571. *Cytotoxic Compounds. Part I.* p-(NN-*Di*-2′-*chloroethyl- and* p-(NN-*Di*-2′-*bromoethyl-amino*)*thiophenol.*

By M. H. Benn, L. N. Owen, and (in part) A. M. Creighton.

p-(*NN*-Di-2-chloroethylamino)thiophenol has been synthesised by several methods; the preferred route involves thiocyanation of *NN*-di-2′-chloroethylaniline, followed by reduction. The bromo-analogue is obtained in a similar way. Derivatives, including mixed disulphides, have been prepared, in some of which the cytotoxic unit is linked to a sugar or steroid.

In contrast to the effect of a *p*-hydroxy-group, which is known to increase the reactivity of the chloroethyl groups in *p*-(*NN*-di-2-chloroethylamino)phenol (in comparison with *NN*-di-2′-chloroethylaniline), the *p*-thiol group causes slight deactivation.

Many of the so-called " nitrogen mustards," $R{\cdot}N(CH_2{\cdot}CH_2Cl)_2$, which have been synthesised in large numbers during the last ten years,[1] show cytotoxic effects on proliferating cells and can inhibit the growth of tumours, but unfortunately their toxicity towards normal tissue renders them unsuitable for prolonged chemotherapeutic use.[2] Danielli [3,4] has pointed out that more favourable selectivity of action on a tumour might be achieved by designing the structure of a " nitrogen mustard " so as to take advantage of certain differences which may exist between neoplastic and healthy cells. The principle of enzymic activation falls into this category, and has already been exploited. Thus [4,5] *NN*-di-2′-chloroethyl-*p*-phenylenediamine (I) is active against the Walker rat carcinoma but has a high general toxicity; the introduction of electron-withdrawing acyl groups, as in the *N*′-acetyl (II) and the *N*′-benzoyl derivative, results in a pronounced reduction both in the reactivity of the halogen atoms and in the toxicity, but only the acetyl derivative retains any significant effect on the tumour. Since the tumour is known to possess an enzyme which can deacylate an acetamido- but not a benzamido-group [4] it is likely that the activity of the acetyl derivative is due to the liberation of the parent " nitrogen mustard " (I) by enzymic fission at the site of the tumour. Several other types of derivative, based on this principle, have been described.[5,6]

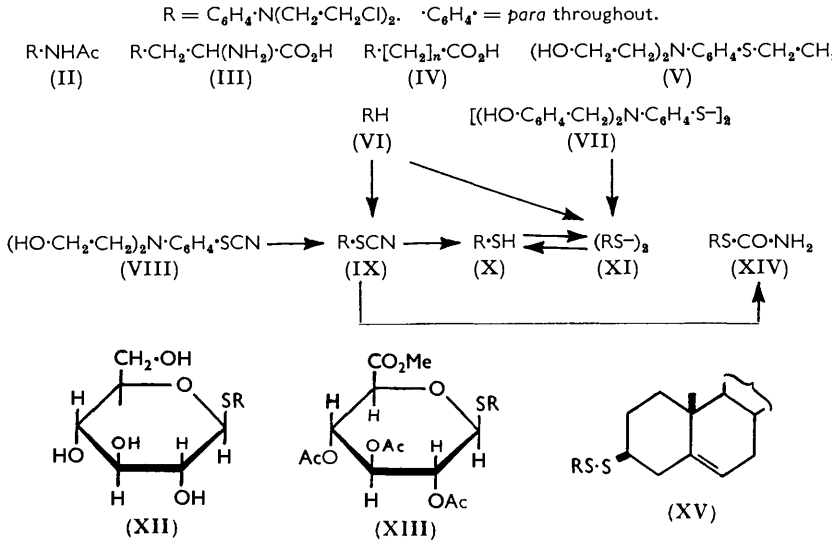
A second possible exploitation of differences in cellular properties lies in the principle of active transport,[3,4] *i.e.*, utilisation of the selectivity which a cell may show for a particular type of molecule, an approach which involves the preparation of compounds in which a " nitrogen mustard " is linked to a physiologically important substance; interesting examples are the phenylalanine derivative (III),[7] the acids (IV),[8] and *NN*-di-2′-chloroethyl-D-glucosamine.[9]

The present series of papers will record syntheses of compounds some of which possess structures based on the principles outlined above. The results of biological tests, carried out under the direction of Professor J. F. Danielli, F.R.S., will be described elsewhere.

Many of the aromatic " nitrogen mustards " hitherto reported have been derivatives of the *p*-amino-compound (I) or of the corresponding phenol. In view of the importance of thiol groups in biological systems, the thiol analogue (X) would be of much interest, and several possible routes to this compound were investigated. *p*-Aminophenol reacts with ethylene oxide in aqueous acetic acid to give *p*-(*NN*-di-2-hydroxyethylamino)phenol,[5] but

[1] See, *inter al.*, Ross, *J.*, 1949, 183, and many subsequent papers; Wilson and Tishler, *J. Amer. Chem. Soc.*, 1951, **73**, 3635.
[2] Klopp and Bateman, *Adv. Cancer Res.*, 1954, **2**, 255; Stock, *ibid.*, p. 425.
[3] Danielli, *Nature*, 1952, **170**, 863.
[4] *Idem*, Ciba Foundation Symposium, " Leukaemia Research," Churchill, London, 1954, p. 263.
[5] Ross, Warwick, and Roberts, *J.*, 1955, 3110.
[6] Ross and Warwick, *J.*, 1956, 1364.
[7] Bergel and Stock, *J.*, 1954, 2409; Bergel, Burnop, and Stock, *J.*, 1955, 1223.
[8] Everett, Roberts, and Ross, *J.*, 1953, 2386.
[9] Vargha, Fehér, and Lendval, *J.*, 1957, 810.

under these conditions *p*-aminothiophenol underwent hydroxyethylation at the thiol group also and gave *NNS*-tri-2-hydroxyethyl-*p*-aminothiophenol (V). Attention was therefore directed to syntheses in which the thiol group could be introduced at a later stage. Hydroxyethylation of di-*p*-aminophenyl disulphide gave the tetrol (VII), but replacement of the hydroxyl groups by chlorine was difficult because of its low solubility in the usual solvents; the method finally adopted was to heat it with phosphorus oxychloride without a solvent, which gave, in poor yield, di-[*p*-(*NN*-di-2-chloroethylamino)phenyl] disulphide (XI). The halogen atoms in this compound were relatively unreactive, as shown by the low rate of hydrolysis in aqueous acetone determined by Ross's method [10,11] (see Table), and no reaction occurred with aromatic amines or with sodium sulphide when attempts were made to characterise it by conversion into a piperazine or a thiazan under the conditions given by Davis and Ross.[12] It was, however, readily reduced by lithium aluminium hydride [13] to the required *p*-(*NN*-di-2-chloroethylamino)thiophenol (X), though the overall yield was unsatisfactory. Reduction of the disulphide (VII) gave *p*-(*NN*-di-2-hydroxyethylamino)thiophenol, which had previously been unsuccessfully sought by hydroxyethylation of *p*-aminothiophenol (see above).



Holzmann [14] prepared di-[*p*-(*NN*-diethylamino)phenyl] disulphide by direct sulphuration of *NN*-diethylaniline with sulphur monochloride, and an attempt was therefore made to prepare the disulphide (XI) by a similar reaction on *NN*-di-2′-chloroethylaniline (VI) in the presence of pyridine (to avoid loss of the amine as hydrochloride). This gave a non-crystalline product in which the presence of the disulphide (XI) was established by reduction with lithium aluminium hydride and characterisation of the resulting thiol (X). The non-crystallinity of the disulphide and of the thiol obtained by this route was probably due to the presence of the *ortho*-isomer; it is significant that the disulphide obtained by Holzmann [14] from *NN*-diethylaniline was less pure than that which can be obtained by oxidation of *p*-*NN*-diethylaminothiophenol (see below).

The production of the thiol group by reduction of a thiocyanate was next investigated. Hydroxyethylation of *p*-thiocyanatoaniline with ethylene oxide in aqueous acetic acid was slow owing to the diminished basic character of the amino-group, and after the normal time

[10] Ross, *J.*, 1949, 183.
[11] Everett and Ross, *J.*, 1949, 1972.
[12] Davis and Ross, *J.*, 1949, 2381; Ross, *J.*, 1950, 815.
[13] Cf. Arnold, Lien, and Alm, *J. Amer. Chem. Soc.*, 1950, **72**, 731.
[14] Holzmann, *Ber.*, 1887, **20**, 1636.

required for bishydroxyethylation only the *N*-2-hydroxyethyl compound was isolated, but by prolonged reaction *NN*-di-2′-hydroxyethyl-*p*-thiocyanatoaniline (VIII) was obtained. A more convenient method, however, was thiocyanation [15] of the readily available *NN*-di-2′-hydroxyethylaniline. Reaction of the diol (VIII) with phosphorus oxychloride in benzene gave a non-crystalline product which contained the desired thiocyanato-compound (IX) since on reduction with lithium aluminium hydride [16] it gave a product from which crystalline derivatives of the thiol (X) could be readily obtained.

It seemed likely that the difficulty in obtaining pure products, with both the disulphide and the thiocyanate route, was due to the rather vigorous conditions under which the halogen atoms were introduced; it would thus be desirable to effect this step at an earlier stage in the synthesis. The problem was finally solved by the use of *NN*-di-2′-chloroethylaniline (VI), which is readily prepared [17] in a pure state from aniline *via* *NN*-di-2′-hydroxyethylaniline. On thiocyanation this dichloride gave an excellent yield of crystalline *NN*-di-2′-chloroethyl-*p*-thiocyanatoaniline (IX), which on reduction with lithium aluminium hydride furnished crystalline *p*-(*NN*-di-2-chloroethylamino)thiophenol (X) in 75% overall yield from aniline.

Oxidation of the thiol with iodine in aqueous sodium hydrogen carbonate gave the disulphide (XI) as an easily crystallisable solid. This is therefore the most satisfactory route to the latter compound, the crystallinity of which is very dependent on a high degree of purity. The *S*-acetyl and the *S*-benzoyl derivative of the thiol were prepared by direct acylation in pyridine, and the *S*-methyl compound by methylation with diazomethane. Condensation of the thiol with acetobromoglucose gave *p*-(*NN*-di-2-chloroethylamino)-phenyl 2 : 3 : 4 : 6-tetra-*O*-acetyl-β-D-thioglucoside, which by base-catalysed solvolysis in methanol gave the thioglucoside (XII). Similarly, with methyl acetobromoglucuronate the thiol gave the methyl ester (XIII) of *S*-*p*-(*NN*-di-2-chloroethylamino)phenyl 2 : 3 : 4-tri-*O*-acetyl-β-D-thioglucuronic acid, but an attempt to obtain the corresponding free acid, by deacetylation followed by hydrolysis with hydrochloric acid (towards which thioglucosides are relatively stable), failed to give a pure product.

Biological tests on certain carbamates having proved interesting,[18] it was desirable to obtain analogues derived from the thiol (X). The formation of thiocarbamates by the hydrolysis of thiocyanates with 95% sulphuric acid has recently been reported,[19] and by this method the thiocyanate (IX) readily gave *S*-*p*-(*NN*-di-2-chloroethylamino)phenyl thiocarbamate (XIV); the *N*′-phenyl derivative of this was obtained by direct interaction of the thiol with phenyl *iso*cyanate.

Since a disulphide is subject to reductive fission *in vivo*, some mixed disulphides derived from the " thiol-mustard " (X) with other thiols were synthesised. Such compounds are commonly prepared [20] by interaction of a thiol with a sulphenyl halide or sulphenyl thiocyanate, though the yields are very variable because often the two symmetrical disulphides are also formed. The sulphenyl halides are normally obtained by direct interaction of halogen and a thiol or disulphide, but although treatment of the disulphide (XI) in carbon tetrachloride with chlorine gave an orange-red solution, indicating the formation of a sulphenyl chloride, the product rapidly decomposed; similar treatment of the thiol (X) was also unsuccessful, possibly because the liberated hydrogen chloride precipitated the base as hydrochloride. Recently the use of *N*-bromo- or *N*-chloro-succinimide in the preparation of sulphenyl halides has been described; [21] this method is useful when the

[15] Cf. Wood, *Organic Reactions*, 1946, **3**, 240.
[16] Cf. Strating and Backer, *Rec. Trav. chim.*, 1950, **69**, 638, 909.
[17] Robinson and Watt, *J.*, 1934, 1536.
[18] Danielli, Hamilton, May, and Barnard, *Ann. Rep. British Empire Cancer Campaign*, 1956, **34**, 398.
[19] Riemschneider, Wojahn, and Orlick, *J. Amer. Chem. Soc.*, 1951, **73**, 5905.
[20] Lecher, *Ber.*, 1920, **53**, 577; Lecher and Simon, *Ber.*, 1921, **54**, 632; Lecher and Wittwer, *Ber.*, 1922, **55**, 1474; Kharasch, Potempa, and Wehrmeister, *Chem. Rev.*, 1946, **39**, 269; Schöberl and Wagner in Houben-Weyl, " Methoden der Organischen Chemie," 4th edn., Georg Thieme Verlag, Stuttgart, 1955, vol. IX, p. 263.
[21] Emde, D.R.-P. 804,572; *Chem. Abs.*, 1952, **46**, 529.

formation of hydrogen halide has to be avoided. Treated in this way the thiol (X) gave red solutions which probably contained the sulphenyl halide, but after further reaction with other thiols (thiophenol, ethanedithiol, or thiocholesterol) only the two symmetrical disulphides could be isolated.

It is recognised [22] that sulphenyl halides may be unstable in the presence of tertiary amines, and this probably accounts for the lack of success in the above experiments; the alternative approach, involving the formation of a sulphenyl halide from the other component, was therefore investigated. Reaction of thiocholesterol (3-β-mercaptocholest-5-ene) * with *N*-chlorosuccinimide in benzene, followed by addition of the " thiol-mustard " (X), gave a mixture which was separated by chromatography on alumina into di(cholest-5-en-3β-yl) disulphide, di-[*p*-(*NN*-di-2-chloroethylamino)phenyl] disulphide (XI), and *p*-(*NN*-di-2-chloroethylamino)phenyl cholest-5-en-3β-yl disulphide (XV), the yield of the mixed disulphide being 30%. Similar treatment of 2 : 3 : 4 : 6-tetra-*O*-acetyl-β-D-thioglucose with *N*-bromosuccinimide, followed by reaction with the " thiol-mustard," gave a 43% yield of 2 : 3 : 4 : 6-tetra-*O*-acetyl-β-D-glucosyl *p*-(*NN*-di-2-chloroethylamino)phenyl disulphide. 2 : 4-Dinitrobenzenesulphenyl chloride reacted smoothly with the " thiol-mustard " to give *p*-(*NN*-di-2-chloroethylamino)phenyl 2 : 4-dinitrophenyl disulphide.

The result of biological tests [18] on the thiol (X) and its derivatives, some of which showed very low toxicities, indicated that a study should be made on compounds of greater reactivity, and some 2-bromoethyl analogues were therefore synthesised. Crystalline *p*-(*NN*-di-2-bromoethylamino)thiophenol [bromo-analogue of (X)] was prepared from *NN*-di-2′-bromoethylaniline, *via* the thiocyanate, by the same methods as those used to effect the conversions (VI) ⟶ (IX) ⟶ (X). Controlled hydrolysis of the thiocyanate gave the thiocarbamate [bromo-analogue of (XIV)]. Oxidation of the bromo-thiol with iodine gave the corresponding disulphide, whilst reaction with diazomethane and with 2 : 4-dinitrobenzenesulphenyl chloride furnished the *S*-methyl ether and *p*-(*NN*-di-2-bromoethylamino)phenyl 2 : 4-dinitrophenyl disulphide, respectively. These bromoethyl compounds are markedly more vesicant than the chloroethyl analogues, and consequently require more careful manipulation.

The extent of hydrolysis of the mustards after 30 min. in boiling 1 : 1 aqueous acetone was determined by Ross's method [10,11] (see Table); when possible, a concentration of *ca.* 0·01M was used, though for sparingly soluble compounds it was necessary to use more dilute solutions and in such cases the results are probably slightly high (cf. refs. 10 and 11). When determinations of both hydrogen ion and halide ion were made the agreement was good, indicating [10] the absence of quaternary halide formation during the hydrolysis of these aromatic " nitrogen-mustards." The parent unsubstituted *NN*-di-2′-chloroethylaniline undergoes 20% hydrolysis under these conditions [10] and it is interesting that the introduction of the *p*-thiol group has a slight deactivating effect; this is in marked contrast to the figure reported [5] for the *p*-hydroxy-analogue (56%), and demonstrates the lack of electron-donation by sulphur towards the aromatic ring. Acylation of the thiol produces the expected reduction in reactivity, and the electron-withdrawing power of the thiocyanato-group is also evident. The figures also show the considerably increased reactivity of the *NN*-di-2′-bromoethyl compounds compared with that of the chloro-analogues, though the deactivating effect of the substituents is again similar since the figure for the parent *NN*-di-2′-bromoethylaniline [10] is 79%.

It was important in the biological tests to establish whether the enzyme systems present in the neoplastic tissue were capable of releasing the " thiol-mustard " (X) from its derivatives. This information is conveniently obtained by the use of model

* We provisionally accept the 3β-configuration proposed by O'Connor and Nace (*J. Amer. Chem. Soc.*, 1953, **75**, 2118), though this may require modification (cf. Pierce, Richards, Shoppee, Stephenson, and Summers, *J.*, 1955, 694).

[22] Moore and Johnson, *J. Amer. Chem. Soc.*, 1935, **57**, 1517; Kharasch, McQuarrie, and Buess, *ibid.*, 1953, **75**, 2658.

(" non-mustard ") compounds, and for this purpose *p*-(*NN*-diethylamino)thiophenol was synthesised by reduction of *NN*-diethyl-*p*-thiocyanatoaniline; it was converted into its disulphide, its *S*-acetyl derivative, and *p*-(*NN*-diethylamino)phenyl 2 : 4-dinitrophenyl disulphide. The thioglucoside has already been described.[23]

R = *p*-(*NN*-Di-2-chloroethylamino)phenyl. R′ = *p*-(*NN*-Di-2-bromoethylamino)phenyl.

Compound	mmole in 25 c.c. of acetone + 25 c.c. of water	Hydrolysis in 30 min. at 66° H^+ (%)	Hal^- (%)	Footnote
R·SH	0·2	15	—	*a*
$(R·S·)_2$	0·04	6	8	*b*, *c*
R·SAc	0·4	3	—	*a*
R·SBz	0·1	2	—	*a*
R·SMe	0·5	13	13	*b*, *c*
R·SCN	0·4	3	3	*b*, *c*
$R·S·CO·NH_2$	0·2	*ca.* 8	—	*a*
R·S· (tetra-acetylglucosyl)	0·5	6	6	*b*, *c*
R·S· (glucosyl)	0·4	7	7	*b*, *c*
R·S·S· (tetra-acetylglucosyl)	0·3	6	5	*b*, *c*
R·S·S· (cholesteryl)	0·2	5	—	*b*
R′·SH	0·1	70	—	*a*
$(R′·S·)_2$	0·03	56	56	*b*, *d*
R′·SCN	0·5	19	19	*b*, *d*
$R′·S·CO·NH_2$	0·2	40	—	*a*

[a] H^+ titrated potentiometrically (glass electrode). [b] H^+ titrated to phenolphthalein indicator. Cl^- titrated to chromate indicator. [d] Br^- titrated to eosin indicator. In 125 c.c. of acetone +25 c.c. of water at 62°.

Experimental

Microanalyses were by Miss J. Cuckney and the staff of the Organic Chemistry Microanalytical Laboratories.

NN-*Di-2′-hydroxyethylaniline*.—Aniline (250 g.), 4N-aqueous acetic acid (150 c.c.) (cf. ref. 8) and ethylene oxide (250 c.c.) were mixed together, with cooling, and stirred overnight. More ethylene oxide (250 c.c.) was then added and stirring continued for a further 24 hr. The mixture was neutralised with aqueous sodium carbonate and extracted with chloroform (3 × 250 c.c.) to give an oil, which was fractionally distilled under nitrogen. *NN*-Di-2′-hydroxyethylaniline was obtained as the fraction, b. p. 170—175°/0·3 mm., 165—168°/0·2 mm., m. p. 57—58° (441 g., 91%) (lit.,[10, 24] b. p. 175—180°/3 mm., m. p. 58°). Yields were invariably excellent, 90—96%. It was converted into *NN*-di-2′-chloroethylaniline, m. p. 45—47°, by Robinson and Watt's [17] method.

p-*Aminophenyl* 2 : 4-*Dinitrophenyl Sulphide*.—Prepared by reaction of *p*-aminothiophenol [25] with chloro-2 : 4-dinitrobenzene in alkaline aqueous ethanol, this crystallised from ethanol in orange-red needles, m. p. 167—168° (Found: C, 49·6; H, 3·3; N, 14·4. $C_{12}H_9O_4N_3S$ requires C, 49·5; H, 3·1; N, 14·4%).

Di-(p-*aminophenyl*) *Disulphide*.—Aerial oxidation of an aqueous alkaline solution of *p*-aminothiophenol, in the dark, gave an almost theoretical yield of the disulphide, which crystallised from aqueous ethanol in yellow needles, m. p. 76—77° (lit.,[26] m. p. 76—77°).

When di-(*p*-aminophenyl) disulphide was tetrazotised in ice-cold 2N-hydrochloric acid, and coupled with β-naphthol in sodium hydroxide solution, *di*-[p-(2-*hydroxy*-1-*naphthylazo*)*phenyl*] *disulphide* was precipitated; recrystallisation from ethyl acetate gave red needles, m. p. 251° (Found: N, 10·1. $C_{32}H_{22}O_2N_4S_2$ requires N, 10·0%).

NNS-*Tri*-(2-*hydroxyethyl*)-p-*aminothiophenol*.—A suspension of *p*-aminothiophenol (10 g.) in 2N-aqueous acetic acid (50 c.c.) was cooled to 0°, ethylene oxide (30 c.c.) was added, and the mixture was stirred overnight. The homogeneous solution was then neutralised with saturated aqueous sodium hydrogen carbonate, and the precipitated oil was separated, taken up in ethanol, and dried (Na_2SO_4); evaporation of the solvent gave a yellow oil, which showed no reaction for a free thiol. A portion was distilled at 240° (bath)/0·02 mm.; the distillate slowly solidified, and on recrystallisation from *iso*propyl alcohol gave NNS-*tri*-(2-*hydroxyethyl*)-p-*aminothiophenol*,

[23] Montgomery, Richtmyer, and Hudson, *J. Org. Chem.*, 1946, **11**, 301.
[24] Gabel, *Ber.*, 1925, **58**, 577.
[25] Gilman and Gainer, *J. Amer. Chem. Soc.*, 1949, **71**, 1749.
[26] Hinsberg, *Ber.*, 1905, **38**, 1133; cf. Schmidt, *Ber.*, 1878, **11**, 1172.

white prisms, m. p. 59° (Found: C, 56·0; H, 7·4; N, 5·1; O, 18·2. $C_{12}H_{19}O_3NS$ requires C, 56·0; H, 7·4; N, 5·4; O, 18·65%).

Di-[p-(NN-*di*-2-*hydroxyethylamino*)*phenyl*] *Disulphide.*—A solution of di-(*p*-aminophenyl) disulphide (30 g.) in ethylene oxide (100 c.c.) and 4N-aqueous acetic acid (20 c.c.) was stirred overnight; the crude product usually separated as an oil or semi-solid mass. Excess of ethylene oxide was removed under reduced pressure, and the residue was neutralised with saturated aqueous sodium hydrogen carbonate, saturated with salt, and extracted with ethyl acetate (4 × 150 c.c.). The extracts were dried (Na_2SO_4) and evaporated to a syrup, which was tested for the presence of primary aromatic amine by diazotisation and coupling with β-naphthol; if positive, the syrup was re-treated with ethylene oxide (65 c.c.) and 4N-aqueous acetic acid (12 c.c.) overnight and worked up as described above. A solution of the product in hot aqueous ethanol (charcoal) deposited feathery yellow crystals (60—75%) of *di*-[p-(NN-*di*-2-*hydroxyethylamino*)*phenyl*] *disulphide*, m. p. 136—138°. Recrystallised from aqueous ethanol it had m. p. 138—140° (Found: C, 56·4; H, 6·9; N, 6·6; S, 15·45; O, 15·35. $C_{20}H_{28}O_4N_2S_2$ requires C, 56·6; H, 6·65; N, 6·6; S, 15·1; O, 15·1%).

The *dipicrate*, which crystallised from methanol–ether as a yellow powder, m. p. 91—93°, readily lost picric acid when heated *in vacuo* (Found, on a sample dried *in vacuo* at 20°: C, 43·6; H, 4·6; N, 12·1. $C_{32}H_{34}O_{18}N_8S_2$ requires C, 43·5; H, 3·9; N, 12·7%). The *tetrabenzoate* (prepared by benzoyl chloride–pyridine) after recrystallisation from pyridine formed an amorphous white powder, m. p. 269—270° (Found: C, 68·25; H, 5·2. $C_{48}H_{44}O_8N_2S_2$ requires C, 68·55; H, 5·3%).

Di-[p-(NN-*di*-2-*chloroethylamino*)*phenyl*] *Disulphide.*—(i) *From di*-[p-(NN-*di*-2-*hydroxyethylamino*)*phenyl*] *disulphide.* The tetrol (10 g.) and phosphorus oxychloride (20 c.c.) were mixed together. There was an immediate exothermic reaction with evolution of hydrogen chloride. After this had ceased the mixture was heated under reflux on a steam-bath for 30 min., and the excess of phosphorus oxychloride was then distilled off under reduced pressure. The residual green syrup was triturated with benzene and saturated aqueous sodium hydrogen carbonate until it had entirely dissolved; the benzene solution was dried (Na_2SO_4) and concentrated under reduced pressure to an oil which was chromatographed on acid-washed alumina, with benzene as eluant. The well-defined yellow band was eluted, and concentration of the eluates gave a yellow oil which deposited crystals (3·4 g., 31%), m. p. 70·5—71·5°, when set aside overnight in benzene–light petroleum (b. p. 40—60°) (1 : 2). Recrystallisation from the same solvent gave *di*-[p-(NN-*di*-2-*chloroethylamino*)*phenyl*] *disulphide*, m. p. 71—72°, raised to 76° on recrystallisation from ether–light petroleum (b. p. 40—60°), as yellow prisms (Found: C, 48·0; H, 5·1; N, 5·5; S, 13·35; Cl, 28·1. $C_{20}H_{24}N_2S_2Cl_4$ requires C, 48·2; H, 4·85; N, 5·6; S, 12·9; Cl, 28·5%). Yields were 19—50% and there was often considerable difficulty in initiating crystallisation.

(ii) *From* NN-*di*-2′-*chloroethylaniline.* To a solution of *NN*-di-2′-chloroethylaniline (40 g.) and pyridine (14·7 g.) in chloroform (100 c.c.), redistilled sulphur monochloride (12·5 g.) in chloroform (100 c.c.) was added dropwise (30 min.), with stirring, at 10°. Stirring was continued for a further 1½ hr., and the mixture was then washed with water, dried (Na_2SO_4), and concentrated to a syrup. Chromatography in benzene on alumina gave crude di-[*p*-(*NN*-di-2-chloroethylamino)phenyl] disulphide as a yellow syrup (49 g.), characterised by reduction to the thiol (see below) and conversion into *p*-(*NN*-di-2-chloroethylamino)phenyl 2 : 4-dinitrophenyl sulphide, m. p. and mixed m. p. 156°.

(iii) *From* p-(NN-*di*-2-*chloroethylamino*)*thiophenol.* The thiol (2·0 g.) (see below) was dissolved in benzene (50 c.c.), and shaken with saturated aqueous sodium hydrogen carbonate (50 c.c.) during the addition of a solution of iodine in aqueous potassium iodide until the iodine colour persisted. The benzene layer was washed with aqueous sodium thiosulphate, dried (Na_2SO_4), and evaporated to a syrup. Chromatography gave the disulphide as yellow needles (1·67 g., 83%), m. p. 78—79° (from ethanol).

Treatment of the disulphide with picric acid in methanol, ethanol, or ether failed to give a crystalline picrate. It was also recovered largely unchanged (m. p. and mixed m. p. *ca.* 72°) after being refluxed for 4 hr. with aniline in aqueous acetone. No product was obtained with *p*-toluidine or with sodium sulphide under the same conditions, though in these cases the unchanged chloroethylamine was not obtained crystalline.

N-2′-*Hydroxyethyl*-p-*thiocyanatoaniline.*—A suspension of *p*-thiocyanatoaniline [27] (17 g.),

[27] Kaufmann and Oehring, *Ber.*, 1926, **59**, 187.

ethylene oxide (25 c.c.), and 2N-aqueous acetic acid (25 c.c.) was stirred at room temperature. After 4 hr. more ethylene oxide (10 c.c.) was added, and the clear solution so obtained was stirred for a further 4 hr.; the excess of ethylene oxide was then removed under reduced pressure. The oil which separated solidified (m. p. 40—70°) but it gave a positive test for primary aromatic amine. It was therefore re-treated with ethylene oxide (40 c.c.) and 2N-aqueous acetic acid (15 c.c.) overnight, and the product, isolated as before, was dissolved in ether, washed with saturated aqueous sodium hydrogen carbonate, and dried (Na_2SO_4). Removal of the solvent gave a solid (16 g., 73%), m. p. 95—100°, which on recrystallisation from aqueous methanol (charcoal) gave N-2′-*hydroxyethyl*-p-*thiocyanatoaniline* as white leaflets, m. p. 104° (Found: C, 55·6; H, 5·2; N, 14·8. $C_9H_{10}ON_2S$ requires C, 55·6; H, 5·2; N, 14·4%).

NN-*Di*-2′-*hydroxyethyl*-p-*thiocyanatoaniline*.—(i) *From* p-*thiocyanatoaniline*. *p*-Thiocyanatoaniline (10 g.) was dissolved in ethylene oxide (50 c.c.), 2N-aqueous acetic acid (20 c.c.) was added, and the solution was stirred for 40 hr., more ethylene oxide (20 c.c.) being added after 20 hr. to replace that lost by evaporation. The excess of ethylene oxide was removed under reduced pressure and the residue neutralised with solid sodium hydrogen carbonate. The oil which separated was dissolved in ethyl acetate and dried (Na_2SO_4), and the solvent removed to yield a solid (12 g., 76%). NN-*Di*-2′-*hydroxyethyl*-p-*thiocyanatoaniline* crystallised from ether in needles, m. p. 63° (Found: C, 55·35; H, 5·95; N, 11·6; S, 13·2. $C_{11}H_{14}O_2N_2S$ requires C, 55·4; H, 5·9; N, 11·8; S, 13·5%).

(ii) *From* NN-*di*-2′-*hydroxyethylaniline*. A solution of *NN*-di-2′-hydroxyethylaniline (18·0 g.) and potassium thiocyanate (30 g.) in acetic acid (200 c.c.) was stirred at *ca.* 12° whilst a solution of bromine (16·0 g.) in acetic acid (50 c.c.) was slowly added (20 min.) below the surface of the solution. Stirring was continued for a further 10 min. and the mixture was filtered to remove polymeric material, the filter-cake being washed with ether–ethanol (2 : 1) (100 c.c.); the combined filtrate and washings were neutralised with saturated aqueous sodium carbonate and extracted with ethyl acetate to give a yellow oil (20 g., 86%), which crystallised on trituration with ether. Recrystallisation from aqueous methanol (charcoal) gave needles of *NN*-di-2′-hydroxyethyl-*p*-thiocyanatoaniline, m. p. 61—63° undepressed with the material prepared as above.

The compound is very soluble in ethanol, methanol, propan-2-ol, and ethyl acetate, sparingly soluble in benzene and ether. It gave a *picrate*, lemon-yellow prisms, m. p. 99—100° (from methanol–ether) (Found: C, 43·75; H, 3·8; N, 14·6. $C_{17}H_{17}O_9N_5S$ requires C, 43·7; H, 3·7; N, 15·0%).

p-(NN-*Di*-2-*hydroxyethylamino*)*thiophenol*.—Di-[*p*-(*NN*-di-2-hydroxyethylamino)phenyl] disulphide (8·0 g.) in concentrated hydrochloric acid (50 c.c.) was heated on a steam-bath and stirred whilst zinc dust was added in portions (5 × 4 g.) during 4 hr.; more concentrated hydrochloric acid (50 c.c.) was added after 2 hr. The hot solution was filtered, made strongly alkaline with 40% aqueous sodium hydroxide (320 g.), and saturated under pressure with hydrogen sulphide. The precipitate was centrifuged down and the supernatant solution was decanted off, adjusted to pH 6—7 with concentrated hydrochloric acid, and extracted with ether, to give crude *p*-(*NN*-di-2-hydroxyethylamino)thiophenol as a yellow oil (2·3 g., 29%) (Found: thiol-S, 12·0. Calc. for $C_{10}H_{15}O_2NS$: thiol-S, 15·05%). Reaction of a portion with chloro-2 : 4-dinitrobenzene in aqueous-ethanolic alkali gave p-(NN-*di*-2-*hydroxyethylamino*)*phenyl* 2 : 4-*dinitrophenyl sulphide*, orange-red prisms, m. p. 152—153° (from methanol) (Found: C, 50·7; H, 4·8; N, 10·8. $C_{16}H_{17}O_6N_3S$ requires C, 50·6; H, 4·5; N, 11·1%).

NN-*Di*-2′-*chloroethyl*-p-*thiocyanatoaniline*.—(i) *From* NN-*di*-2′-*hydroxyethyl*-p-*thiocyanatoaniline*. The diol (5 g.) was heated with phosphorus oxychloride (8 c.c.) on the steam-bath for 30 min. and the mixture was then cooled and poured into ice-water (100 c.c.). The solution was neutralised with saturated aqueous sodium hydrogen carbonate and extracted with benzene, to give an oil which was chromatographed in benzene on alumina to give *NN*-di-2′-chloroethyl-*p*-thiocyanatoaniline as a yellow oil (2·3 g., 39%), which did not crystallise but was characterised by reduction to the thiol (see below) and formation of the 2 : 4-dinitrophenyl sulphide, m. p. and mixed m. p. 155°.

(ii) *From* NN-*di*-2′-*chloroethylaniline* (with A. M. Creighton). A solution of bromine (14·7 g.) and sodium bromide (5 g.) in dry methanol (50 c.c.) was added (5—10 min.) to a stirred solution of *NN*-di-2′-chloroethylaniline (20 g.) and "AnalaR" ammonium thiocyanate (15·0 g.) in dry methanol (300 c.c.) at *ca.* 5°. Stirring was continued for a further 10 min., and the

mixture was then poured into water (1 l.) to precipitate NN-*di*-2′-*chloroethyl*-p-*thiocyanatoaniline* as a colourless solid (25·3 g., 100%), m. p. 40—41°. Recrystallisation from ethanol gave leaflets, m. p. 49° (Found: C, 48·1; H, 4·6; N, 9·9. $C_{11}H_{12}N_2SCl_2$ requires C, 48·0; H, 4·4; N, 10·2%).

The use of acetic acid in place of methanol gave a crude product which after chromatography on alumina gave the same material (74%), m. p. 47—48°.

p-(NN-*Di*-2-*chloroethylamino*)*thiophenol*.—(i) *From* NN-*di*-2′-*chloroethyl*-p-*thiocyanatoaniline*. The thiocyanate (27 g.) in anhydrous ether (300 c.c.) was added dropwise to a stirred suspension of finely powdered lithium aluminium hydride (3·0 g.) in anhydrous ether at such a rate (30 min.) as to maintain gentle reflux. The mixture was then stirred and refluxed for a further 30 min. (by which time the initial pale yellow colour of the solution had disappeared), then cooled before the excess of reagent was decomposed by ice-water. The precipitated complex was decomposed by ice-cold 6N-aqueous hydrochloric acid, the pH then brought to *ca.* 6 with saturated aqueous sodium hydrogen carbonate, the ether layer separated, and the aqueous solution re-extracted with ether (3 × 100 c.c.). The combined, dried (Na_2SO_4) extracts were evaporated under nitrogen to yield an oil which crystallised on trituration with a little methanol. The solid was washed with light petroleum (b. p. 40—60°) and dried *in vacuo* (22·4 g., 90%; m. p. 57—58°). p-(NN-*Di*-2′-*chloroethylamino*)*thiophenol* on recrystallisation from methanol formed pale cream prisms, m. p. 59—60° (Found: C, 47·8; H, 5·4; N, 5·4; thiol-S, 12·8. $C_{10}H_{13}NSCl_2$ requires C, 48·0; H, 5·2; N, 5·6; thiol-S, 12·8%). The crystalline thiol became blue-green when exposed to the air for a few hours; this occurred more rapidly when it was moistened with methanol or ethanol and was presumably due to oxidation.

Reaction of the thiol with chloro-2 : 4-dinitrobenzene in ethanol–benzene containing the theoretical amount of alkali gave p-(NN-*di*-2′-*chloroethylamino*)*phenyl* 2 : 4-*dinitrophenyl sulphide*, which crystallised from ethanol–benzene in orange-red prisms, m. p. 156—157° (Found: C, 46·5; H, 4·0; N, 10·1. $C_{16}H_{15}O_4N_3SCl_2$ requires C, 46·2; H, 3·6; N, 10·1%).

(ii) *From di*-[p-(NN-*di*-2-*chloroethylamino*)*phenyl*] *disulphide*. The disulphide (27·0 g.) in dry ether (250 c.c.) was reduced with lithium aluminium hydride (2·5 g.) in ether (200 c.c.), and the mixture worked up as described above for the reduction of the thiocyanate, except that refluxing was continued for 40 min. and 2N-hydrochloric acid was used to decompose the complex. The thiol was obtained as a waxy solid (26·7 g., 98%) (Found: thiol-S, 10·9%). It readily gave the 2 : 4-dinitrophenyl derivative described above, m. p. and mixed m. p. 156—157°.

p-(NN-*Di*-2-*chloroethylamino*)*phenyl Thiolacetate*.—A solution of *p*-(*NN*-di-2-chloroethylamino)thiophenol (6·0 g.) in acetic anhydride (35 c.c.) and pyridine (3 c.c.) was heated for 3 hr. on a steam-bath under nitrogen, then cooled and poured into water (800 c.c.). The precipitated solid on recrystallisation from ethanol (charcoal) gave the *thiolacetate*, pale yellow prisms (4·2 g., 60%), m. p. 63—64°, raised to 64—65° on further recrystallisation from ethanol (Found: C, 49·6; H, 5·4; Cl, 24·4. $C_{12}H_{15}ONSCl_2$ requires C, 49·3; H, 5·2; Cl, 24·3%).

p-(NN-*Di*-2-*chloroethylamino*)*phenyl Thiolbenzoate* (with A. M. Creighton).—A solution of *p*-(*NN*-di-2-chloroethylamino)thiophenol (5·0 g.) and benzoic anhydride (5·0 g.) in pyridine (30 c.c.) was set aside overnight and then diluted with water (100 c.c.). The precipitated solid, on recrystallisation from acetone–ethanol, gave the S-*benzoyl derivative*, pale yellow prisms (6·1 g., 86%), m. p. 109—110° (Found: C, 57·4; H, 5·1; Cl, 20·1. $C_{17}H_{17}ONSCl_2$ requires C, 57·6; H, 4·8; Cl, 20·0%).

NN-*Di*-2′-*chloroethyl*-p-(*methylthio*)*aniline*.—A solution of diazomethane (from 4 g. of α-nitrosomethylurea) in dry ether (35 c.c.) was added, with swirling, to *p*-(*NN*-di-2′-chloroethylamino)thiophenol (5·0 g.) in dry ether (20 c.c.). There was quiet effervescence until about three-quarters of the diazomethane had been added after which there was no apparent reaction. The mixture was kept at 0° overnight, then allowed to warm to room temperature; charcoal, moistened with concentrated hydrochloric acid, was added, and after the effervescence had ceased the mixture was shaken for 5 min., then neutralised with aqueous sodium hydrogen carbonate and filtered; the charcoal residue was washed with warm ether, and the aqueous phase extracted with ether. The combined ether solutions were dried (Na_2SO_4) and evaporated to a pasty solid which was chromatographed in benzene on alumina to give NN-*di*-2′-*chloroethyl*-p-(*methylthio*)*aniline* (1·8 g., 26%), m. p. 58—60° raised to 62—63° on recrystallisation (prisms, from methanol) (Found: C, 50·1; H, 5·95; Cl, 27·05. $C_{11}H_{15}NSCl_2$ requires C, 50·0; H, 5·7; Cl, 26·8%).

p-(NN-*Di*-2-*chloroethylamino*)*phenyl Thiolcarbamate*.—A solution of *NN*-di-2-chloroethyl-*p*-thiocyanatoaniline (2·8 g.) in ice-cold 94% sulphuric acid (28 c.c.) was kept overnight at 0°,

then poured on crushed ice (300 g.). The precipitated solid was collected, washed with water (200 c.c.), and recrystallised from ethanol to give p-(NN-*di*-2-*chloroethylamino*)*phenyl thiolcarbamate* as pale yellow prisms (1·53 g., 52%), m. p. 158° (Found: C, 45·1; H, 5·0; N, 9·3. $C_{11}H_{14}ON_2SCl_2$ requires C, 45·05; H, 4·8; N, 9·55%).

p-(NN-*Di*-2-*chloroethylamino*)*phenyl* N-*Phenylthiolcarbamate*.—Phenyl *iso*cyanate (0·61 g.) and *p*-(*NN*-di-2-chloroethylamino)thiophenol (1·25 g.) were heated together in a sealed tube at 100° for 3 hr. and then set aside overnight. The solid product on recrystallisation from acetone–ethanol gave p-(NN-*di*-2-*chloroethylamino*)*phenyl* N-*phenylthiolcarbamate* (1·03 g., 56%), m. p. 143°, raised to 146—147° on recrystallisation from ethanol (Found: C, 55·4; H, 5·2; N, 7·35. $C_{17}H_{18}ON_2SCl_2$ requires C, 55·3; H, 4·9; N, 7·6%).

p-(NN-*Di*-2-*chloroethylamino*)*phenyl* 2 : 3 : 4 : 6-*Tetra*-O-*acetyl*-β-D-*thioglucoside*.—α-Acetobromoglucose (8·22 g.) was added to a solution of *p*-(*NN*-di-2-chloroethylamino)thiophenol (5·63 g.) and potassium hydroxide (1·10 g.) in chloroform–ethanol (1 : 2) (90 c.c.). The solution was refluxed for 30 min., then cooled and washed with water; the chloroform layer was removed and the aqueous phase was re-extracted with chloroform. The combined extracts were washed with saturated aqueous sodium hydrogen carbonate and with water, dried (Na_2SO_4), and concentrated to give a solid (7·2 g., 62%), m. p. 110—112°. Recrystallisation from ethanol gave p-(NN-*di*-2-*chloroethylamino*)*phenyl* 2 : 3 : 4 : 6-*tetra*-O-*acetyl*-β-D-*thioglucoside*, colourless needles, m. p. 110—111°, $[\alpha]_D^{22}$ −44° (*c* 2 in $CHCl_3$) (Found: C, 49·7; H, 5·4; Cl, 12·1. $C_{24}H_{31}O_9NSCl_2$ requires C, 49·7; H, 5·4; Cl, 12·2%).

p-(NN-*Di*-2-*chloroethylamino*)*phenyl* β-D-*Thioglucoside*.—The above tetra-acetate (2·02 g.) was suspended in anhydrous methanol, and a small piece of sodium (*ca.* 5 mg.) was added. The flask was sealed, swirled until all the solid had dissolved, and then kept overnight at 0°. The solution was neutralised with carbon dioxide and evaporated to an oil which on crystallisation from ethanol–pentane gave p-(NN-*di*-2-*chloroethylamino*)*phenyl* β-D-*thioglucoside*, needles, (1·09 g., 76%), m. p. 85—87°, $[\alpha]_D^{22}$ −40° (*c* 2 in pyridine) (Found: C, 46·8; H, 5·7; Cl, 17·4. $C_{16}H_{23}O_5NSCl_2$ requires C, 46·6; H, 5·6; Cl, 17·2%).

Methyl Ester of S-p-(NN-*Di*-2-*chloroethylamino*)*phenyl* 2 : 3 : 4-*Tri*-O-*acetyl*-β-D-*thioglucuronic Acid*.—Potassium (0·80 g.) was dissolved in anhydrous methanol (175 c.c.), *p*-(*NN*-di-2-chloroethylamino)thiophenol (5·50 g.) was added, the air in the flask was displaced with nitrogen, and the solution was warmed to dissolve the thiophenol and then cooled to 0°. Methyl α-acetobromoglucuronate [28] (8·20 g.) was added, and the mixture was shaken, with continued cooling, until all the acetobromo-sugar had dissolved. The solution was kept at 0° for 20 min. and then allowed to warm to room temperature; it had then become neutral. It was filtered and concentrated under reduced pressure to an oil, which was dissolved in chloroform (100 c.c.), washed with saturated aqueous sodium hydrogen carbonate, and recovered. It was then dissolved in pyridine (25 c.c.) and acetic anhydride (25 c.c.), kept at 0° overnight, poured into water (400 c.c.), stirred for 10 min., then acidified with 2N-sulphuric acid (200 c.c.), and extracted with chloroform to give an oil. On crystallisation from methanol this gave the *thioglucuronate*, colourless prisms (6·79 g., 55%), m. p. 134—136°, $[\alpha]_D^{25}$ −43° (*c* 2 in $CHCl_3$) (Found: C, 48·85; H, 5·35; Cl, 13·0. $C_{23}H_{29}O_9NSCl_2$ requires C, 48·8; H, 5·2; Cl, 12·5%).

Cholest-5-*en*-3β-*yl* p-(NN-*Di*-2-*chloroethylamino*)*phenyl Disulphide*.—3β-Mercaptocholest-5-ene [29] (4·83 g.) in dry benzene (30 c.c.) was added dropwise with stirring to a solution of *N*-chlorosuccinimide (1·60 g.) in dry benzene (100 c.c.) at 5—8°, with exclusion of light and moisture. Stirring was continued for 15 min. after addition was complete, and the mixture was allowed to attain room temperature before being filtered to remove succinimide (0·9 g.; m. p. 124—126°). A solution of *p*-(*NN*-di-2-chloroethylamino)thiophenol (3·0 g.) in dry benzene (10 c.c.) was then added. A reaction occurred and hydrogen chloride was evolved; a pasty solid, presumably a mixture of hydrochlorides, separated. The mixture was heated to 50—60° for 10 min., then cooled, washed with saturated aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated under reduced pressure to an oil, which was chromatographed on alumina (6 cm. × 30 cm.) with benzene–light petroleum (b. p. 60—80°) (1 : 1) as eluant.

The initial colourless eluates (1·25 l.) yielded di-(cholest-5-en-3β-yl) disulphide, which crystallised from acetone in pearly leaflets (2·1 g.), m. p. 143—144° (lit.,[30] m. p. 144·5°). The

[28] Bollenback, Long, Benjamin, and Lindquist, *J. Amer. Chem. Soc.*, 1955, **77**, 3310.
[29] O'Connor and Nace, *J. Amer. Chem. Soc.*, 1953, **75**, 2118.
[30] Wagner-Jauregg and Lennartz, *Ber.*, 1941, **74**, 27.

very pale yellow eluates (500 c.c.) that followed yielded an oil, a solution of which in propan-2-ol–ether slowly deposited colourless crystals of *cholest-5-en-3β-yl* p-(NN-*di-2-chloroethylamino*)-*phenyl disulphide* (2·02 g., 26%), m. p. 71—72°, $[\alpha]_D^{25}$ −36° (*c* 2 in $CHCl_3$) (Found: C, 68·2; H, 8·9; Cl, 11·2. $C_{37}H_{57}NS_2Cl_2$ requires C, 68·3; H, 8·8; Cl, 10·9%). A further small quantity of the mixed disulphide (0·5 g.) was obtained from the next eluates (500 c.c.).

Elution of the residual slowly moving yellow band gave di-[*p*-(*NN*-di-2-chloroethylamino)-phenyl] disulphide (1·4 g., 46%), m. p. 75—76° after recrystallisation from ethanol.

2 : 3 : 4 : 6-*Tetra*-O-*acetyl*-β-D-*thioglucose*.—Prepared in 79% yield by Schneider and Bansa's method,[31] this initially had m. p. 70—72°, but m. p. 112—113° after being kept for 6 months under nitrogen at room temperature. A second preparation had m. p. 116—117°, undepressed with the previous sample (m. p. 112—113°). Wrede [32] gives m. p. 75°, Richtmyer *et al.*[33] 74—75°, Schneider and Bansa [31] 113—114°.

p-(NN-*Di-2-chloroethylamino*)*phenyl* 2 : 3 : 4 : 6-*Tetra*-O-*acetyl*-β-D-*glucosyl Disulphide*.—2 : 3 : 4 : 6-Tetra-*O*-acetyl-β-D-thioglucose (3·64 g.) in dry benzene (50 c.c.) was added dropwise to a cold (10—12°), stirred, suspension of *N*-bromosuccinimide (1·78 g.) in dry benzene (100 c.c.), with exclusion of light and moisture. The mixture was stirred for a further 10 min., then filtered from succinimide. *p*-(*NN*-Di-2-chloroethylamino)thiophenol (2·50 g.) in dry benzene (10 c.c.) was added to the solution; there was an immediate reaction, with evolution of hydrogen bromide and precipitation of a yellow, sticky paste. The mixture was warmed to 60° for 10 min. and then cooled; the supernatant liquid was decanted off from the pasty solid {which from its yellow colour was judged to be mainly the hydrobromide of di-[*p*-(*NN*-di-2-chloroethyl-amino)phenyl] disulphide}, washed with saturated sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated to an oil, which on crystallisation from ethanol gave the mixed *disulphide* (2·63 g., 43%), m. p. 100—102°, $[\alpha]_D^{22}$ −273° (*c* 2 in $CHCl_3$) (Found: C, 47·4; H, 5·4; Cl, 11·2. $C_{24}H_{31}O_9NS_2Cl_2$ requires C, 47·1; H, 5·1; Cl, 11·6%).

The yellow solid remaining after decantation of the benzene solution was triturated with benzene and saturated sodium hydrogen carbonate. The benzene layer furnished di-[*p*-(*NN*-di-2-chloroethylamino)phenyl] disulphide, m. p. 76° (from ethanol).

NN-*Di-2′-bromoethylaniline*.—Phosphorus pentabromide (86 g.) was added in small portions to a solution of *NN*-di-2′-hydroxyethylaniline (30 g.) in chloroform (150 c.c.), the vigour of the reaction being controlled by external cooling. The homogeneous solution was then refluxed for 30 min. (colourless needles, possibly of a hydrobromide, separated after about 20 min.), cooled, stirred with ice-water (600 c.c.), and neutralised by the cautious addition of solid sodium hydrogen carbonate. The aqueous phase was re-extracted with chloroform and the combined extracts were dried (Na_2SO_4) and evaporated to an oil, which was distilled (b. p. 136—138°/1 mm.). The distillate solidified (m. p. 50—52°), and on recrystallisation from light petroleum (b. p. 40—60°) gave colourless prisms (41 g., 80%), m. p. 53—54° (lit.,[10] m. p. 53—55°).

NN-*Di-2′-bromoethyl*-p-*thiocyanatoaniline* (with A. M. CREIGHTON).—A stirred solution of *NN*-di-2′-bromoethylaniline (30 g.) and ammonium thiocyanate (16 g.) in dry methanol (650 c.c.) was treated, as described for the chloro-analogue, with bromine (15·7 g.) and sodium bromide (8 g.) in methanol (80 c.c.). The mixture was stirred for a further 20 min. and then poured into water. NN-*Di-2′-bromoethyl*-p-*thiocyanatoaniline* was precipitated as a colourless solid (33·8 g., 95%), m. p. 64—65°; recrystallisation of a portion from ethanol gave needles, m. p. 68° (Found: C, 36·5; H, 3·65; N, 7·4. $C_{11}H_{12}N_2SBr_2$ requires C, 36·3; H, 3·3; N, 7·7%).

p-(NN-*Di-2-bromoethylamino*)*phenyl Thiolcarbamate*.—Controlled hydrolysis of *NN*-di-2-bromoethyl-*p*-thiocyanatoaniline (2 g.) with 95% ice-cold sulphuric acid, as described for the chloro-compound, gave p-(NN-*di-2-bromoethylamino*)*phenyl thiolcarbamate*, platelets (from benzene) (1·8 g., 85%), m. p. 165—166° (Found: C, 34·7; H, 3·9; N, 6·95. $C_{11}H_{14}ON_2SBr_2$ requires C, 34·6; H, 3·7; N, 7·3%).

p-(NN-*Di-2-bromoethylamino*)*thiophenol* (with A. M. CREIGHTON).—*NN*-Di-2-bromoethyl-*p*-thiocyanatoaniline (10·0 g.) in warm ether (200 c.c.) was reduced by addition to a suspension of lithium aluminium hydride (1·0 g.) in ether (100 c.c.), and the product isolated, as described for the reduction of the chloro-compound. Recrystallisation from ethanol–light petroleum

[31] Schneider and Bansa, *Ber.*, 1931, **64**, 1321.
[32] Wrede, *Z. physiol. Chem.*, 1922, **119**, 46.
[33] Richtmyer, Carr, and Hudson, *J. Amer. Chem. Soc.*, 1943, **65**, 1477.

(b. p. 40—60°) gave almost colourless prisms of p-(NN-*di*-2-*bromoethylamino*)*thiophenol* (8·95 g., 95%), m. p. 84—86°, raised to 88—89° on recrystallisation from ethanol (Found: C, 36·0; H, 4·1; Br, 47·4; thiol-S, 9·3. $C_{10}H_{13}NSBr_2$ requires C, 35·4; H, 3·9; Br, 47·4; thiol-S, 9·5%). It became blue-green on exposure to air. Reaction of the thiol with chloro-2 : 4-dinitrobenzene failed to give a satisfactory derivative.

Di-[p-(NN-*di*-2-*bromoethylamino*)*phenyl*] *Disulphide*.—*p*-(*NN*-Di-2-bromoethylamino)thiophenol (2·0 g.) was oxidised with iodine as described for the chloro-compound. The product, after chromatography and recrystallisation from acetone–ethanol, gave the *disulphide* (1·4 g., 71%), m. p. 78—79° (Found: C, 36·0; H, 3·95; Br, 47·1. $C_{20}H_{24}N_2S_2Br_4$ requires C, 35·5; H, 3·6; Br, 47·3%).

NN-*Di*-2′-*bromoethyl*-4-(*methylthio*)*aniline*.—*p*-(*NN*-Di-2-bromoethylamino)thiophenol (4·0 g.) in dry ether (50 c.c.) was treated with diazomethane (from 4·0 g. of α-nitrosomethylurea) in dry ether, and the product isolated as described for the chloro-compound. The S-*methyl derivative* was obtained as colourless prisms (1·27 g., 30%), m. p. 57° (from ethanol) (Found: C, 37·5; H, 3·5; Br, 45·8. $C_{11}H_{15}NSBr_2$ requires C, 37·4; H, 4·3; Br, 45·3%).

p-(NN-*Diethylamino*)*thiophenol*.—*NN*-Diethyl-*p*-thiocyanatoaniline, b. p. 145°/0·5 mm. (lit.,[34] b. p. 138°/1 mm.), was prepared from *NN*-diethylaniline by the method [35] described for the *NN*-dimethyl compound. It was reduced with lithium aluminium hydride, as described above for the di-2′-chloroethyl analogue, to *p*-(*NN*-diethylamino)thiophenol (50—70%), b. p. 97—98°/0·06 mm., 124°/0·14 mm., n_D^{20} 1·5942. No physical constants were recorded by Montgomery *et al.*[23]

Reaction of the thiol with chloro-2 : 4-dinitrobenzene in aqueous alkaline ethanol gave p-NN-*diethylaminophenyl* 2 : 4-*dinitrophenyl sulphide*, orange-red plates (from ethyl acetate), m. p. 147° (Found: C, 55·6; H, 5·0; N, 11·9. $C_{16}H_{17}O_4N_3S$ requires C, 55·3; H, 4·9; N, 12·1%).

Acetylation of the thiol with acetic anhydride and a trace of sulphuric acid for 30 min. at 100° gave the S-*acetyl derivative* (3·5 g., 71%), b. p. 152—153°/0·75 mm., n_D^{21} 1·5920 (Found: C, 64·4; H, 7·9; N, 6·2. $C_{12}H_{17}ONS$ requires C, 64·55; H, 7·7; N, 6·3%).

Oxidation of the thiol with iodine, as described for the di-2′-chloroethyl analogue gave the disulphide, which crystallised from ethanol in yellow prisms, m. p. 74°. Holzmann [14] gives m. p. 69—72° for the product obtained by reaction of sulphur monochloride with diethylaniline.

Mixed Disulphides Derived from 2 : 4-*Dinitrobenzenesulphenyl Chloride* (with A. M. Creighton).—A solution of *p*-(*NN*-di-2-bromoethylamino)thiophenol (2·6 g.) in dry benzene (30 c.c.) was added slowly to 2 : 4-dinitrobenzenesulphenyl chloride [36] (1·8 g.) in dry benzene (30 c.c.). The mixture was shaken for a few minutes and set aside overnight. It was then poured into saturated aqueous sodium hydrogen carbonate (150 c.c.), the benzene layer was removed, washed with water, dried, and concentrated, and the residue transferred to a column of alumina. Elution with benzene gave p-(NN-*di*-2-*bromoethylamino*)*phenyl* 2 : 4-*dinitrophenyl disulphide* as golden-yellow needles (2·4 g.), m. p. 119—120°, transformed by recrystallisation from acetone–ethanol into deep red prisms (2·1 g.), m. p. 132—133° (a mixture of the two forms had m. p. 132—133°) (Found: C, 35·9; H, 3·0; N, 7·9. $C_{16}H_{15}O_4N_3S_2Br_2$ requires C, 35·8; H, 2 8; N, 7·8%).

Prepared in a similar way p-(NN-*di*-2-*chloroethylamino*)*phenyl* 2 : 4-*dinitrophenyl disulphide* formed red prisms, m. p. 140° (from acetone–ethanol) (Found: C, 43·0; H, 3·8; N, 9·3. $C_{16}H_{15}O_4N_3S_2Cl_2$ requires C, 42·9; H, 3·4; N, 9·4%); and p-NN-*diethylaminophenyl* 2 : 4-*dinitrophenyl disulphide* formed purple prisms, m. p. 109° (from acetone–ethanol) (Found: C, 50·8; H, 4·9; N, 11·0. $C_{16}H_{17}O_4N_3S_2$ requires C, 50·6; H, 4·9; N, 11·1%).

This investigation was supported by a grant from the British Empire Cancer Campaign. We thank Mr. C. C. Harris, Brown and Polson Ltd., for a generous gift of glucuronolactone.

Department of Organic Chemistry,
Imperial College of Science and Technology,
South Kensington, London, S.W.7. [*Received, March 7th*, 1958.]

[34] Fichter and Schonmann, *Helv. Chim. Acta*, 1936, **19**, 1411.
[35] *Org. Syntheses*, Coll. Vol. II, p. 574.
[36] Kharasch, Gleason, and Buess, *J. Amer. Chem. Soc.*, 1950, **72**, 1796.