculation made was shown by hydrolysing the sodium salt of the acid (I; $n = 3$) whilst carefully titrating the solution with sodium hydroxide in 50% acetone. Under these conditions the carboxyl groups will always be ionised and the observed value of 46% for the liberation of chloride ions compares well with the calculated value of 45%. Even the corrected values may be a little low since allowance has not been made for any acid which has been esterified in the reaction. 4-2′-Chloroethyl-2 : 3 : 4 : 7-tetrahydro-7-keto-5 : 6-benz-1 : 4-oxazepine (II; X = Cl) is hydrolysed at the rate of 8% in 30 min. under the standard conditions.

In order to assess the rate of reaction of the acidic chloroethylamines under physiological conditions the rate of elimination of chloride ions at 37° in a solution buffered with sodium hydrogen carbonate was determined. The percentage liberation of chloride ion in one hour was as follows: acid (I; $n = 0$), 10·2; ($n = 1$), 75; ($n = 2$), 77·5; ($n = 3$), 75; and *NN*-di-2-chloroethyl-*m*-aminobenzoic acid, 34. At 20° the phenylacetic acid derivative (I; $n = 1$) is hydrolysed at the rate of 12% in one hour in bicarbonate buffer; this value may be compared with the figure of 60% in one hour at 25° under similar conditions



Graph showing the relationship between log k and σ.

given for *NN*-di-2-chloroethylmethylamine (Golumbic, Fruton, and Bergmann, *J. Org. Chem.*, 1946, **11**, 518).

Hammett has shown that the equation $\log k = \log k_0 + \sigma\rho$ expresses the relation between the rate or equilibrium constants of a general reaction for a series of substituted aromatic compounds, k and k_0 being the constant of the substituted and unsubstituted members respectively, σ a constant for the substituting group, and ρ a constant for the general reaction. If the unimolecular rate constants for the hydrolysis of a series of substituted aryldi-2-chloroethylamines are plotted against the σ values assigned by Hammett (" Physical Organic Chemistry," McGraw Hill Book Co., New York, 1940, p. 188), the graph shown is obtained. In view of the complexity of the hydrolysis reaction involving, as it does, the elimination of two halogen atoms the general agreement between the experimentally determined points and the straight line obtained by the method of least squares is considered satisfactory.

The value of $\log k_0$ calculated from the twelve points is −3·95, in good agreement with the experimental value of −3·91. The value of ρ for the reaction is −2·10. When the hydrolysis constant of the appropriate compound is known the equation $\sigma = \log k - 3{\cdot}95/2{\cdot}10$ enables the σ values for other substituents to be calculated. Table 2 gives some values of σ calculated from rates determined in studies of aryldi-2-chloroethylamines. In two instances the values may be compared with those assigned by Hammett and the agreement is satisfactory.

Biological Results (personal communication from Professor A. Haddow).—All the acids (I) inhibit the growth of transplanted Walker rat carcinoma; moderate activity is shown by the acids ($n = 0$, 2, and 4), and compound ($n = 1$) shows high activity, whilst

(I; $n = 3$) is outstanding. The methyl and ethyl esters of the acids (I) are all active, except the ethyl benzoate derivative ($n = 0$): the corresponding methyl ester shows slight activity, probably because it is more easily hydrolysed to the free acid *in vivo*. *NN*-Di-2-chloroethyl-*m*- and -*o*-aminobenzoic acids and their methyl esters are effective compounds. The monofunctional lactone (II; X = Cl) and chlorohydrin (V) are inactive, thus confirming the greater effectiveness of bifunctional compounds (Goldacre, Loveless, and Ross, *Nature*, 1949, **163**, 667; Loveless and Ross, *ibid.*, 1950, **166**, 1112). Diethyl *NN*-di-2′-chloroethyl-4-aminophthalate and *NN*-di-2′-chloroethyl-4-aminobenzylmalonic acid are inactive.

TABLE 2.

Substituent	Hydrolysis (%) *	log *k* (sec.$^{-1}$)	σ (calc.)	Substituent	Hydrolysis (%) *	log *k* (sec.$^{-1}$)	σ (calc.)
m-CO_2Et	4·5	−4·592	0·306	*p*-$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2Et$	23	−3·838	−0·054
p-Br	6	−4·46	0·244 [a]	*p*-$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$	33	−3·653	−0·143
p-$CH_2{\cdot}CO_2Et$	15	−4·044	0·045	*p*-Bu^n	36	−3·606	−0·164
p-$CH_2{\cdot}CO_2H$	17	−3·985	0·017	*p*-NHAc	42	−3·519	−0·205
p-CH:CHPh	18	−3·958	0·004	*p*-OEt	53	−3·38	−0·27 [b]
p-$CH_2{\cdot}CH_2{\cdot}CO_2Et$	21	−3·883	−0·030	*p*-OH	56	−3·317	−0·301

* For conditions see Table 1. [a] Hammett gives 0·231. [b] Hammett gives − 0·25.

The especial activity of *NN*-di-2-chloroethyl-γ-*p*-aminophenylbutyric acid cannot be ascribed to exceptional chemical reactivity or physical properties (see Table 1) but appears to be connected with its molecular structure. The pK_a values given in Table 1 were determined in 50% acetone; since the values are probably about 1·5 pH units lower in purely aqueous solutions (Cavill, Gibson, and Nyholm, *J.*, 1949, 2466), all the acids are practically wholly dissociated under physiological conditions. It is interesting that outstandingly high trypanocidal activity was demonstrated in the phenylbutyric acid derivative in a series of arsenoso-compounds of structure (VIII) (Eagle, *Science*, 1945, **101**, 69).

(VIII) $CO_2H{\cdot}[CH_2]_n$—C$_6$H$_4$—As→O

p-Butyl-*NN*-di-2-chloroethylaniline is a moderately active inhibitor, not being as effective as the acidic derivatives, and of the three azo-compounds (VII) only the 2′-carboxylic acid is active.

EXPERIMENTAL

NN-*Di*-2-*chloroethyl*-p-*aminophenylacetic Acid*.—Ethyl *p*-aminophenylacetate (18 g.), ethylene oxide (12 ml.), and benzene (10 ml.) were heated at 150° for 14 hr. The resulting *ethyl* NN-*di*-2-*hydroxyethyl*-p-*aminophenylacetate* formed stout prisms, m. p. 70·5—71·5°, from benzene–light petroleum (b. p. 40—60°) (Found: C, 63·1; H, 8·0. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9%). A solution of the hydroxyethyl ester (15 g.) in benzene (40 ml.) containing phosphorus oxychloride (15 ml.) was heated for 1 hr., the two layers becoming homogeneous. The solution was poured on ice, the benzene layer dried (Na_2SO_4), shaken with activated alumina, and evaporated, affording *ethyl* NN-*di*-2-*chloroethyl*-p-*aminophenylacetate*, prismatic needles, m. p. 52—53°, from pentane (Found: C, 54·8; H, 6·5. $C_{14}H_{19}O_2NCl_2$ requires C, 55·3; H, 6·3%). When the ester was heated with five times its weight of concentrated hydrochloric acid the *chloroethyl-acid* was obtained. It formed fine needles, m. p. 105°, from benzene–pentane (Found: C, 52·0; H, 5·7. $C_{12}H_{15}O_2NCl_2$ requires C, 52·2; H, 5·5%).

NN-*Di*-2-*chloroethyl*-p-*aminophenylpropionic Acid*.—*Ethyl* NN-*di*-2-*hydroxyethyl*-p-*aminophenylpropionate*, prepared from ethyl *p*-aminophenylpropionate as described above, was an oil, b. p. 160—170°/1 mm. (Found: C, 64·0; H, 8·6. $C_{15}H_{23}O_4N$ requires C, 64·0; H, 8·2%). It was similarly converted into the *di*-2-*chloroethyl* ester, which formed oblong plates, m. p. 40·5°, from light petroleum (b. p. 40—60°) (Found: C, 56·3, 56·9; H, 6·7, 6·5. $C_{15}H_{21}O_2NCl_2$ requires C, 56·6; H, 6·7%) and into the *acid*, which crystallised from benzene–light petroleum (b. p. 40—60°) as fine needles, m. p. 114—115° (Found: C, 53·7; H, 6·2. $C_{13}H_{17}O_2NCl_2$ requires C, 53·8; H, 5·9%).

Nitration of γ-*Phenylbutyric Acid*.—(*a*) γ-Phenylbutyric acid (Truce and Olsen, *J. Amer. Chem. Soc.*, 1952, **74**, 4721) (59 g.) was added to a well-stirred mixture of concentrated nitric acid (118

ml.) and concentrated sulphuric acid (118 ml.) at −5° during 1½ hr. The mixture was allowed to reach room temperature (2 hr.), then poured on ice and filtered. The *dinitro-acid* crystallised from *iso*propyl ether as prisms, m. p. 97—98° (Found : C, 47·3; H, 4·0%; equiv., 256. $C_{10}H_{10}O_6N_2$ requires C, 47·2; H, 4·0%; equiv., 254).

(*b*) The crude nitration product obtained by the method of van der Scheer (*ibid.*, 1934, **56**, 744) had an equivalent weight of 209. After several crystallisations from *iso*propyl ether γ-*p*-nitrophenylbutyric acid, m. p. 92°, was obtained (van der Scheer gives m. p. 92—93°).

Methyl p-*Aminophenylbutyrate*.—Acetyl chloride (3 ml.) was cautiously added to a solution of the nitro-acid (18 g.) in methanol (50 ml.) and the solution was heated under reflux for 1 hr. After the addition of acetyl chloride (3 ml.) to the cooled solution heating was continued for a further 2 hr. and then one-half of the solvent was removed by distillation. The residue was diluted with water and extracted with ether. After washing of the extract with 2N-sodium carbonate evaporation gave *methyl* γ-p-*nitrophenylbutyrate*, m. p. 39—41°, rhombs from light petroleum (b. p. 40—60°) or prismatic needles from methanol (Found : C, 59·3; H, 5·8. $C_{11}H_{13}O_4N$ requires C, 59·2; H, 5·9%). A solution of the nitro-ester (18 g.) in methanol (100 ml.) containing palladium–calcium carbonate (2 g.; 2%) was shaken in hydrogen, the theoretical volume of hydrogen for the reduction of the nitro-group being rapidly taken up. Evaporation of the filtered solution gave an oil which crystallised from light petroleum (b. p. 40—60°). *Methyl* p-*aminophenylbutyrate* formed needles, m. p. 42° (Found : C, 68·7; H, 7·7. $C_{11}H_{15}O_2N$ requires C, 68·4; H, 7·8%).

NN-*Di*-2-*chloroethyl*-γ-p-*aminophenylbutyric Acid*.—(*a*) The amino-ester may be converted into the di-2-chloroethyl-acid as described above, but the following method gave higher yields.

(*b*) Ethylene oxide (25 ml.) was added to a stirred suspension of the amino-ester (10 g.) in N-acetic acid (50 ml.). After 4 hr. the solution became homogeneous and the excess of ethylene oxide was removed under reduced pressure. The oily hydroxyethyl compound which separated was extracted with ether and washed with 2N-sodium carbonate. The residue obtained after evaporation of the dried solution was dissolved in benzene (150 ml.), and the remaining water was removed by distilling off 50 ml. of solvent. After addition of phosphorus oxychloride (15 ml.) the mixture was heated under reflux for 1 hr. The solvent and excess of oxychloride were then removed under reduced pressure and the residue heated for 1 hr. with concentrated hydrochloric acid (40 ml.). The cooled solution was diluted with water (200 ml.) and extracted with ether (4 × 150 ml.). Evaporation of these extracts gave NN-*di*-2-*chloroethyl*-γ-p-*aminophenylbutyric acid* which formed flattened needles, m. p. 64—66°, from light petroleum (b. p. 40—60°) (Found : C, 55·5; H, 6·1. $C_{14}H_{19}O_2NCl_2$ requires C, 55·3; H, 6·3%).

NN-*Di*-2-*chloro*-n-*propyl*-γ-p-*aminophenylbutyric Acid*.—A mixture of the *p*-aminophenylbutyric ester (5 g.), propylene oxide (12·5 ml.), and N-acetic acid (25 ml.) was stirred for 7 hr. and then heated under reflux for 1 hr.; two clear layers were formed. The hydroxypropyl compound was isolated as an oil which was converted into the chloride as above. The non-crystalline chloroethyl ester was hydrolysed with concentrated hydrochloric acid. NN-*Di*-2-*chloropropyl*-γ-p-*aminophenylbutyric acid* formed prisms (from pentane), m. p. 67—69° (Found : C, 58·2; H, 7·1%; equiv., 330. $C_{16}H_{23}O_2NCl_2$ requires C, 57·9; H, 7·0%; equiv., 332).

p-n-*Butyl*-NN-*di*-2-*chloroethylaniline*.—*p*-*n*-Butylaniline was treated with ethylene oxide in benzene in the usual manner. The *di*-2-*hydroxyethyl* derivative formed plates, m. p. 52°, from light petroleum (b. p. 60—80°) (Found : C, 70·8; H, 9·9. $C_{14}H_{23}O_2N$ requires C, 70·8; H, 9·8%); it formed a *picrate*, m. p. 67°, yellow plates from benzene (Found : C, 51·1; H, 5·9; N, 12·1. $C_{20}H_{26}O_9N_4$ requires C, 51·5; H, 5·6; N, 12·0%). Phosphorus oxychloride converted the hydroxy-compound into the *di*-2-*chloroethyl* derivative, an oil, b. p. 180°/4 mm., 155—160°/1 mm. (Found : Cl, 25·5. $C_{14}H_{21}NCl_2$ requires Cl, 25·8%).

NN-*Di*-2-*chloroethyl*-δ-p-*aminophenylvaleric Acid*.—Cinnamylidenemalonic acid (Liebermann, *Ber.*, 1895, **28**, 1439) was decarboxylated by heat in pyridine, and the product was reduced in ethanol at a platinum catalyst. The phenylvaleric acid was nitrated by Freedman and Doak's method (*J. Amer. Chem. Soc.*, 1949, **71**, 779), giving the nitro-acid (45%), m. p. 83°. Esterification and reduction, as described for nitrophenylbutyric acid, gave the amino-ester, characterised by hydrolysis to the known δ-*p*-aminophenylvaleric acid, m. p. 111—113°. The amino-ester was converted directly into the chloroethyl acid in the usual manner. NN-*Di*-2-*chloroethyl*-δ-p-*aminophenylvaleric acid* formed prismatic needles, m. p. 87°, from light petroleum (b. p. 60—80°) (Found : C, 56·7; H, 6·1; Cl, 22·4%; equiv., 318. $C_{15}H_{21}O_2NCl_2$ requires C. 56·6; H, 6·6; Cl, 22·3%; equiv., 318).

NN-*Di*-2-*chloroethyl*-m-*aminobenzoic Acid*.—By heating with ethylene oxide in benzene solution and then treatment with phosphorus oxychloride, methyl *m*-aminobenzoate was converted

into *methyl* NN-*di*-2-*chloroethyl*-m-*aminobenzoate*, m. p. 61—62°, thick rectangular plates from light petroleum (b. p. 60—80°) (Found: C, 52·4; H, 5·7. $C_{12}H_{15}O_2NCl_2$ requires C, 52·2; H, 5·5%). Hydrolysis with concentrated hydrochloric acid afforded the *chloroethyl-acid*, m. p. 177—178°, flattened needles from benzene (Found: C, 50·3; H, 5·1%; equiv., 265. $C_{11}H_{13}O_2NCl_2$ requires C, 50·4; H, 5·0%; equiv., 262).

Diethyl NN-*Di*-2′-*chloroethyl*-4-*aminophthalate*.—The non-crystalline product (18 g.) obtained by heating diethyl 4-aminophthalate with ethylene oxide in benzene was heated for ½ hr. with thionyl chloride (12 ml.) in benzene (75 ml.). The mixture was poured on ice, and the benzene layer washed with 2N-sodium carbonate and evaporated. A solution of the residue in light petroleum (b. p. 60—80°) was passed down a column of activated alumina. Further elution of the column with light petroleum did not remove any material but washing with *cyclo*hexane gave *diethyl* NN-*di*-2′-*chloroethyl*-4-*aminophthalate*, which formed fine needles, m. p. 82—83°, from light petroleum (b. p. 40—60°) (Found: C, 53·3; H, 6·1. $C_{16}H_{21}O_4NCl_2$ requires C, 53·1; H, 5·9%). When the diester (1 g.) was heated under reflux for 2 hr. with concentrated hydrochloric acid (20 ml.) *NN*-di-2-chloroethyl-*m*-aminobenzoic acid, m. p. 177° not depressed by admixture with a specimen prepared as above, was produced (Found: C, 50·8; H, 4·7%; equiv., 264).

Action of Ethylene Oxide on Methyl Anthranilate.—(*a*) Methyl anthranilate (15·1 g.), ethylene oxide (10 ml.), and benzene (10 ml.) were heated at 150° for 16 hr. The residue obtained after the evaporation of the solvent was crystallised from benzene–light petroleum (b. p. 60—80°). 2 : 3 : 4 : 7-*Tetrahydro*-4-2′-*hydroxyethyl*-7-*keto*-4 : 6-*benz*-1 : 4-*oxazepine* formed plates, m. p. 80° [Found: C, 63·8; H, 6·5%; equiv., 206; *M* (Rast), 200. $C_{11}H_{13}O_3N$ requires C, 63·8; H, 6·3%; equiv. and *M*, 207]. In one run the main product was *methyl* N-2-*hydroxyethylanthranilate*, m. p. 70°, platelets from light petroleum (b. p. 40—60°) (Found: C, 61·6; H, 6·8. Calc. for $C_{10}H_{13}O_3N$: C, 61·3; H, 6·7; N, 7·2%).

(*b*) When methyl anthranilate was treated for 24 hr. at 20° with an excess of ethylene oxide in N-acetic acid the monohydroxyethyl derivative, m. p. 71—72°, long needles from light petroleum (b. p. 40—60°) (Found: C, 61·6; H, 6·8; N, 7·2%), was the only product. It formed a *hydrochloride*, m. p. 162—164°, hard prisms from methanol–ether (Found: C, 51·5; H, 6·2; N, 6·3; Cl, 15·2. $C_{10}H_{14}O_3NCl$ requires C, 51·8; H, 6·1; N, 6·0; Cl, 15·3%). Kiprianov (*loc. cit.*) described a monohydroxyethyl derivative, m. p. 70·5—71·5°, which gave a hydrochloride, m. p. 100°.

N-2-*Chloroethyl*-N-2-*hydroxyethylanthranilic Acid*.—The tetrahydrohydroxyoxazepine (5 g.), phosphorus oxychloride (20 ml.), and benzene (200 ml.) were heated under reflux for ½ hr. The benzene layer was poured on ice, and the tarry residue was treated with cold saturated sodium hydrogen carbonate solution and then extracted with benzene. The combined benzene extracts were dried and evaporated, and the residue was passed in light petroleum (b. p. 60—80°) down a column of activated alumina. The early eluates afforded 4-2′-*chloroethyl*-2 : 3 : 4 : 7-*tetrahydro*-7-*keto*-5 : 6-*benz*-1 : 4-*oxazepine*, m. p. 55°, plates from *cyclo*hexane [Found: C, 59·1; H, 5·6; Cl, 15·7%; *M* (Rast), 200. $C_{11}H_{12}O_2NCl$ requires C, 58·5; H, 5·4; Cl, 15·7%; *M*, 225·7]. When hydrolysed by hot concentrated hydrochloric acid the oxazepine yielded N-2-*chloroethyl*-N-2-*hydroxyethylanthranilic acid*, m. p. 80°, prisms from ether (Found: C, 54·5; H, 5·7; Cl, 14·6%; equiv., 241. $C_{11}H_{14}O_3NCl$ requires C, 54·2; H, 5·8; Cl, 14·6%; equiv., 243·7). The non-crystalline ester, formed by the action of ethereal diazomethane on the acid, gave a *picrate*, m. p. 132—133°, prisms from methanol (Found: C, 44·7; H, 4·1. $C_{18}H_{19}O_{10}N_4Cl$ requires C, 44·4; H, 3·9%).

NN-*Di*-2-*chloroethylanthranilic Acid*.—A solution of the tetrahydrohydroxyethyloxazepine (3 g.) in methanol (50 ml.) was saturated with dry hydrogen chloride at 0°. After ½ hr. under reflux the solution was cooled, resaturated with hydrogen chloride, and heated for a further ½ hr. The residue, obtained after removal of the methanol by distillation, solidified and was crystallised from acetone–methanol. The *hydrochloride* of methyl *NN*-di-2-hydroxyethylanthranilate formed plates, m. p. 137—138° (decomp.) (Found: C, 52·1; H, 6·7. $C_{12}H_{18}O_4NCl$ requires C, 52·3; H, 6·6%). Kiprianov (*loc. cit.*) records m. p. 160° for a compound claimed to be this hydrochloride. The ester formed a *picrate*, m. p. 133°, prisms from ethanol (Found: C, 46·1; H, 4·5. $C_{18}H_{20}O_{11}N_4$ requires C, 46·1; H, 4·3%). The hydrochloride (1 g.) and phosphorus oxychloride (4 ml.) were heated under reflux for ½ hr., cooled, and poured on ice. Sodium acetate was added and the mixture extracted with benzene. The benzene extract yielded methyl *NN*-di-2-chloroethylanthranilate as an oil which formed a *picrate*, m. p. 103°, plates from methanol (Found: C, 43·2; H, 3·9. $C_{18}H_{18}O_9N_4Cl_2$ requires C, 42·8; H, 3·6%). When the ester was heated with concentrated hydrochloric acid NN-*di*-2-*chloroethylanthranilic acid*,

m. p. 85°, needles from benzene–light petroleum (b. p. 60—80°) (Found: C, 50·6; H, 5·4. $C_{11}H_{13}O_2NCl_2$ requires C, 50·4; H, 5·0%), was obtained.

N-2-*Chloroethylanthranilic Acid*.—Treatment of methyl *N*-2-hydroxyethylanthranilate with phosphorus oxychloride in the usual manner gave *methyl* N-2-*chloroethylanthranilate*, m. p. 35°, platelets from pentane (Found: C, 55·9; H, 5·7. $C_{10}H_{12}O_2NCl$ requires C, 56·2; H, 5·7%). On hydrolysis the corresponding *acid*, m. p. 115—116°, platelets from light petroleum (b. p. 60—80°) (Found: C, 54·7; H, 5·3. $C_9H_{10}O_2NCl$ requires C, 54·2; H, 5·1), was obtained.

NN-*Di*-2′-*chloroethyl*-4-*aminobenzylmalonic Acid*.—Condensation of diethyl sodiomalonate with *p*-nitrobenzyl chloride gave a product, m. p. 166°, which is a bisnitrobenzyl derivative and not the required mononitro-compound (the m. p. 168·5° given in Heilbron's "Dictionary of Organic Compounds" for *p*-nitrobenzylmalonic ester is incorrect). The required mononitrobenzyl derivative was prepared by nitrating diethyl benzylmalonate; it formed prisms, m. p. 60° from benzene (lit., m. p. 63°). The amino-ester (m. p. 60°; lit., 64°), obtained by catalytic (palladium–calcium carbonate in ethanol) reduction was treated with ethylene oxide in N-acetic acid as described above. After 24 hr. the non-crystalline product was isolated and treated with phosphorus oxychloride in benzene. The chloro-compound was submitted to a chromatographic purification but did not crystallise.

When the chloro-ester (750 mg.) was heated under reflux for ½ hr. with concentrated hydrochloric acid (2 ml.) the product obtained formed needles, m. p. 112° (from benzene) alone or mixed with *NN*-di-2-chloroethyl-*p*-aminophenylpropionic acid (m. p. 114°).

Evaporating a solution of the chloro-ester (3 g.) in concentrated hydrochloric acid (15 ml.) on a steam-bath until no further reduction in bulk occurred, and cooling, gave a pasty solid. This was collected, washed with a little ice-cold hydrochloric acid, and crystallised from ethyl acetate, affording the *hydrochloride* of *NN*-di-2-chloroethyl-*p*-aminobenzylmalonic acid as small prisms, m. p. 163° (decomp.) (Found: C, 45·5; H, 5·2%; equiv., 126. $C_{14}H_{18}O_4NCl_3$ requires C, 45·4; H, 4·9%; equiv., 123·5). The free acid did not crystallise.

4-(NN-*Di*-2″-*chloroethylamino*)*azobenzenecarboxylic Acids*.—The diazonium salt prepared by adding sodium nitrite (1·4 g.) in water (10 ml.) to a solution of *p*-aminobenzoic acid (2·8 g.) in water (40 ml.) containing concentrated hydrochloric acid (6 ml.) was added to a solution of *NN*-di-2-chloroethylaniline (4·4 g.) in ethanol (150 ml.). After ½ hr. at room temperature the red precipitate was collected and crystallised from 2-methoxyethanol. 4-(NN-*Di*-2″-*chloroethylamino*)*azobenzene*-4′-*carboxylic acid* formed small orange-red plates, m. p. 212—214° (Found: C, 56·2; H, 5·1; N, 11·1, 11·6. $C_{17}H_{17}O_2N_3Cl_2$ requires C, 55·8; H, 4·7; N, 11·5%).

The 3′-*carboxylic acid*, deep yellow flattened needles (from methanol), m. p. 162—164° (Found: C, 55·9; H, 4·9; N, 11·7%), and the 2′-*carboxylic acid*, deep orange-red plates (from acetone), m. p. 179—180° (Found: C, 56·1; H, 5·0; N, 11·5%), were similarly prepared.

Rate of Hydrolysis of Chloroethylamino-acids in Bicarbonate Solution.—Since the acids dissolved only with difficulty in N/6("isotonic")-sodium hydrogen carbonate, even when finely divided, they were dissolved in N/6-sodium hydroxide and this solution was saturated with carbon dioxide, phenolphthalein being used as indicator. Nitrogen was passed through to remove the excess of carbon dioxide—unless this was done precipitation of the acid occurred—and the solution was kept at the required temperature, after which the liberated chloride ion was titrated with silver nitrate (potassium chromate).

This investigation was supported by grants to the Royal Cancer Hospital and to The Institute of Cancer Research from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service, and was carried out during the tenure by one of the authors (W. C. J. R.) of a British Empire Cancer Campaign Fellowship. The authors thank Professor A. Haddow for permission to quote the results of tumour inhibition studies.

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