Cytotoxic Compounds. Part XVII.[1] *o*-, *m*-, and *p*-(Bis-2-chloroethylamino)phenol, *p*-[*N*-(2-Chloroethyl)methylamino]phenol, *NN*-Bis-2-chloroethyl-*p*-phenylenediamine, and *NN*-Bis-2-chloroethyl-*N'*-methyl-*p*-phenylenediamine as Sources of Biologically Active Carbamates

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New or improved syntheses are reported of the nitrogen mustards named in the title, and of methyl *m*-(bis-2-chloroethylamino)-*p*-hydroxybenzoate. By reactions of the amines with aryl chloroformates, or of the phenols with aryl isocyanates or with isocyanates derived from α-amino-esters, carbamates containing alkyl, chloro-, alkoxy-, hydroxy-, methoxycarbonyl, carboxy-, acetyl, and sulphamoyl groups are obtained. Some of these have shown marked anti-tumour activity. *p*-(Bis-2-chloroethylamino)phenyl chloroformate has been prepared: it provides an alternative route to mustard carbamates. Some carbamates of the lachrymator type have been synthesised from *p*-aminophenacyl chloride.

For reasons explained in Part II,[2] aromatic nitrogen mustards carrying a urethane substituent on the aryl group have attracted attention as cytotoxic agents having favourable biological characteristics. The carboxylic acid (4) (I.C. 140), prepared from the phenol (3)[2] to provide a urethane which would have the clinical advantage of being water-soluble in buffered solution, showed the unexpectedly high chemotherapeutic index ($L.D._{50}/E.D._{90}$) of 17.[3] The 'reversed' carbamate (5) (I.C. 213)[4] and its methyl ester gave indices of 46 and 137 respectively, and a figure of 150 was recorded for the isomeric methyl ester (6). These are remarkable values, since the clinically useful nitrogen mustard Chlorambucil has an index of 12 measured under the same conditions.[5] Mustard urethanes are therefore compounds of considerable pharmacological interest, and analogues in which the aryl rings carry a range of substituents in a variety of orientations are evidently worthy of investigation.

Syntheses of many such urethanes have now been effected by reactions of a mustard phenol with an isocyanate, or of a mustard amine with a chloroformate. These condensations were conventional, and apart from a few instances when special circumstances arose, are described (together with the physical properties of the urethanes) only in a Supplementary Publication.* It was necessary to establish satisfactory conditions for the preparation of isocyanates from some amino-esters, and these are recorded in the Experimental section of this Paper, but details concerning other isocyanates, and the syntheses of many chloroformates, are given in the Supplementary Publication.

All the parent aromatic mustards were prepared by new or improved routes. The best method for the synthesis of the phenol (3) involves protection of the phenolic group in the intermediate *p*-(bis-2-hydroxyethylamino)phenol by *O*-benzylation.[2] After conversion of the resulting diol into the dichloride, the benzyl group has hitherto been removed by hydrogenolysis,[2,6] but this can be more conveniently effected with hydrochloric acid, giving an almost quantitative yield of the phenolic mustard.

The *o*-isomer (1), previously synthesised by Artico and Ross,[6] was prepared by a modified route, since, in contrast to their report, *o*-aminophenol reacted readily with ethylene oxide to afford the triol (7), which by selective benzylation gave the ether (8), and thence, with phosphorus pentachloride, the dichloride (9). In this instance, hydrochloric acid failed to effect a smooth debenzylation, so the phenol (1) was obtained by hydrogenolysis.

m-Aminophenyl benzyl ether with ethylene oxide gave the diol analogous to (8), from which the corresponding dichloride was obtained. Artico and Ross[6] synthesised this product by a different method, but failed to achieve the necessary hydrogenolysis. We have confirmed this difficulty, which can be surmounted by adoption of the new procedure using hydrochloric acid, to give the required *m*-(bis-2-chloroethylamino)phenol (2).

Compounds in which the nitrogen atom carries only one 2-chloroethyl substituent are generally much less active as anti-tumour agents than those of the true nitrogen mustard type,[7] but a few have shown properties of potential value[8,9] and the preparation of some urethanes derived from such a system was desirable. As a parent of such compounds, *p*-[*N*-(2-chloroethyl)-methylamino]phenol (10) was synthesised from benzyl

* Supplementary Publication No. SUP 20806 lists the sources of the known isocyanates and chloroformates used, outlines the syntheses of new chloroformates, and describes the general methods of preparation, the physical properties, and the analyses of the carbamates. Some ureas and hydantoins, prepared by reaction of isocyanates with *NN*-bis-2-chloroethyl-*p*-phenylenediamine, are also included. [For details of Supplementary Publications, see Notice to Authors No. 7, in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.]

[1] Part XVI, G. Durrant, P. D. Edwards, and L. N. Owen, *J.C.S. Perkin I*, 1973, 1271.

[2] M. H. Benn, A. M. Creighton, L. N. Owen, and G. R. White, *J. Chem. Soc.*, 1961, 2365.

[3] T. J. Bardos, Z. F. Chmielewicz, and P. Hebborn, *Ann. New York Acad. Sci.*, 1969, **163**, 1006; and personal communication.

[4] L. N. Owen and R. Sridhar, *J. Chem. Soc.* (*C*), 1970, 472.

[5] T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Medicin. Chem.*, 1965, **8**, 167.

[6] M. Artico and W. C. J. Ross, *Biochem. Pharmacol.*, 1968, **17**, 893.

[7] W. C. J. Ross, 'Biological Alkylating Agents,' Butterworths, London, 1962, p. 113.

[8] M. Artico and W. C. J. Ross, *Biochem. Pharmacol.*, 1968, **17**, 873.

[9] J. K. Chakrabarti and O. M. Friedman, *Chem. and Ind.*, 1965, 898.

p-(methylamino)phenyl ether by a route analogous to that used for the phenol (2).

Condensation of the four phenols with aryl isocyanates gave the carbamates (11; R^2 = *p*-CO_2Me), (12; R^2 = *m*- and *p*-CO_2Me), (13; R^2 = *o*-, *m*-, and *p*-Me, *o*-, *m*-, and *p*-Cl, *o*-, *m*-, and *p*-OMe, *o*- and *m*-CO_2Me, *p*-$SO_2{\cdot}NH_2$), and (14; R^2 = *o*- and *p*-CO_2Me); by selective hydrolysis [2,4] of the methyl esters, the acids (11; R^2 = *p*-CO_2H), (12; R^2 = *m*-CO_2H), (13; R_2 = *m*-CO_2H), and (14; R^2 = *p*-CO_2H) were obtained. The sulphonamide was included because *p*-(bis-2-bromoethylamino)benzenesulphonamide [10] showed the property, unusual for a nitrogen mustard, of a delayed toxicity associated with inhibition of folic acid utilisation.[11] Synthesis by reaction of the phenolic mustard (3) with *p*-sulphamoylphenyl isocyanate gave a product very difficult to purify. The phenol was therefore converted, by reaction with carbonyl chloride, into the chloroformate (15) which reacted with sulphanilamide to give the same urethane, but of better quality. The chloroformate (15), the first mustard derivative of this type to be described, is a useful reagent because it provides an alternative method for the synthesis of mustard urethanes. In particular, it allows the preparation of derivatives fully substituted on the carbamate nitrogen atom (important for biological comparison with those containing the –CO·NH– system) which cannot be made by the isocyanate route. For example, reaction of this chloroformate with methyl *p*-(methylamino)benzoate gave the carbamate (16).

The incorporation of alkylating functions into biologically important molecules has been exemplified earlier by compounds in which a nitrogen mustard is attached by a urethane linkage to the hydroxy-functions of serine, threonine, and tyrosine.[4] One urethane was reported in which the linkage involved the amino-group of glycine,[2] and several more examples (17; R^2 = Me, $PhCH_2$, *p*-$MeO{\cdot}C_6H_4{\cdot}CH_2$, $MeS{\cdot}CH_2{\cdot}CH_2$, and $EtS{\cdot}CH_2{\cdot}CH_2$) have now been prepared by condensation of the phenol (3) with α-isocyanato-esters; the corresponding carboxylic acids were obtained by brief acidic hydrolysis.

As a potential source of urethanes in which a carboxy-function is attached to the aromatic ring carrying the nitrogen mustard, methyl *m*-(bis-2-chloroethylamino)-*p*-hydroxybenzoate (18) was synthesised from methyl *m*-amino-*p*-hydroxybenzoate. The procedure was unusual in that protection of the phenolic group was not necessary; the chlorination was effected by direct treatment of the intermediate triol with thionyl chloride.

[10] M. H. Benn, A. M. Creighton, B. J. Johnson, L. N. Owen, and G. R. White, *J. Chem. Soc.*, 1964, 3395.

[11] R. Hawkins, L. N. Owen, and J. F. Danielli, *J. Theor. Biol.*, 1963, **5**, 236.

For the synthesis of ' reversed ' urethanes, analogous to compound (5), a mustard amine is required. The *p*-amino-compound (19) has hitherto been prepared by nitrosation of *NN*-bis-2-chloroethylaniline, followed by reduction,[12] but a much cleaner product (which gives derivatives which are easier to purify) is obtained by conversion of *p*-aminoacetanilide, *via* *p*-(bis-2-hydroxyethylamino)acetanilide, into the dichloride (20), followed by deacetylation with hydrochloric acid. In the synthesis of nitrogen mustards, the most uncertain stage is usually the conversion of the diol into the dichloride, and the nature of the best reagent is unpredictable. After many trials, thionyl chloride in dimethylformamide was found to be best for the direct preparation of the dichloride (20), but the yield was poor, and better results were obtained by the conversion of the diol into the bistoluene-*p*-sulphonate and reaction of this with calcium chloride in 2-ethoxyethanol.[13]

Reaction of the *p*-amino-mustard with aryl chloroformates gave the carbamates (21; R^2 = *o*-, *m*-, and *p*-Me, *p*-Bu^t, *o*-, *m*-, and *p*-Cl, *o*-, *m*-, and *p*-OMe, *p*-SMe, *o*-, *m*-, and *p*-O·CH_2Ph, *m*- and *p*-Ac, *p*-NO_2, *m*-NMe_2, and $[CH_2]_n$·CO_2Me where n = 1—3). By selective hydrolysis of the methyl esters, the carboxylic acids (21; R^2 = $[CH_2]_n$·CO_2H where n = 1—3) were obtained; these and the original *p*-carboxy-compound (5) form a homologous series and were prepared because considerable differences exist in the anti-tumour action within a series of homologues of *p*-(bis-2-chloroethylamino)benzoic acid.[14] The phenols (21; R^2 = *o*-, *m*-, and *p*-OH) were obtained by hydrogenolysis of the benzyl ethers. Two doubly substituted carbamates (22; R^2 = Me and CO_2Me) and the *m*-mustard derivative (23) were also prepared.

As a source of urethanes in which the carbamate nitrogen atom is fully substituted, *NN*-bis-(2-chloroethyl)-*N'*-methyl-*p*-phenylenediamine (26) was synthesised. *p*-Amino-*N*-methylacetanilide was converted into the diol (24), but with thionyl chloride–dimethylformamide this gave the cyclic sulphite (28) as the only identifiable product. Bosshard and his co-workers[15] have suggested that the active species in this reagent is chloromethylenedimethylammonium chloride (29), which they have isolated, but more recently the intermediate chloro-sulphite (30) has been obtained,[16] and the formation of the ester (28) proves that the chloro-sulphite persists even under conditions claimed to give the iminium chloride (29). The required dichloride (25) was eventually obtained from the diol (24) by the use of thionyl chloride alone; acidic hydrolysis then gave the secondary amine (26). Although there is one reference to this compound,[17] no indications of its source or properties were given, and its mention was probably erroneous.[18] It was converted into the carbamate (27).

α-Halogeno-ketones are biological alkylating agents,[19] and for comparison with the analogous nitrogen mustards, the carbamates (31; R = *o*-, *m*-, and *p*-CO_2Me) were prepared from *p*-aminophenacyl chloride. The corresponding acids were made by selective hydrolysis of the *m*- and the *p*-ester, but conditions could not be established for similar preparation of the *o*-acid.

In preliminary biological tests on the Walker 256 carcinoma in rats or mice,[20] some of the carbamates have shown high activity combined with low toxicity. Of particular interest is the finding that, in the series comprising the acid (5) and its three side-chain homologues, the compound with the best chemotherapeutic index, 140, is the propionic acid; in the series studied by Ross,[14] propionic and butyric side chains have been found to give optimum activity, though of a lower order. Other very effective products are the chloro- and methoxy-derivatives (19; R^2 = *p*-Cl, *o*-OMe, and *m*-OMe) which have indices of 70, 140, and 120, respectively. Carbamates derived from the mustard phenols are generally less effective than the isomers prepared from mustard amines, but there are exceptions; the indices for the compounds (13; R^2 = *m*-Cl) and (19; R^2 = *m*-Cl) are, respectively, 52 and 9.

EXPERIMENTAL

Unless otherwise specified, i.r. spectra of liquids were measured for liquid films, and those of solids for solutions in chloroform; ^{1}H n.m.r. spectra were recorded for solutions in deuteriochloroform on a Varian A60 instrument. Petroleum refers to the fraction of b.p. 40—60°.

Methyl 2-Isocyanato-4-(methylthio)butyrate.—DL-Methionine (10 g) and methanol (100 ml), saturated at 0° with hydrogen chloride, were boiled together for 24 h. The solution was then concentrated, and the methyl ester hydrochloride was precipitated by addition of dry ether. The salt was collected and suspended in dry toluene (400 ml) previously saturated with carbonyl chloride at 0°, and the mixture was boiled under reflux for 24 h. Distillation gave the *isocyanate* (7·7 g), b.p. 86° at 10^{-2} mmHg, $\nu_{max.}$ 2250 and 1730 cm^{-1} (Found: C, 44·6; H, 5·9; N, 7·6. $C_7H_{11}NO_3S$ requires C, 44·4; H, 5·9; N, 7·4%). It was characterised by heating with water, to give the *urea*, m.p. 92° (from aqueous methanol) (Found: N, 8·1; O, 22·8. $C_{13}H_{24}N_2O_5S_2$ requires N, 8·0; O, 22·7%).

Methyl 2-Isocyanato-4-(ethylthio)butyrate.—DL-Ethionine methyl ester[21] (6·8 g) under the same conditions gave the *isocyanate* (6·5 g), b.p. 108—110° at 1·5 mmHg, n_D^{20} 1·4806, $\nu_{max.}$ 2230 cm^{-1} (Found: C, 46·8; H, 6·2; N, 6·7. $C_8H_{13}NO_3S$ requires C, 47·2; H, 6·4; N, 6·9%).

Methyl 2-Isocyanato-3-(p-methoxyphenyl)propionate.—L-*p*-Methoxyphenylalanine methyl ester hydrochloride[22] (10·0 g) was similarly converted into the *isocyanate* (5·1 g), b.p.

[12] J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, 1949, 1972.
[13] *Cf.* W. Werner, *J. prakt. Chem.*, 1972, **314**, 577.
[14] J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 1953, 2386.
[15] H. H. Bosshard, R. Mory, M. Schmid, and H. Zollinger, *Helv. Chim. Acta*, 1959, **42**, 1653.
[16] G. Ferré and A. L. Palomo, *Tetrahedron Letters*, 1969, 2161; *Anales de Quim.*, 1969, **65**, 163.
[17] A. R. Crathorne and G. D. Hunter, *Biochem. J.*, 1957, **67**, 37.
[18] W. C. J. Ross, personal communication.
[19] Ref. 7, pp. 17 and 176; D. J. Cooper and L. N. Owen, *J. Chem. Soc.* (*C*), 1966, 533; L. N. Owen and R. Sridhar, *ibid.*, 1970, 564.
[20] P. Hebborn, personal communication.
[21] U.S.P. 2,840,595 (*Chem. Abs.*, 1959, **53**, 13075).
[22] B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Amer. Chem. Soc.*, 1955, **77**, 1.

126—128° at 0·2 mmHg (Found: C, 60·6; H, 6·0; N, 5·85. $C_{12}H_{13}NO_4$ requires C, 61·1; H, 5·6; N, 5·95%). Reaction of a sample with aniline gave *methyl* 3-(p-*methoxyphenyl*)-2-(3-*phenylureido*)*propionate*, m.p. 120° (from aqueous methanol) (Found: C, 65·7; H, 6·3; N, 8·7. $C_{18}H_{20}N_2O_4$ requires C, 65·8; H, 6·1; N, 8·5%).

Benzyl 2-*Isocyanato*-3-(p-*methoxyphenyl*)*propionate*.—A mixture of L-*p*-methoxyphenylalanine [22] (12 g), toluene-*p*-sulphonic acid (17·5 g), benzyl alcohol (50 ml), and benzene (400 ml) was boiled under reflux, water being continuously removed with a Dean–Stark separator, and finally by use of a Soxhlet tube containing silica gel. The cooled solution was washed with aqueous sodium hydrogen carbonate then dried and treated with hydrogen chloride. The precipitated benzyl ester hydrochloride, (13·5 g), m.p. 200°, was collected and treated in the usual way with carbonyl chloride in toluene (but heated for only 90 min) to give the *isocyanate* (9·1 g), m.p. 36°, b.p. 170° at 0·2 mmHg, $[\alpha]_D^{22}$ −88° (*c* 9 in acetone), n_D^{19} 1·5540 (Found: C, 69·1; H, 5·6; N, 4·5. $C_{18}H_{17}NO_4$ requires C, 69·4; H, 5·5; N, 4·5%). When warmed with water it gave the *urea*, m.p. 148—149° (from ethanol) (Found: C, 71·0; H, 6·25; N, 4·7. $C_{35}H_{36}N_2O_7$ requires C, 70·4; H, 6·1; N, 4·7%).

Methyl 2-*Isocyanatopropionate*.—Treatment of DL-alanine (15 g) in dry dioxan (225 ml) with carbonyl chloride and with hydrogen chloride gave 2-isocyanatopropionyl chloride (12·6 g), b.p. 64° at 12 mmHg (lit.,[23] 67—68° at 47 mmHg). To a solution of this product (9·8 g) in dry ether (45 ml), dry methanol (2·7 g) in dry ether (8 ml) was added dropwise (1 h), and the solution was set aside for 24 h. Evaporation left a solid residue, presumably the carbamoyl chloride, which was distilled to give a main fraction (5·1 g), b.p. 46—48° at 0·5 mmHg, n_D^{17} 1·4220, $\nu_{max.}$ 2260 and 1730 cm^{-1}, which was the *isocyanato-ester* (Found: C, 46·3; H, 5·5; N, 10·8. $C_5H_7NO_3$ requires C, 46·5; H, 5·5; N, 10·85%). The *urea*, formed by stirring the isocyanate with water, had m.p. 163—164° (from aqueous methanol) (Found: C, 46·9; H, 7·1; N, 12·0. $C_9H_{16}N_2O_5$ requires C, 46·5; H, 7·0; N, 12·1%).

p-(*Bis*-2-*chloroethylamino*)*phenol Hydrochloride*.—Benzyl *p*-(bis-2-chloroethylamino)phenyl ether [2] (30 g), acetic acid (150 ml), and concentrated hydrochloric acid (150 ml) were boiled together for 1 h under reflux. The cooled solution was treated with an excess of sodium carbonate, followed by sufficient water to dissolve the salts, and then extracted with chloroform. Hydrogen chloride (4 g) in ethanol (50 ml) was then added to the dried extract, which on evaporation gave a solid residue of the phenol hydrochloride (21 g), m.p. 180—181°, $\nu_{max.}$ (mull) 3200 cm^{-1} (lit.,[2] m.p. 170—173°).

o-(*Bis*-2-*hydroxyethylamino*)*phenol*.—Ethylene oxide (105 ml) was added to a mixture of *o*-aminophenol (21 g), acetic acid (70 ml), and water (140 ml) at 0°. After being stirred at 0° for 6 h and then overnight at ambient temperature, the solution was neutralised with solid sodium carbonate, sufficient water being added to dissolve the salts. Extraction with chloroform gave the crude product as an oil (18 g), a portion of which was distilled to afford the triol, b.p. 150° at 10^{-4} mmHg, m.p. 49—50° (from chloroform–petroleum) (lit.,[8] m.p. 49—50°), τ 2·7—3·3 (4H, m, aromatic), 4·42 (3H, s, OH), 6·44 (4H, t, CH_2N), and 6·96 (4H, t, CH_2O). It was characterised as the *tris*-p-*nitrobenzoate*, m.p. 179° (from acetic acid) (Found: C, 57·5; H, 3·9; N, 8·5. $C_{31}H_{24}N_4O_{12}$ requires C, 57·75; H, 3·75; N, 8·7%).

[23] Y. Iwakura, K. Uno, and S. Kang, *J. Org. Chem.*, 1965, **30**, 1158.

Benzyl o-(*Bis*-2-*hydroxyethylamino*)*phenyl Ether*.—Benzyl bromide (15·4 g) was added to a solution of the preceding triol (17·7 g) and potassium hydroxide (5·1 g), in ethanol (250 ml). After being boiled under reflux for 3 h, the mixture was poured into water and extracted with ether to give an oil. T.l.c. showed this to be a mixture containing a major component, which was separated by column chromatography in ethyl acetate on alumina. The benzyl ether (21·4 g) was characterised as the *bis*-p-*nitrobenzoate*, m.p. 99—101° (from chloroform–petroleum) (Found: C, 63·3; H, 5·0; N, 7·0. $C_{31}H_{27}N_3O_9$ requires C, 63·6; H, 4·7; N, 7·2%).

Benzyl o-(*Bis*-2-*chloroethylamino*)*phenyl Ether*.—The preceding diol (21·0 g), phosphorus pentachloride (34·3 g), and chloroform (140 ml) were boiled together under reflux for 15 h. The cooled mixture was poured onto ice, and the chloroform layer was separated and washed with aqueous sodium hydrogen carbonate and with water, then dried and evaporated to give the dichloride as an oil; the i.r. spectrum showed no hydroxy-absorption. The product was characterised as the *picrate*, m.p. 115—118° (from ethanol) (Found: C, 50·1; H, 4·2; Cl, 12·8; N, 10·0. $C_{23}H_{22}Cl_2N_4O_8$ requires C, 49·9; H, 4·0; Cl, 12·8; N, 10·1%).

o-(*Bis*-2-*chloroethylamino*)*phenol Hydrochloride*.—The preceding dichloride (10·0 g) in methanol (100 ml) was hydrogenated at ambient temperature and pressure overnight, in the presence of 10% palladised charcoal (0·25 g) and a few drops of concentrated hydrochloric acid. The solution was then filtered and evaporated to a red oil, which was dissolved in benzene and passed through a column of silica. The colourless eluate was concentrated and treated with gaseous hydrogen chloride, which precipitated an oil. The supernatant liquor was removed by decantation, and the oil was triturated with petroleum, whereupon it crystallised. Recrystallisation from ethanol–petroleum afforded the colourless phenol hydrochloride (4·0 g), m.p. 102—105° (Found: C, 44·6; H, 5·4; Cl, 39·1; N, 5·2. Calc. for $C_{10}H_{14}Cl_3NO$: C, 44·4; H, 5·2; Cl, 39·3; N, 5·2%) (lit.,[6] m.p. 134—135° for a sample not analytically pure).

Benzyl m-(*Bis*-2-*hydroxyethylamino*)*phenyl Ether*.—*m*-Aminophenyl benzyl ether,[24] m.p. 62° (2·0 g), with ethylene oxide (10 ml) in acetic acid (5 ml) and water (5 ml), under the conditions described above, gave the *diol* (2·0 g), m.p. 58—59° (from benzene–petroleum) (Found: C, 70·8; H, 7·1; N, 4·7. $C_{17}H_{21}NO_3$ requires C, 71·0; H, 7·4; N, 4·9%).

Benzyl m-(*Bis*-2-*chloroethylamino*)*phenyl Ether*.—The preceding diol (8·8 g) was heated with phosphoryl chloride (4·5 g) in boiling benzene (150 ml) for 18 h. Isolation of the product, as described for the *o*-isomer, gave the *dichloride* (6·0 g), b.p. *ca.* 230° at 10^{-4} mmHg (Found: C, 63·3; H, 6·1; Cl, 21·5; N, 4·4. $C_{17}H_{19}Cl_2NO$ requires C, 63·0; H, 5·9; Cl, 21·9; N, 4·3%).

m-(*Bis*-2-*chloroethylamino*)*phenol*.—A solution of the preceding benzyl ether (1·0 g) in acetic acid (5 ml) and concentrated hydrochloric acid (5 ml) was boiled for 3·5 h, then poured onto ice and neutralised with an excess of sodium hydrogen carbonate. The mixture was extracted with chloroform, but t.l.c. indicated the presence of a small amount of the benzyl ether; the chloroform solution was therefore extracted twice with aqueous 10% sodium hydroxide and then discarded. Acidification of the alkaline solution with dilute hydrochloric acid, followed by extrac-

[24] E. Cortes and F. Walls, *Bol. Inst. Quim. Univ. nac. auton. Mexico*, 1964, **16**, 3 (*Chem. Abs.*, 1965, **63**, 564); J. Sova, A. Sekera, and C. Vrba, *Chem. listy*, 1957, **51**, 2339; A. A. Morton and W. R. Slaunwhite, *J. Biol. Chem.*, 1949, **179**, 259.

tion with chloroform, then afforded the phenol (0·6 g), τ 2·7—3·9 (4H, aromatic), 5·0br (1H, s, OH; removed by D_2O), and 6·4s (8H, 4 × CH_2). It was characterised by conversion into the urethanes I.C. 262, 264, and 265 (Supplementary Publication).

Benzyl p-[N-(2-*Hydroxyethyl*)*methylamino*]*phenyl Ether*.—Benzyl *p*-(methylamino)phenyl ether [25] (1·0 g), ethylene oxide (10 ml), acetic acid (10 ml), and water (20 ml), under the conditions described above, gave the alcohol (0·2 g), m.p. 67—68° (from benzene–petroleum) (Found: C, 74·8; H, 7·35; N, 5·3. $C_{16}H_{19}NO_2$ requires C, 74·7: H, 7·4; N, 5·45%).

Benzyl p-[N-(2-*Chloroethyl*)*methylamino*]*phenyl Ether*.—The alcohol (1·5 g) was treated with phosphoryl chloride (0·32 g) in boiling benzene overnight. The product, isolated as described above, was the *chloride* (1·0 g) which after crystallisation from aqueous methanol, and then from petroleum, had m.p. 121—122° (Found: C, 70·0; H, 6·3; Cl, 12·9; N, 4·8. $C_{16}H_{18}ClNO$ requires C, 69·7; H, 6·6; Cl, 12·85; N, 5·1%).

p-[N-(2-*Chloroethyl*)*methylamino*]*phenol Hydrochloride*.—The preceding benzyl ether (1·0 g) was hydrogenolysed overnight at ambient temperature and pressure in methanol (20 ml), over 5% palladised charcoal (0·1 g). The product, an oil, was dissolved in benzene and treated with gaseous hydrogen chloride. The solid precipitate, after recrystallisation from ethanol–petroleum, afforded the *hydrochloride* (0·4 g), m.p. 135—140° (Found: C, 48·4; H, 6·1; Cl, 31·85; N, 6·5. $C_9H_{13}Cl_2NO$ requires C, 48·7; H, 5·9; Cl, 31·9; N, 6·3%).

p-(*Bis*-2-*chloroethylamino*)*phenyl Chloroformate*.—A suspension of *p*-(bis-2-chloroethylamino)phenol hydrochloride (5·0 g) in pure chloroform (100 ml) was shaken with an excess of aqueous sodium carbonate. The chloroform layer was then separated and dried, before being slowly added to a stirred saturated solution of carbonyl chloride in benzene (50 ml) containing quinoline (2·5 g). A slow stream of carbonyl chloride was passed through the mixture during the addition. Next day the solution was filtered and then washed with water and dried. Evaporation gave the *chloroformate* (2·5 g), b.p. 130° at 10^{-1} mmHg, $\nu_{max.}$ (CCl_4) 1780 cm^{-1} (Found: C, 44·5; H, 4·2; Cl, 36·0; N, 4·6. $C_{11}H_{12}Cl_3NO_2$ requires C, 44·5; H, 4·1; Cl, 35·9; N, 4·7%).

p-(*Bis*-2-*chloroethylamino*)*phenyl* N-(p-*Methoxycarbonylphenyl*)-N-*methylcarbamate* (16).—A solution of the preceding chloroformate (1·7 g), methyl *p*-(methylamino)benzoate (0·9 g), and pyridine (0·5 g) in pure chloroform (30 ml) was boiled under reflux for 7 h, then cooled and washed successively with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and water. Evaporation of the dried solution then gave the *urethane* (1·2 g), m.p. 92—93° [from benzene–petroleum (b.p. 80—100°)], $\nu_{max.}$ (mull) 1720 cm^{-1} (Found: C, 56·4; H, 5·4; Cl, 16·7; N, 6·4. $C_{20}H_{22}Cl_2N_2O_4$ requires C, 56·45; H, 5·2; Cl, 16·7; N, 6·6%).

p-(*Bis*-2-*chloroethylamino*)*phenyl* N-(p-*Sulphamoylphenyl*)-*carbamate*.—A solution of the same chloroformate (0·7 g) in dry acetone (10 ml) was slowly added to a stirred solution of sulphanilamide (0·4 g) and pyridine (0·2 g) in dry acetone (20 ml). After 90 min the mixture was poured into water and extracted with ethyl acetate to give the *urethane* (0·4 g), m.p. 185—202° (from ethyl acetate–carbon tetrachloride), $\nu_{max.}$ (mull) 1725 cm^{-1}; τ [$(CD_3)_2CO$] 0·50 (1H, s, NH), 2·0—3·5 (8H, m, aromatic), 6·19 (8H, s, CH_2), and 6·83 (2H, s, $SO_2{\cdot}NH_2$) (Found: C, 47·2; H, 4·5; Cl, 16·3; N, 9·85. $C_{17}H_{19}Cl_2N_3O_4S$ requires C, 47·2; H, 4·5; Cl, 16·4; N, 9·7%).

Methyl m-(*Bis*-2-*hydroxyethylamino*)-p-*hydroxybenzoate*.—Ethylene oxide (16 ml) was added to a suspension of methyl *m*-amino-*p*-hydroxybenzoate (3·3 g) in water (15 ml) and acetic acid (50 ml). The mixture was shaken for 24 h and was then concentrated under reduced pressure and treated with an excess of aqueous sodium hydrogen carbonate. Extraction with ethyl acetate (3 × 30 ml) gave the *triol* (3·1 g), m.p. 79° (from benzene), $\nu_{max.}$ 1700 cm^{-1} (Found: C, 56·6; H, 6·8; N, 5·5. $C_{12}H_{17}NO_5$ requires C, 56·5; H, 6·7; N, 5·5%).

Methyl m-(*Bis*-2-*chloroethylamino*)-p-*hydroxybenzoate* (18).—Thionyl chloride (12 ml) was added to a solution of the preceding triol (3·4 g) in dry benzene (25 ml). The mixture was boiled under reflux for 30 min, then cooled, diluted with chloroform, and washed with aqueous sodium hydrogen carbonate. Evaporation of the dried organic layer gave an oil, which was purified by chromatography (alumina; chloroform); it then solidified. Recrystallisation from acetone–petroleum gave the *dichloride*, m.p. 76°, $\nu_{max.}$ 1700 cm^{-1} (Found: C, 49·5; H, 5·4; Cl, 24·4; N, 4·8. $C_{12}H_{15}Cl_2NO_3$ requires C, 49·3; H, 5·2; Cl, 24·3; N, 4·8%).

p-(*Bis*-2-*chloroethylamino*)*acetanilide* (20).—(i) Thionyl chloride (13 g) was added to a solution of *p*-(bis-2-hydroxyethylamino)acetanilide [26] (11·7 g) in dry dimethylformamide (100 ml). After being kept at 90° for 30 min, the mixture was poured into water and extracted with chloroform. The extracts were washed with aqueous sodium hydrogen carbonate, and with water, then dried ($MgSO_4$) and evaporated to a solid residue. On crystallisation from benzene–petroleum (charcoal) this gave the dichloride (3·4 g), m.p. and mixed m.p. 122—125° (lit.,[12] m.p. 124—126°).

(ii) A stirred mixture of the same diol (24 g) and toluene-*p*-sulphonyl chloride (42 g) in pyridine (200 ml) was kept at *ca.* 0° for 1 h. A further portion (42 g) of the acid chloride was added, and stirring was maintained for 1 h at 0° and then for 1 h at ambient temperature. The mixture was then again cooled and treated dropwise with sufficient water to decompose the excess of chloride. More water (500 ml) was then added, and the resulting suspension was stirred overnight to yield a pale yellow solid, which was collected, washed with water, and dried *in vacuo* to give the crude bis-toluene-*p*-sulphonate (48 g), m.p. 78—85°, $\nu_{max.}$ 1670 and 1365 cm^{-1}, τ 2·2—3·7 (12H, m, aromatic), 5·95 and 6·50 (8H, two t, $CH_2{\cdot}CH_2$), 7·58 (6H, s, CH_3Ar), and 7·90 (3H, s, $CH_3{\cdot}CO$).

A solution of this product (48 g) and anhydrous calcium chloride (22 g) in 2-ethoxyethanol (250 ml) was heated at 120° for 20 min and allowed to cool. When poured into water (500 ml) it gave the same dichloride (22 g), m.p. 122—125°, τ 2·5—3·5 (4H, q, aromatic), 6·35 (8H, s, $CH_2{\cdot}CH_2$), 7·34 (1H, s, NH), and 7·90 (3H, s, $CH_3{\cdot}CO$).

NN-*Bis*-2-*chloroethyl*-p-*phenylenediamine Hydrochloride*.—A solution of the preceding acetyl compound (4·3 g), in concentrated hydrochloric acid (40 ml), was boiled under reflux until t.l.c. ($CHCl_3$) showed absence of starting material (2 h). After dilution with water (50 ml) and neutralisation with solid sodium hydrogen carbonate, the mixture was extracted with ether (4 × 25 ml); the extracts were dried and mixed with saturated methanolic hydrogen chloride (*ca.* 10 ml). The precipitate was collected, dissolved in the minimum amount of cold methanol, and reprecipitated by addition of ether to give the amine hydrochloride (2·9 g) as

[25] B. Robinson, *J. Chem. Soc.*, 1965, 3336.
[26] J. I. DeGraw, L. O. Ross, L. Goodman, and B. R. Baker, *J. Org. Chem.*, 1961, **26**, 1933.

an almost white powder, decomp. 230°, which showed an i.r. spectrum (mull) identical with that of material prepared by the older method.[12]

p-(*Bis*-2-*hydroxyethylamino*)-N-*methylacetanilide*.— Reaction of *p*-amino-*N*-methylacetanilide, m.p. 70—71° (lit.,[27] 63°) (20 g) and ethylene oxide (42 g) in acetic acid (70 ml) and water (130 ml), under the conditions described for the *o*-phenol, gave, by extraction of the neutralised mixture with ethyl acetate, the diol (21 g), $\nu_{max.}$ 3400 and 1650 cm^{-1}, τ 3·2 (4H, q, aromatic), 5·39 (2H, s, OH), 6·18 and 6·42 (8H, two t, $CH_2 \cdot CH_2$), 6·82 (3H, s, $CH_3 \cdot N$), 8·2 (3H, s, $CH_3 \cdot CO$), and 7·8 (*ca.* 2H, impurity). It could not be purified, but was characterised as the *dibenzoate* (treatment with benzoyl chloride–aqueous sodium hydroxide), m.p. 141—143° (from ethanol) (Found: C, 70·3; H, 6·2; N, 6·0. $C_{27}H_{28}N_2O_5$ requires C, 70·4; H, 6·1; N, 6·1%).

p-(*Bis*-2-*chloroethylamino*)-N-*methylacetanilide* (25).—A solution of the crude diol (17 g) in pure chloroform (50 ml) was added slowly (90 min) to a solution of thionyl chloride (26 g) in chloroform (100 ml) at 60—70°. The temperature was maintained for a further hour, and the mixture was then cooled and neutralised with aqueous sodium hydrogen carbonate. The washed and dried chloroform layer on evaporation gave a dark oil which was purified by column chromatography (silica; ethyl acetate) to give the *dichloride* (5·4 g), m.p. 93—94° (from carbon tetrachloride–petroleum) (Found: C, 53·95; H, 6·2; Cl, 24·5; N, 9·6. $C_{13}H_{18}Cl_2N_2O$ requires C, 54·0; H, 6·2; Cl, 24·5; N, 9·7%).

When the diol (2 g) in chloroform (15 ml) was mixed at 0° with the reagent prepared by heating a mixture of thionyl chloride (2 g) and dimethylformamide at 70° for 1 h, the product (after 1 h at 0°), obtained by evaporation under reduced pressure and chromatography of the residual oil, was the *cyclic sulphite* (28) (0·5 g), m.p. 136—138° (from carbon tetrachloride–petroleum), τ 3·2 (4H, q, aromatic), 5·1—6·5 (8H, m, $CH_2 \cdot CH_2$), 6·8 (3H, s, $CH_3 \cdot N$), and 8·15 (3H, s, $CH_3 \cdot CO$) (Found: C, 52·05; H, 6·0; N, 9·2; S, 10·7. $C_{13}H_{18}N_2O_4S$ requires C, 52·3; H, 6·1; N, 9·5; S, 10·7%).

NN-*Bis*-2-*chloroethyl*-N′-*methyl*-p-*phenylenediamine* (26).—A solution of the preceding *N*-acetyl dichloride (6 g) in concentrated hydrochloric acid (50 ml) was boiled under reflux for 3 h, then cooled, diluted with water (200 ml), neutralised with powdered sodium carbonate, and extracted with ether (3 × 100 ml). The dried extracts were treated with methanolic hydrogen chloride, and the precipitate was recrystallised from cold methanol by addition of ether, to give the *amine hydrochloride* (4·5 g), m.p. 150—160° (Found: C, 46·5; H, 6·1; Cl, 37·4; N, 9·7. $C_{11}H_{17}Cl_3N_2$ requires C, 46·6; H, 6·0; Cl, 37·5; N, 9·9%).

p-(2-*Methoxycarbonylethyl*)*phenyl* N-p-(*Bis*-2-*chloroethylamino*)*phenyl*-N-*methylcarbamate* (27).—*p*-(2-Methoxycarbonylethyl)phenylchloroformate (see Supplementary Publication) (1·4 g) was added to the preceding amine hydrochloride (1·5 g) and pyridine (0·5 g) in benzene (100 ml). The mixture was boiled under reflux for 1 h, then cooled, and washed successively with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and water. Evaporation then gave an oil, which was purified by column chromatography (silica; chloroform) to give the *urethane* (1·9 g) (Found: C, 58·5; H, 6·0; Cl, 15·5; N, 6·15. $C_{22}H_{26}Cl_2N_2O_4$ requires C, 58·3; H, 5·8; Cl, 15·6; N, 6·2%).

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