A Thesis entitled

SYNTHESES OF SOME ALKYLATING AGENTS WITH POTENTIAL CYTOTOXIC PROPERTIES

submitted by

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in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the faculty of science, University of London.

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January 1970.

A B S T R A C T

In the introduction, a review of biological alkylating agents is presented, including their application in the field of cancer chemotherapy. particular stress is laid on those agents which are derived from nitrogen mustards. The problems connected with the design of potentially cytotoxic compounds are briefly discussed.

Syntheses are described of a series of carbamates formed by interaction of p-(NN-di-2-chloroethylamino)phenol and isocyanates derived from the esters of (a) essential amino acids, and (b) aromatic amino acids. Three novel nitrogen mustard systems have been synthesised. They are, respectively, the meta and ortho analogues of p-(NN-di-2-chloro-ethylamino)phenol, and the monofunctional agent p-(N-methyl-N-2-chloroethylamino)phenol. In a number of cases, urethanes containing a methyl ester function were selectively hydrolysed to the corresponding free carboxylic acid, so that both ester and acid were available for comparative biological tests.

α-Halo-ketones are known enzyme inhibitors, and the syntheses are described of a series of compounds containing a carbamate bridge linking the amino group of p-aminophenacyl chloride to hydroxy-esters.

TO

ANNETTE AND DYLAN

ACKNOWLEDGMENTS

I would like to take this opportunity of expressing my deepest thanks to Professor L.N. Owen for his helpful encouragement during the course of this work, and for his patient guidance at all times, which was especially valuable whenever difficulties arose. I am indebted to Professor D.H.R. Barton for providing the opportunity to work in this department, and to Imperial College for awarding me generous financial support. My colleagues in the Armstrong Laboratory and in Room 99 deserve special mention for their friendship and helpfulness.

I wish to thank Ron Carter and his technical staff, and also the staff of the microanalytical department for their assistance during the past three years. I am very grateful to Mrs. M. Morris for typing the manuscript.

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INTRODUCTION

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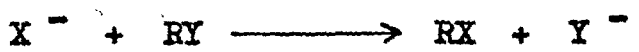
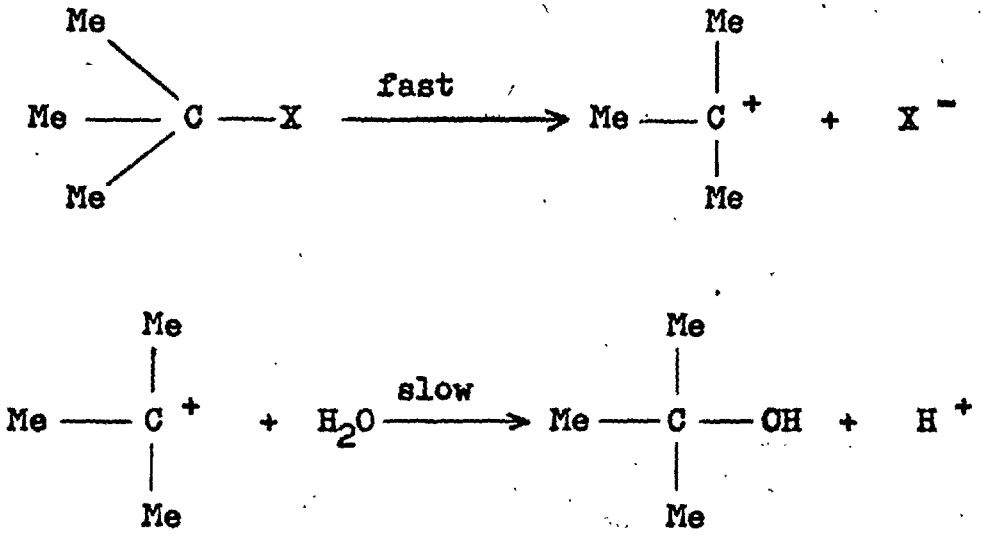
C H A P T E R 1

Biological Alkylating Agents

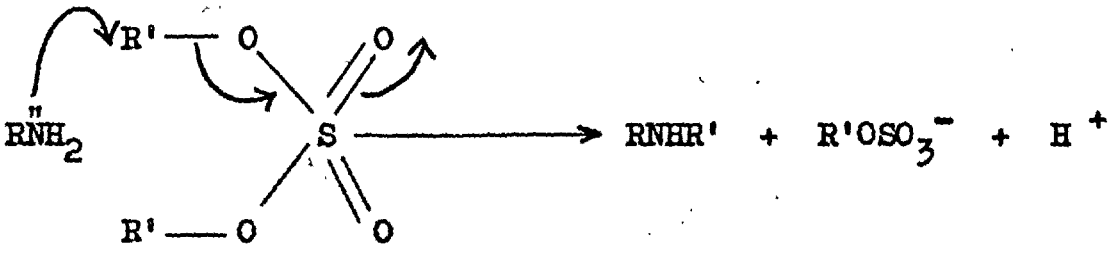
Of the many different types of organic compounds which have been found to show cytotoxic properties, the alkylating agents are a class of major importance. The replacement of a hydrogen atom in a molecule by an alkyl group, or the addition of the group to a molecule containing an atom in a lower valency state, is known as alkylation, and any compound which can effect this replacement or addition is known as an alkylating agent. Thus, in the process shown in fig. 1, RY is the alkylating agent, and we can consider that the entity Y becomes detached from the alkyl group to a greater or lesser extent during the course of the reaction. The resulting electron-deficient alkyl fragment will therefore seek the electrons of any available nucleophile.



(fig. 1)



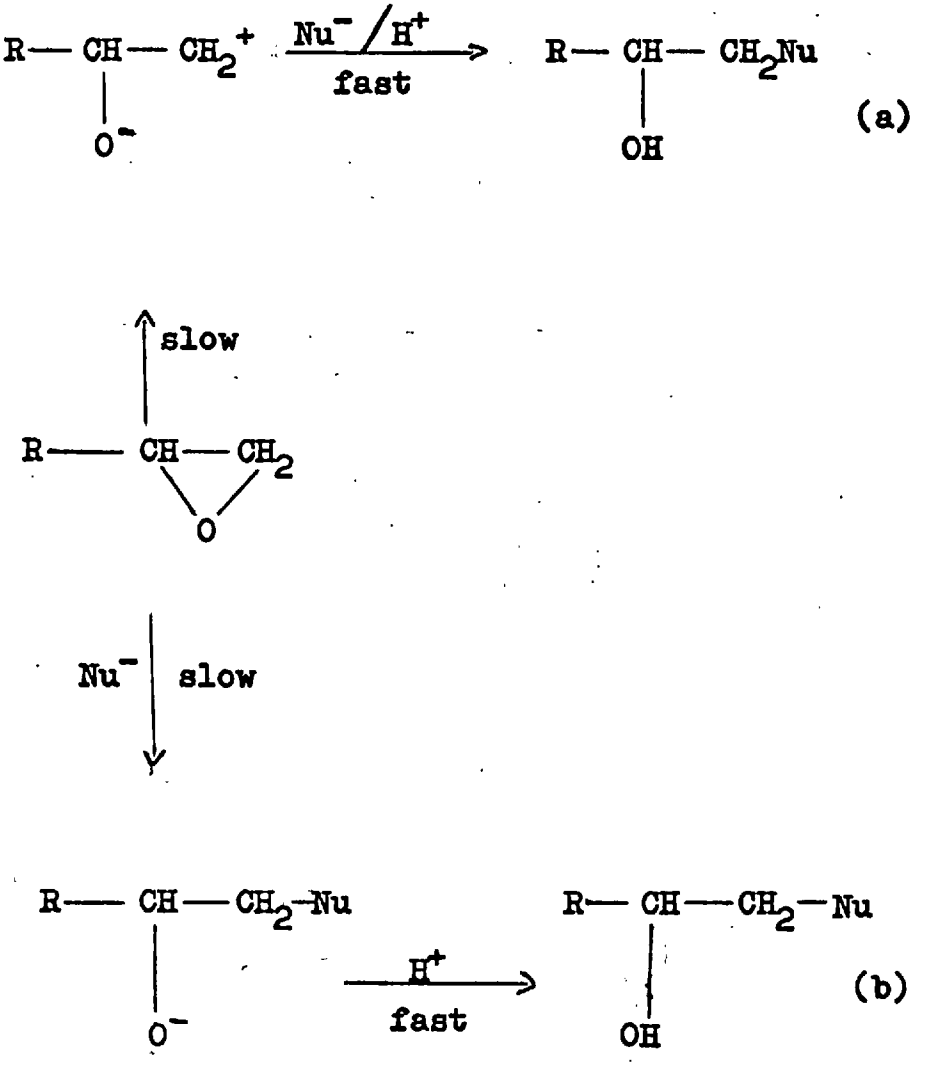
(fig. 2)



(fig. 3)

A typical group of compounds which are able to function under physiological conditions of temperature and pH, and in aqueous solution, are the alkyl halides. Of these, primary and secondary alkyl halides tend to react by an S_N2 mechanism, except in solvents of high dielectric constant, whereas tertiary alkyl halides tend to undergo S_N1 reactions. Consider, for example, the reaction of a tertiary butyl halide, shown in fig. 2. Such a compound will be of little interest as a biological alkylating agent, because the tertiary carbonium ion, once formed, is more likely to react with surrounding water molecules than with other functional groups. Also effective under very mild conditions in aqueous solution are the esters of sulphuric acids (fig. 3). Note that only one of the alkyl groups on the ester is available for efficient alkylation, since the mono alkyl sulphate anion, once formed, is much less likely to react with another nucleophilic centre.

Epoxides have been shown to be useful biological alkylating agents. They react with nucleophiles (Nu^-) in neutral solution by two possible mechanisms (fig. 4). Mechanism (a) is unimportant biologically, since, as mentioned earlier, the carbonium ion formed from an S_N1 ring-opening process is more likely to react with surrounding water molecules than with reactive nucleophilic centres. Such a

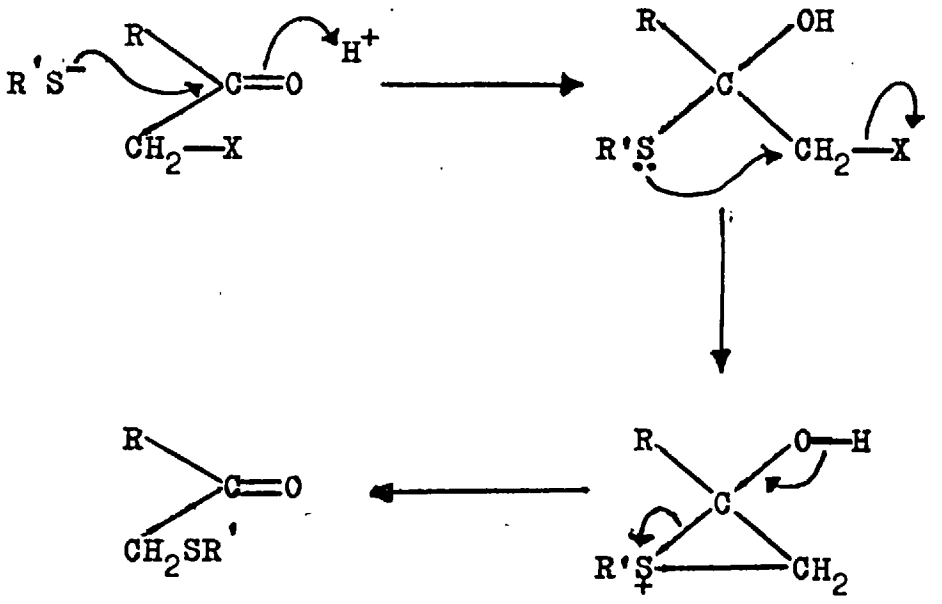

R— CH — CH2+
O-
Nu- / H+
fast
R— CH — CH2Nu
OH
(a)
slow
R— CH — CH2
O
Nu-
slow
R— CH — CH2—Nu
O-
H+
fast
R— CH — CH2—Nu
OH
(b)

(fig. 4)

mechanism will normally only operate on epoxides having a terminally substituted carbon atom. Those epoxides whose terminal carbon atoms are unsubstituted will tend to react by the S_N2 process outlined in mechanism (b), and in these cases the rate of reaction will be increased by the number of reactive nucleophilic sites present.

The attachment of an acyl group to the α-carbon atom of an alkyl halide provides an extremely effective alkylating agent, especially towards thiol groups. The enhanced reactivity is attributed to the strong attraction between the attacking nucleophile and the electron-deficient carbon atom of the carbonyl group (see fig. 5).

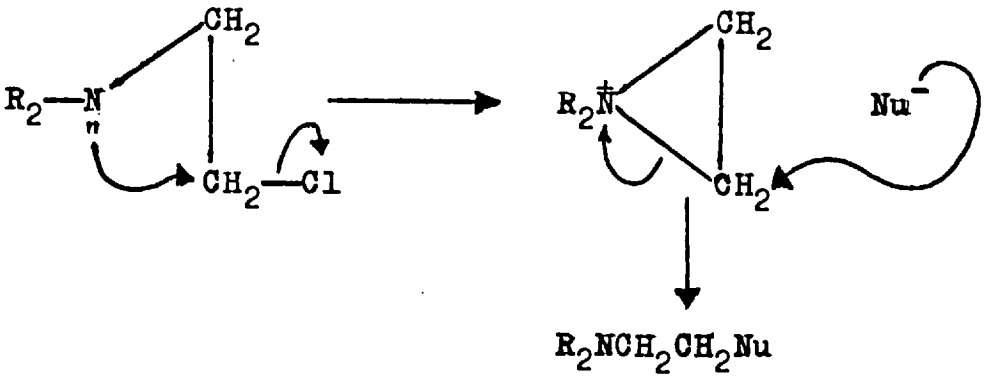
Perhaps the most widely studied of the biological alkylating agents are the class of compounds known as 'nitrogen mustards', of which two main types are most frequently encountered (1 and 2, where R=H, alkyl, or aryl). In aqueous solution, a typical aliphatic nitrogen mustard (1, R= Me) will initially undergo an intramolecular S_N1 cyclization to form an ethyleneimmonium ion (fig. 6), which will, in turn, react with a nucleophile Nu^- by an S_N2 mechanism.



(fig. 5)

$R_2NCH_2CH_2Cl$ (1)

$R—N(CH_2CH_2Cl)_2$ (2)



(fig. 6)

Because of their wide range of activity and because they have been studied so extensively, the 2-haloethylamines or 'nitrogen mustards' will be discussed later in greater detail.

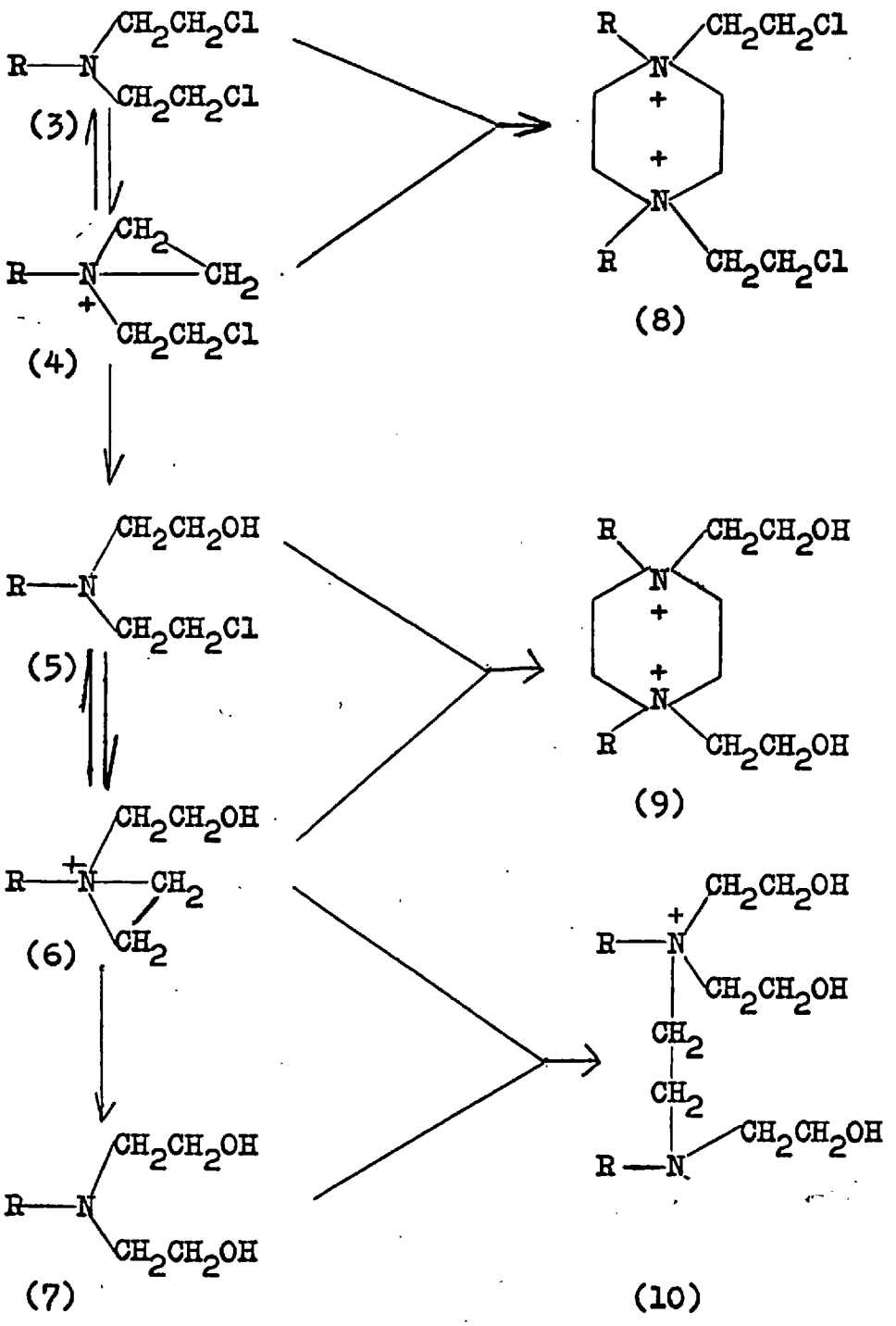
Other classes of compound that may possess alkylating ability in biological systems include chloromethyl ethers, ammonium compounds, 2-chloroethyl sulphides (sulphur mustards), and methanesulphonates. It was the wide variety of structures capable of acting as biological alkylating agents, that prompted Ross [1] to observe that it was unlikely that such compounds operate by a process of physical adsorption or competitive inhibition.

CHAPTER 2

Reactions of Nitrogen Mustards

With Water

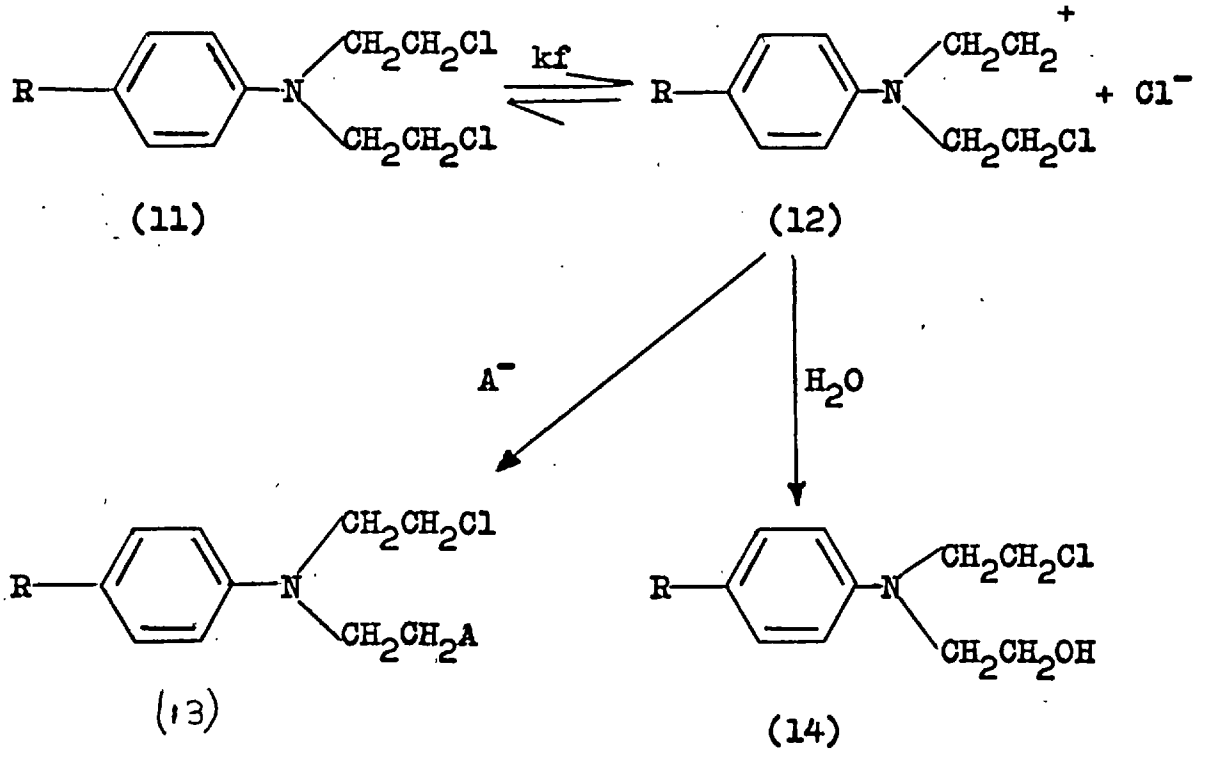
When methyl-bis(β-chloroethyl)amine (3, R=Me) is shaken with water it rapidly goes into solution, liberating one equivalent of Cl^- ion and a very small amount of H^+ ions. This observation is in accordance with the first step of the mechanism indicated in fig. 7 whereby the ethyleneimmonium ion (4) is formed. As the hydrolysis proceeds, both H^+ ions and Cl^- ions are liberated at about equal rates indicating both the formation of (5) and the quaternary cyclic compound (8); the latter has been shown to be formed to an appreciable extent in such solutions [2]. The formation of the analogous dihydroxy dimer (9) has been shown by Golumbic et al.[3] to occur in a bicarbonate-buffered solution of pH8. The increase in acidity of the reaction mixture during the hydrolysis suggests that the chlorohydrin (5) is stabilised due to salt formation. This is borne out by the fact that (5) is the major product of the reaction. The liberation of Cl^- ions does not exceed one equivalent, indicating that compounds (6) and (7) are not produced to any extent. In a solution buffered at pH8 however, the hydrolysis proceeds further to furnish the ethyleneimmonium ion (6) and finally the diethanolamine (7).



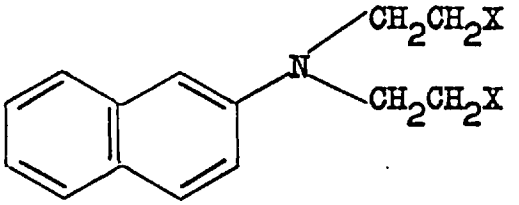
(fig. 7)

The reactions of aryldi-2-halogenoalkylamines in pure water have not been studied due to their low solubility, but aqueous acetone has been found to be an effective medium for such studies.[4] Hughes[5] demonstrated that the hydrolyses are S_N1 in character (fig. 8), but that as the reaction proceeds, the value of k_f gradually falls due to the increasing importance of the recombination of the ions. The addition of anions causes an increase in the velocity of reaction whereas the addition of chloride ions causes a large decrease in the rate.

Further evidence for the S_N1 nature of the reactions of aryl nitrogen mustards is furnished by the fact that the relative amounts of ester (13) and hydroxy-derivative (14) formed from the parent mustard (11) should be independent of the nature of the halogen atom provided that a carbonium ion of the type (12) is produced. This has been shown experimentally in the case of the naphthalene derivative (15). Moreover, the presence of electron donating substituents in the aromatic ring of (11), e.g. R=OMe, should increase the rate of an S_N1 hydrolysis, whereas those substituents that are electron attracting, e.g. R=CO_2Et, should inhibit the hydrolysis. Such has in fact been shown to be the case[6].



(fig. 8)



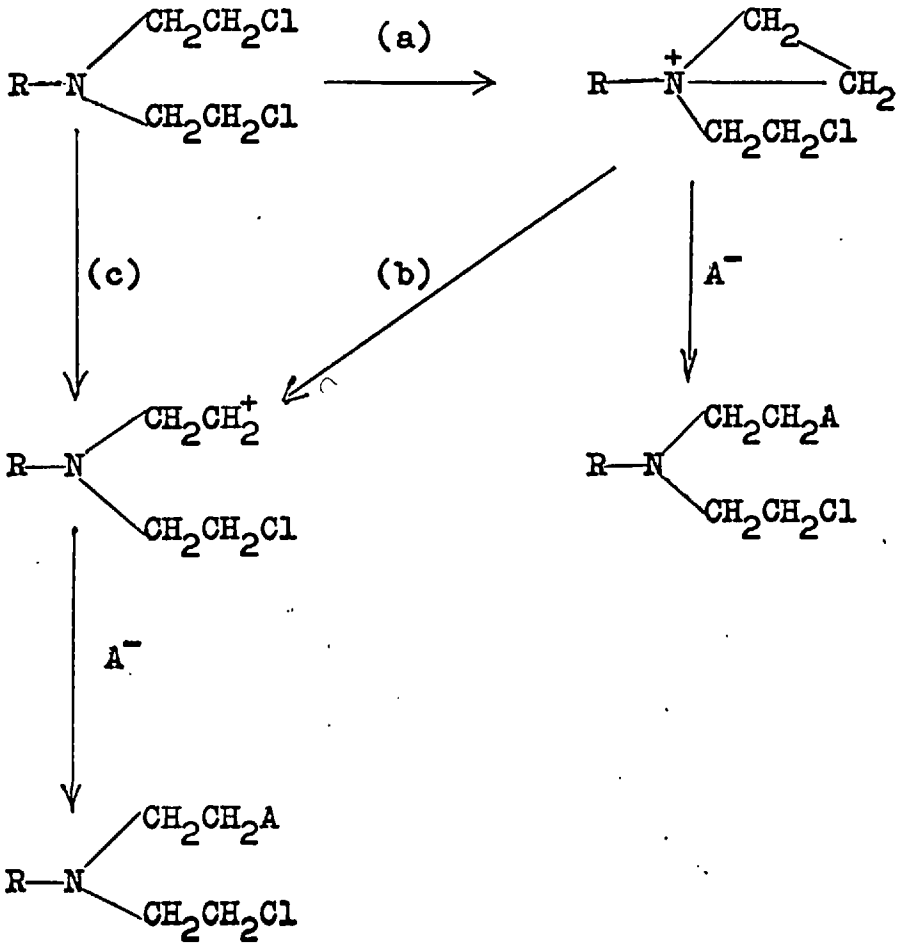
(X = Cl or Br)

(15)

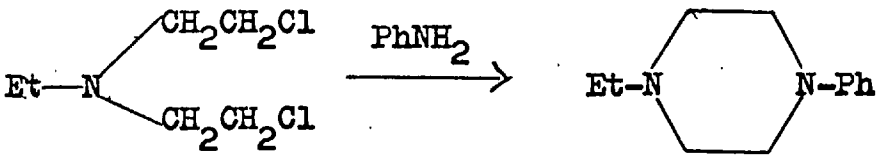
Since aromatic amines are much weaker bases than the aliphatic counterparts, one would imagine that the tendency of aryl halogenoalkylamines to cyclize to form ethyleneimmonium ions should be considerably less than it is in the case of analogous aliphatic compounds. In fact, no evidence for this process occurring has been found.

With Anions

When aliphatic nitrogen mustards are placed in a solution containing an anion, a substituted aminoethyl ester is formed. The reaction may be considered as taking place by one of the following mechanisms (fig. 9). Powerful nucleophiles will tend to follow path (c) whereas those anions derived from organic acids will probably attack after cyclization, since their electron-donating ability will be comparable with or less than that of the nitrogen atom. It has been found that esters derived from acetate and hippurate are not formed from the mustard (2, R=Me or Et) in slightly alkaline solution [7]. This is in accordance with the observations of Cohen et al.[8] that simple esters of methyl-di-2-hydroxyethylamine (7, R=Me) are easily hydrolysed. The reactivity of aryl-di-2-chloroalkylamines towards various anions has been studied by Ross [9] who showed that for a given


CH2CH2Cl
R—N
CH2CH2Cl
(a)
CH2
+
N
CH2
CH2CH2Cl
(c)
(b)
A−
CH2CH2+
CH2CH2A
A−
CH2CH2A
CH2CH2Cl

(fig. 9)


CH2CH2Cl
Et—N
CH2CH2Cl
PhNH2
Et-N
N-Ph

(16) (17)

series of anions, the order of reactivity was the same for both aromatic nitrogen mustards and di-2-chloroethyl sulphide.

With Bases

Prelog and Stephan[10] found that by heating an aliphatic nitrogen mustard such as (16) with an ethanolic solution of aniline, the 4-ethyl-1-phenyl piperazine (17) was formed. Similar products were obtained from aryl-di-2-halogenoalkylamines, the reaction being a good synthetic route for the preparation of NN-disubstituted piperazines[11].

Compounds of biological importance

When one wishes to consider the reactions of nitrogen mustards in biological systems, it is important to decide which functional groups in the systems will be nucleophilic under physiological conditions. The centres of high electron density which are likely to be encountered are, carboxylate anions, inorganic anions, e.g. phosphate, alkoxy ions, and thiolate anions, although amines and thioethers are also important. Whether or not a particular functional group is in a reactive form at physiological pH will obviously depend on the dissociation constant of that group. The two main classes of compound which are likely

to interact with alkylating agents in biological systems are proteins and nucleic acids, and in fig. 10. a selection of the more common functional groups in proteins and nucleic acids is presented, together with their pK's and the fraction (f) of the group which will be in its reactive form at pH 7.5.

Studies on the susceptibility of proteins to attack by alkylating agents have, for the most part, been concerned with di-2-chloroethyl sulphide, the extent of reaction being determined by noting the increase in the number of bound sulphur atoms, either by elemental analysis or by employing mustard gas containing radioactive sulphur. [12, 13, 14]. Among proteins proved to react with sulphur mustard are ovalbumin, serum proteins, insulin and gelatin, [15, 16, 17] while Fruton et al.[18] observed that methyl-di-2-chloroethylamine blocked amino groups in both egg albumin and gelatin. As is to be expected, enzymes are also inhibited by treatment with alkylating agents, and early work by Dixon and Needham[19] demonstrated that phosphokinases are particularly sensitive to such treatment.

Some early experiments on nucleic acids were performed by Gjessing and Chanuti[20] who established that

GROUP	pKa	f
PROTEINS		
α-Carboxyl	3.0-3.2	0.9999
Carboxyl (aspartyl)	3.0-4.7	0.9999-0.999
Carboxyl (glutamyl)	4.4	0.999
Phenolic hydroxyl(tyrosine)	10.4	0.001
Thiol(terminal cysteine)	7.9-8.4	0.1-0.06
Thiol(non-terminal cysteine)	10.8	5×10^{-4}
Imidazolinium(histidine)	5.6-7.0	0.99-0.76
Guanidinium	11.6-12.6	10^{-4}-10^{-5}
NUCLEIC ACIDS		
Primary phosphoryl	2.0	0.9999
Secondary phosphoryl	6.0	0.96
Aromatic hydroxyl(uracil,thymine)	10.2	0.002
Aromatic hydroxyl(guanine)	10.1	0.0025
Sugar hydroxyl	13	10^{-5}
Aromatic amino(guanine)	2.3	0.9999
Aromatic amino(adenine,cytosine)	3.7-4.2	0.999

(fig. 10)

methyl-di-2-chloroethylamine exerted a depolymerizing action on sodium thymonucleate, as evidenced by the fact that competing anions inhibited the effect. Investigations of this nature are considered further in the next chapter.

CHAPTER 3

Alkylating Agents and Cytotoxicity

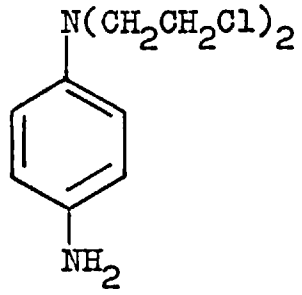
The outstanding systemic action of the nitrogen mustards and related alkylating agents is that which causes the death of cells. Guthrie[21] who first synthesised sulphur mustard in 1860 noticed its vesicant action, but it was probably Krumbhaar[22] who first appreciated the extent to which the hematopoietic system is affected by these compounds. The cytotoxic effect of alkylating agents, i.e. an effect in which cells are so severely damaged that they cannot survive, is in essence a more pronounced manifestation of their cytostatic effect, whereby cell-mitosis is delayed or inhibited, and the predominance of one effect over the other is probably determined more by dose rate than by the nature of the agent.

The observation that sulphur mustard, like X-rays, inhibited mitotic activity in the vaginal epithelium in mice, led Auerbach and Robson[23] to test the compound for other radiomimetic effects. The breakage of chromosomes, in the process of cell division, following treatment with relatively high doses of alkylating agents has frequently

been encountered, especially in plants containing a few large chromosomes in the cell[24, 25]. Rinaldini[26], in work concerned with p-amino-NN-di-2-chloroethylaniline (18), found that high concentrations of the mustard killed cells of frontal bone chick osteoblasts grown in culture medium, while at low dosages a cytostatic effect was exerted.

The relationship between the cytotoxic effects of alkylating agents and their potential usefulness in the treatment of neoplastic diseases was recognized in 1931 by Adair and Bagg[27] who demonstrated that the local application of sulphur mustard to superficial tumours in mice would induce regressions. Subsequent work established that several types of tumour in both rats and mice were susceptible to this mustard[28, 29], lymphoid tumours being particularly sensitive[30].

Although nitrogen and sulphur mustards have been the subject of most chemotherapeutic work in relation to cancer, other specific compounds have received attention. Triethylenemelamine (19) for example which is a cross-linking agent for wool has been tested and found to possess activity against mouse sarcoma and leukaemia[31, 32], and against the Walker carcinoma 256 [33]. Epoxides such as (20) have also proved effective inhibitors of the Walker 256 rat carcinoma, but only at toxic levels[34].



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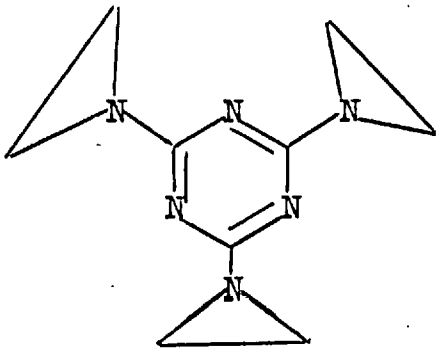

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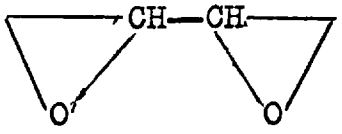
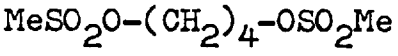


(20)

As mentioned in chapter 1, sulphonic esters of which (21) is representative are also effective alkylating agents, and their activity againt the Walker 256 carcinoma[35], and other solid tumours[36] has been demonstrated. The compound nitromin (22) first synthesised by Stahmann and Bergmann[37] in 1946 has been reported to have greater efficacy and less toxicity than its precursor, methyl-bis(2-chloroethyl)amine, when administered to rats with Yoshida carcinoma[38]. Moreover, the compound was found to have no vesicant action on the skin. The above remarks serve merely to illustrate the fact that biological alkylating agents have found, and are still finding, enormous application in the chemotherapy of cancer. Of perhaps more importance to those engaged in the design of new drugs, is an understanding of their fate and mode of action in vivo.

In 1949, Haddow[39], who thought that the cytotoxic activity of nitrogen mustards depended on the presence of at least two β-halogenoethyl groups in the molecule, put forward the hypothesis that one haloalkylamino group becomes anchored to a reactive centre of a protein fibre in a chromosome just before mitosis, while the second group approaches and reacts with another centre on the same protein chain or an adjacent one. Such bridge formation he concluded, could lead to chromosome fragmentation.



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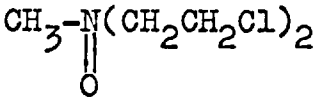

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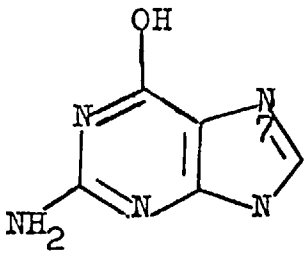
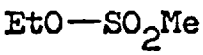


(23)

Since then, however, several alkylating agents possessing only one reactive group have been synthesised and found to possess both anti-tumour activity and the ability to effect chromosomal abnormalities[40, 41]. As a continuation of the studies on the connection between proteins and alkylating agents, Roberts and Warwick[42] administered ethyl methanesulphonate (24) to a rat and isolated the compound as derivatives of S-ethyl cysteine (25). It can be seen from fig. 10 that the thiol group in terminal cysteine units of protein will be more reactive towards alkylation than the thiol groups of glutathione or mid-cysteinyl units. That alkyl methanesulphonates will react <u>in vivo</u> with the thiol groups of cysteine was later confirmed by Roberts and Warwick[43] who administered Myleran (21) to rats, and isolated the drug as thiophen derivatives containing a cysteinyl sulphur atom.

The early work of Gjessing and Chanuti[20] on nucleic acids was pursued by Butler and co-workers[44] who showed that methyl-di-2-chloroethylamine at pH 7-9 destroyed the structural viscosity of thymus D.N.A., this being accompanied by a decrease in molecular weight[45]. Butler later observed that when D.N.A. was treated with the same nitrogen mustard, the molecular size decreased



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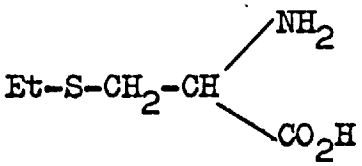
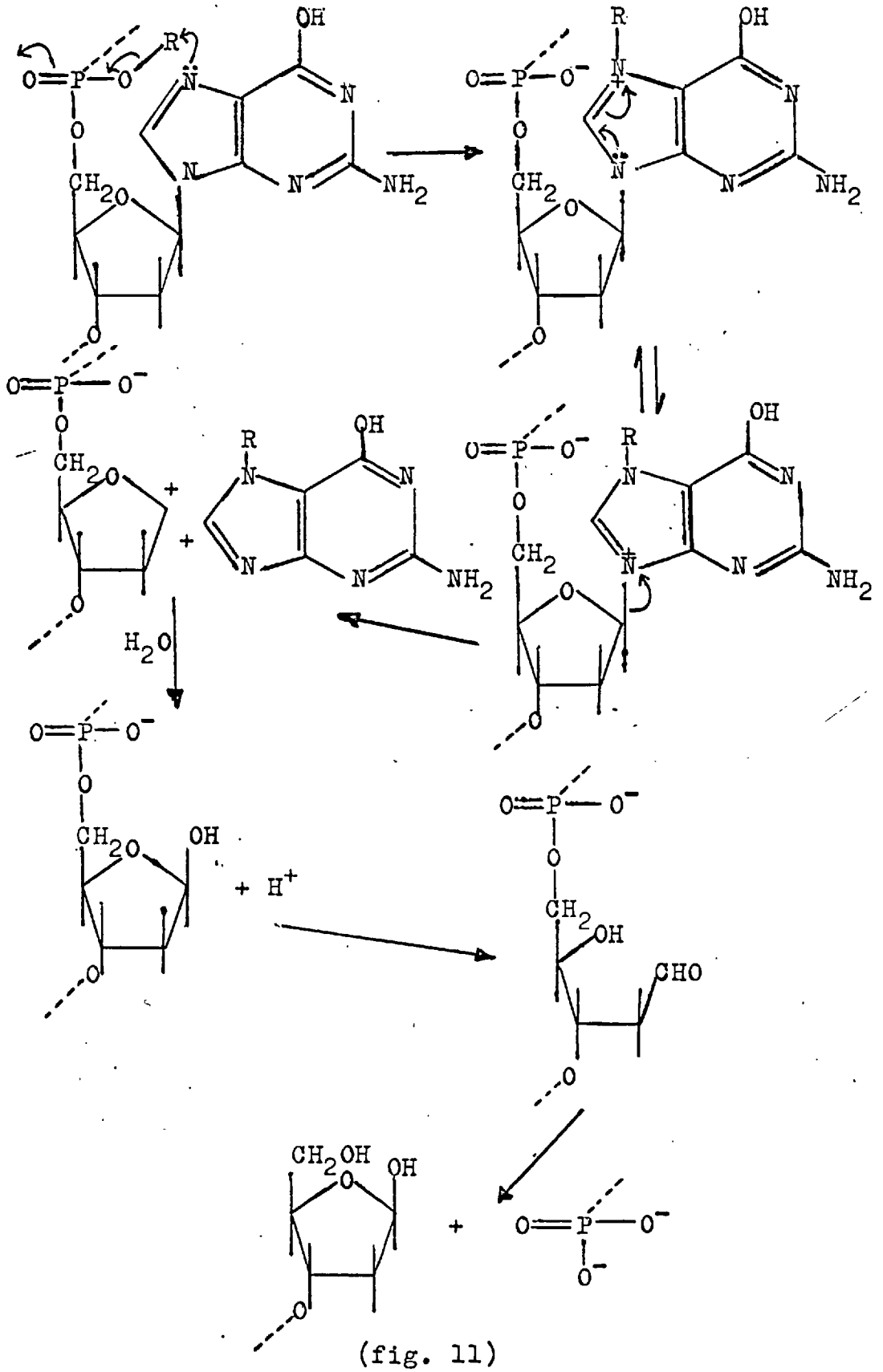


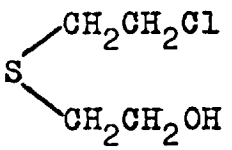
(25)

for a considerable time after the initial reaction[46]. The accompanying change in viscosity was dependent on this effect, and was ascribed to changes of interaction and configuration consequent on the breakage of hydrogen bonds owing to the alkylation of the primary amino groups in the bases. From a chemical point of view, a reaction with primary and secondary phosphoryl groups would be expected (see fig. 10), and indirect evidence for this has been obtained[47, 48]. In the Crick-Watson model of D.N.A., the N_7 position in the guanine moiety (23) has been shown to be the most nucleophilic site of those centres not involved in hydrogen bonding[49]. The reactivity of this nitrogen atom was established in 1961 by Brookes and Lawley[50] who alkylated guanosine with Myleran (21) and obtained 7-alkyl-guanines. Lett and Parkins[51] observed that the treatment of D.N.A. with nitrogen mustards resulted initially in decreased viscosity, due to molecular coiling, but not in decreased molecular weight. The molecular weight did, however, fall after the reaction mixture was allowed to stand at 37°. The post-reaction changes were explained by the hypothesis that the inital site of reaction in D.N.A. is the secondary phosphoryl group, and that the triester so formed alkylates the ring nitrogen atom in the purine base. The quaternized

purine splits off D.N.A. leaving a sugar phosphate residue. A plausible mechanism for the above sequence is presented in fig. 11. Further light on the problem was shed by Brookes and Lawley[52] in 1965 in a study of the effect of Myleran (21) and the monofunctional sulphur mustard (26) on protein, R.N.A. and D.N.A. in the liver, transplanted hepatoma, and leukaemic spleen of mice in vivo. All three cell constituents reacted, the product of (26) and nucleic acids being 7-(2-hydroxy etmylthioethylguanine). More recent work by Brookes and Lawley[53] has substantiated the idea that cellular D.N.A. is the most susceptible target to alkylating agents, one of their arguments being that strains of escherichia coli were inactivated by mustard gas at doses so low that on the basis of the number of alkylations per molecule, most of the smaller macromolecules such as R.N.A. and protein would escape alkylation, unless some considerable specificity of attack on particular macromolecules occurred, for which there is, as yet, no evidence.



(fig. 11)



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Side Effects of Alkylating Agents

While many biological alkylating agents have found clinical application in cancer chemotherapy, their usefulness is limited due to their high toxicity and the many deleterious side-effects to which they give rise. Hendry et al.[54] concluded that tumour inhibition was a manifestation of an action directed against dividing cells in general rather than against tumour cells in particular. The attainment of specificity, by means of structural modification and enzymic potentiation will be discussed later. Neurological disturbances which rapidly became fatal were observed when small animals were administered with large doses of methyl di-2-chloroethylamine(bis-nitrogen mustard). Both parasympathomimetic and paralytic phenomena were apparent[55], the former being related to the cyclisation of the first chloroethylamino group, whereas the latter phenomenon was attributed to the methyl β hydroxyethyl-ethyleneimmonium ion[56].

A reduction in the size of the spleen, in addition to serious bone-marrow effects, after the addition of the bis-mustard was observed (as early as 1947) by Kindred[57] who noticed that mitotic division of primitive

red or white blood cells was inhibited. Later, Osgood et al.[58] established that the cells which failed to divide in the bone-marrow, were actually killed.

Perhaps the most serious shortcoming of the biological alkylating agents is their tumorigenic action on body tissues. Boyland and Horning[59] in 1949 discovered that an injection of the bis nitrogen mustard may be followed after a considerable interval by the development of a tumour which may or may not appear at the site of injection. Such neoplasms may also appear in the lungs[60] or in a variety of areas[61]. Since then, Walpole et al.[62] using a variety of agents, have established the tumour-inducing role of these substances.

The relationship between nitrogen mustards and X-rays is demonstrated not only in their anti-carcinogenic behaviour but also in their harmful side-effects. As Koller[63] pointed out, the end results of heavy exposure to X-rays and to chemical agents of the nitrogen mustard series, are similar (e.g. skin blistering, whitening of hair, chromosome injuries, and induction of tumours) but the intermediate changes leading to these end-results differ for the radiations and the chemical compounds.

C H A P T E R 4

Drug Design

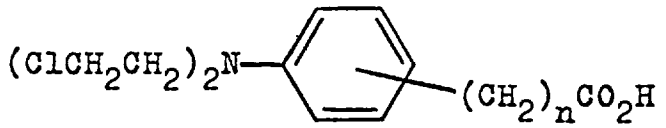
As has already been mentioned, most of the compounds so far described are highly toxic to both normal and neoplastic tissue. To achieve specificity of action coupled with low toxicity towards host cells is the major problem in the chemotherapy of cancer. The problem is made the more difficult by the fact that there are virtually no qualitative chemical differences between normal and neoplastic cells[64]. The recent discovery, however, that asparagine cannot be synthesised by cancer cells[65], indicates a line of research which may prove most profitable, since synthetically modified asparagine molecules may impair the metabolism of the amino acid in the proliferating cells.

Quantitative differences between healthy and neoplastic cells, do exist, and it is by exploiting these differences that much of the progress in the field of cancer chemotherapy has been achieved. The greater the number of variables on which a drug depends, suggested Danielli[66], the greater will be the selectivity of the drug. These variables will be discussed below in some detail.

A useful parameter for measuring the efficiency of a particular drug, has been its chemotherapeutic index (C.I.) which is generally taken to be the ratio of the median lethal dose to the minimum dose required to cause 90% tumour regression i.e. LD_{50}/ED_{90}.

Effect of Solubility

In order to increase the solubility of aromatic nitrogen mustards, Everett et al.[67] prepared a series of carboxylic acid derivatives (27). When n was equal to 0, the most effective tumour inhibitor was found to be the ortho-derivative, while the most effective compound in the whole series was found to be the one corresponding to n=3 with the carboxyl group in the para position. This drug, known as chlorambucil has been used extensively against chronic lymphocytic leukaemia[68], and ovarian carcinoma[69]. The carboxylic acids probably diffuse undissociated through cell membranes[70], and since the pKa of these acids in aqueous media is between 5 and 6, a considerable fraction of these will be in the anionic form. Thus the enhancement of reactivity, by conferring solubility on the mustards, is somewhat offset by their deactivation due to dissociation. As an example of this factor it may be mentioned that the derivative (28) is a tumour inhibitor whereas the more strongly acidic compound (29) is inactive[71].



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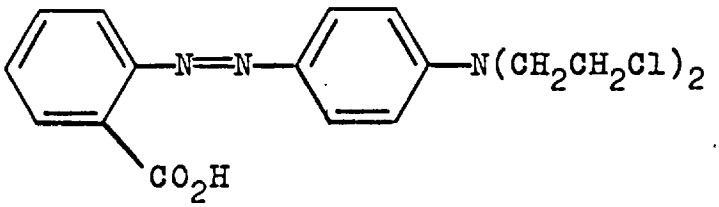

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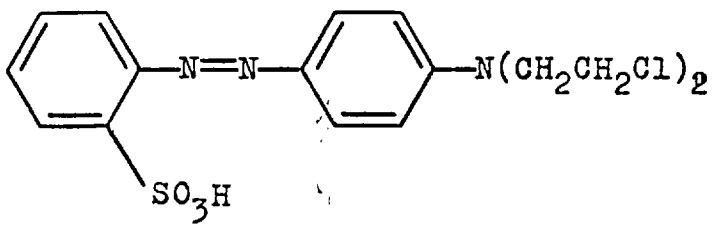
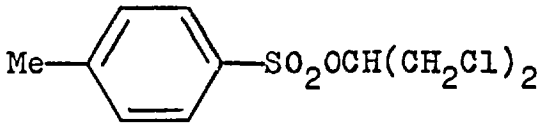


(29)

Effect of Electronic Charge

Although the anionic forms of the agents (27) are more reactive than the non-ionized forms, it has been shown that the latter will diffuse more easily through cell membranes. Moreover an equilibrium between the two forms will be set up inside the cell, so that the more reactive species will predominate. Thus, the conversion of the alkylating agent (30) into its carboxyl derivative (31) introduced a tumour inhibitory effect in the latter which was completely absent in the former[72].

Amine derivatives of nitrogen mustards which are sufficiently basic will be protonated at physiological pH, but again there will be an equilibrium between the two forms, the neutral species being the more readily diffused, and the more reactive, as in the case of compound (32), since a positive charge will have a deactivating effect on the mustard[73]. Cationic agents, however, will have the ability to interact with macromolecules of opposite charge. An example of the phenomena is illustrated by the drugs chloroquine and quinacrine whose protonated forms associate with D.N.A.[74], and are selectively concentrated within certain tissues[75]. Nitrogen mustard derivatives (33) and (34) of these compounds were synthesised[76] and tested biologically by Jones et al[77].



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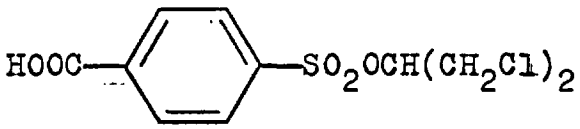

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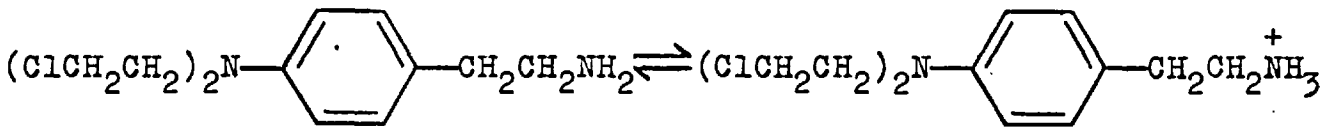

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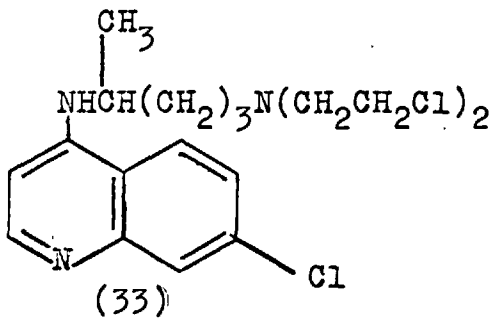

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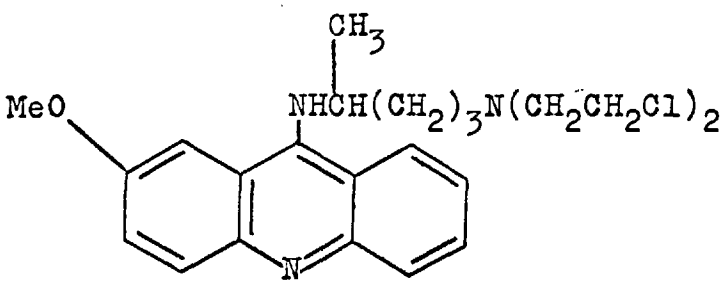
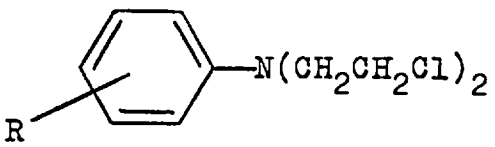


(34)

who found that they were capable of inhibiting the growth of some mouse ascites tumours and several rat leukaemias.

Effect of Varying Aromatic Ring System.

Aromatic nitrogen mustards of the general type (35) lend themselves readily to modification of the ring system by employing a variety of functional groups (R) in all three of the available substitution positions. Since aliphatic mustards are so highly toxic it was thought that some aromatic derivatives might be found which are sufficiently tumour inhibiting without being as hazardous as their aliphatic counterparts. A series of such compounds was studied by Haddow[78] and co-workers. Thus, for R=p-CHO, the compound (35) was found to be inactive against the Walker 256 carcinoma, whereas for R=p-CH_3CONH, the mustard (35) was found to be an effective inhibitor of the same tumour. For R=Me, all three isomers were found to be effective inhibitors, but the m-isomer was shown to have the least chemical reactivity, taking the percentage hydrolysis in ½ hr. in 50% acetone as a measure of chemical reactivity. The most successful of the compounds studied by these workers was the β-naphthylamine derivative (15, X=Cl) (page 17) which has received extensive trials against Hodgkins disease,



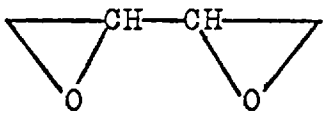
(35)

and chronic leukaemias of both myeloid and lymphatic origin[79, 80].

Influence of Functionality

In the case of epoxides, it has been established that difunctional derivatives may or may not have an anti-tumour action, whereas similar activity in the case of monofunctional derivatives has never been found. For example the diepoxide (36) has shown promise as a chemotherapeutic agent[81], whereas the mono-epoxide (37), having a greater chemical reactivity, has been found inactive biologically[82].

Methanesulphonates, as mentioned in chapter 1, can function as alkylating agents and a typical example is afforded by ethyl methanesulphonate (24) which, at a daily dose of 100 mg. per kg., will inhibit the growth of the Walker tumour. The chlorinated derivative (38) is, however, far more biologically active and is to be regarded as difunctional, since it reacts *in vivo* with cysteine to produce 2-chloroethylcysteine[83] (39). Both compounds have been reported by Fahmy and Fahmy[84] to produce mutagenic effects in Drosophilia. The lack of tumour-inhibiting power of (39) is probably due to its high reactivity,



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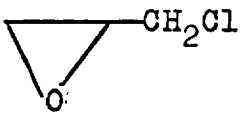


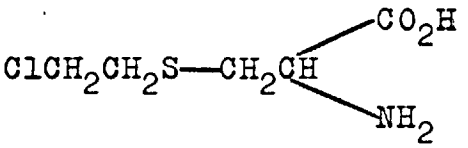
(37)

$EtOSO_2CH_3$

(24)

$ClCH_2CH_2OSO_2Me$

(38)



(39)

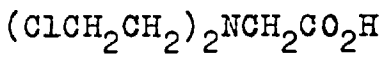
and therefore the effectiveness of (38) implies that it must diffuse into a neoplastic cell prior to its reaction with cysteine. It has been noted earlier that monofunctional nitrogen mustards, while displaying mutagenic effects were not in the past considered capable of tumour inhibition, but that later experiments conducted by Biesele et al.[40] and Loveless and Ross[41] disproved this hypothesis. It is however clear that difunctional nitrogen mustards are in most cases more effective anti-carcinogenic agents than their mono-functional counterparts, although recently there have been cases of monofunctional compounds having higher cytotoxicity[85].

Effect of Active Transport

Any feature in the structure of a potentially cytotoxic compound which will assist its diffusion through the cell membrane, should enhance its activity, since it is generally accepted that such diffusion is a necessary process prior to the occurrence of a biologically significant reaction. It has even been suggested recently that differences between normal and neoplastic cell membranes exist, and may facilitate the attack of active agents through the latter[86]. Much work has been done, therefore, in incorporating alkylating moieties in structures which are

important in cell metabolism, such as amino acids, carbohydrates, and steroids, since it is known that processes of active transport into cells are normally available to these compounds[70].

Derivatives of glycine (40) and alanine (41) were prepared[87] and although not capable of active transport, strictly speaking, (41) was claimed to induce remissions in various human leukaemias[88]. S-2-Chloroethylcysteine (39) which is capable of active transport has no tumour-inhibitory power as has already been stated, although it can exert a mutagenic action in Drosophilia. The most successful compound in this class has been the L-phenylalanine derivative (42) known as melphalan. An intraperitoneal administration at a dose of 1 mg. per kg. caused a complete inhibition of the growth of the Walker rat carcinoma[89]. The o-isomer of the DL-derivative (merphalan) is even more active and has been found to cause 80% inhibition of the same carcinoma at a single dose of 0.5 mg. per kg. using the identical method of administration. Other compounds in this class which have been tested for their anti-carcinogenic ability are derivatives of serine[90], threonine[91], and tyrosine[92].



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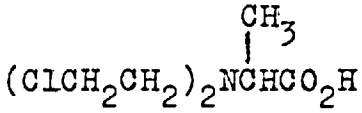

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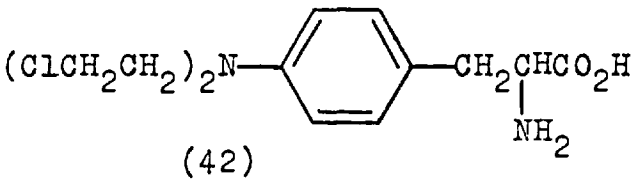


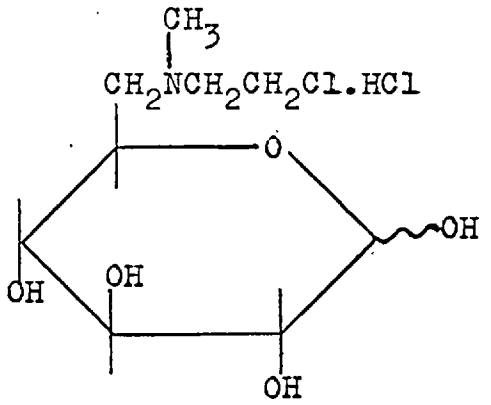
(42)

There have been many syntheses of carbohydrate derivatives containing nitrogen mustard groups, an effective example of these being the monofunctional glucose compound (43) which showed considerable activity against the L1210 leukaemia[93]. Benn and Owen[94] prepared the aromatic mustard derivative (44) which should be less toxic than aliphatic analogues.

A few steroid nitrogen mustards have been synthesised and tested but they have not produced any outstanding results[95]. The work described in this thesis has made use of the principle of active transport in combination with the idea of enzymic activation, a concept which will be outlined below.

Latent Activity

Rapidly growing cells which are actively synthesising proteins and nucleic acids are especially sensitive to alkylating agents. Thus, if one can relate the extent of alkylation to the enzyme make-up of the cell, one should be able to increase the specificity of action. Complete specificity could be obtained by administering a drug which was ineffective itself but which could be transformed in



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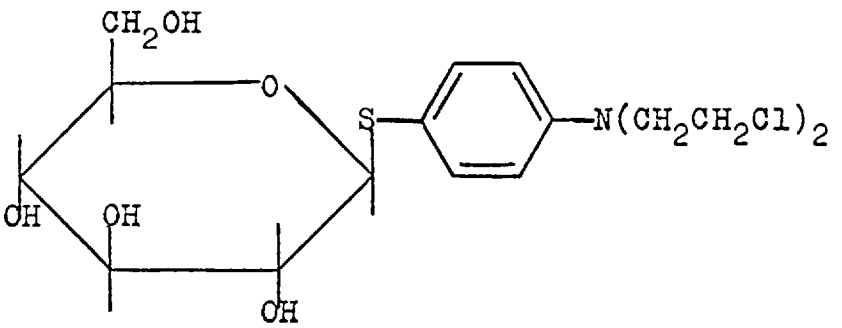
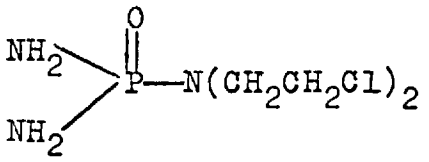


(44)

neoplastic cells to a reactive form by means of an enzyme present only in the neoplastic tissue. An important further point is that the cytotoxic form of a drug once potentiated should react rapidly at the site of its formation, for low reactivity and high permeability will render it ineffective. It must be stated that this ideal case has never be attained, and yet the exploitation of those differences in levels of enzyme activity that do exist, between healthy and neoplastic cells, has afforded considerable progress in the field of cancer chemotherapy.

Early attempts in this direction were based on the report that phosphoramidase activity was higher in malignant tumours than in healthy tissues[96]. Accordingly, the tri-amide (45) was prepared[97], which had one seventh the toxicity of di-2-chloroethylamine, but for which no useful chemotherapeutic properties were reported. More recently, a successful compound of this type (46) was prepared by Ross et al.[98]. Enzymic hydrolysis of this compound would give rise to the cytotoxic aromatic mustard (47), and in fact the drug was found to exhibit anti-tumour activity. Endoxan (48), a further example of an effective agent in this class, liberates bis(β-chloroethyl)amine by



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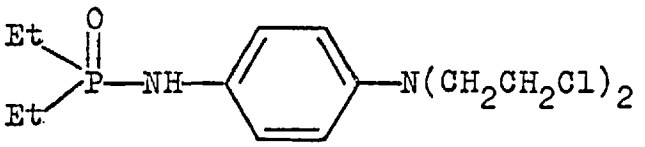

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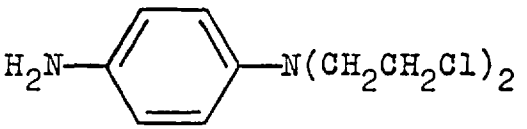

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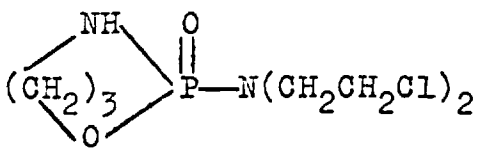
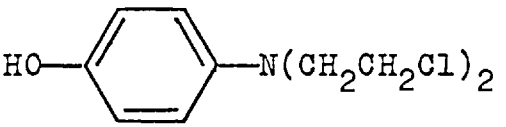


(48)

enzymic hydrolysis[99]. Gerhartz[100] in a review of its application concluded that the compound showed relatively superior action of the lymphatic reticulum and the plasma-cell reticular systems.

The phenolic nitrogen mustard (49) is tumour inhibitory but also highly toxic to host tissues. The formation of the ester (50) greatly reduces the reactivity of the parent mustard to which it can be converted <u>in vivo</u> by a process of enzymic hydrolysis, such enzymes having been found in the Walker carcinoma[101]. That this type of enzymic activation enhances the anti-tumour effect of alkylating agents is demonstrated by the fact that the chemotherapeutic index of (50) is far higher than that of (49).

Another group of compounds studied from the point of view of enzymic activation, were amides based on di-2-chloroethylamine. Acylation of the nitrogen atom reduced its electron density and hence its ability to form the reactive ethyleneimmonium ion. The derivatives, of which (51) is a general example (R=alkyl or aryl), were prepared by Ross and Wilson[102] and they are unlikely to be biologically active unless they can be hydrolysed back to the parent nitrogen mustard. Evidence for this process



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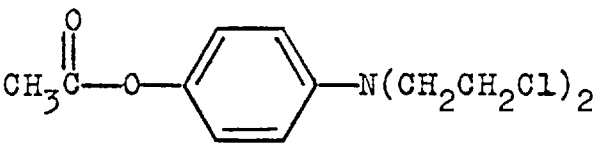

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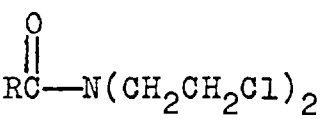
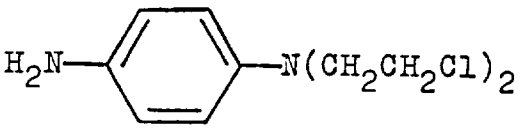


(51)

occurring was shown by the fact that a number of the compounds were tumour inhibitory, in particular the derivative with R=CF_3 which was seen to induce complete inhibition of the Walker rat carcinoma when administered intraperitoneally as a single dose of 250 mg. per kg. in arachis oil. Acylation of the primary amino group of the p-phenylenediamine derivative (47) which is highly reactive, had the same effect of reducing its chemical reactivity and toxicity, compounds of the form (52) being produced. As an example, the agent having R=Me was found to be anti-carcinogenic and to have a higher chemotherapeutic index than that of its precursor (47)[101].

Drug Resistance

Closely related to the concept of latent activity, for the cancer chemotherapist, is the phenomenon of adaptive resistance of tumour cells to administered drugs. In the case of alkylating agents it is probable that resistance involves the ability to prevent sufficient alkylating potential from reaching the cell nucleus. Another possible mechanism for resistance could be the synthesis of detoxifying enzymes by the neoplastic cell. Danielli[103] suggested that the development of resistance in tumour cells might be exploited in the design of more



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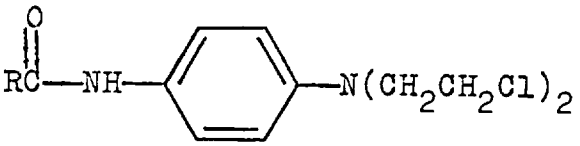
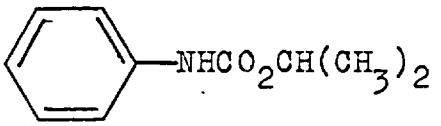


(52)

specific drugs. He later found that the Walker tumour readily develops a resistance to urethane[104], and after administering the aromatic urethane (53) to a rat carrying a Walker tumour, he demonstrated the presence of an enzyme, in the tumour, capable of liberating aniline from the derivative[105]. This was followed by the administration of the nitrogen mustard urethane (54), synthesised by Benn et al.[106]. The results showed that in terms of complete regression of tumour, the combination of (53) followed by (54) was eight times more effective than the application of (53) alone. Moreover, on no occasion did the application of (54) alone cause complete regression. Since then numerous studies on urethane derivatives of alkylating agents has been carried out. Duvăz[107] in 1966 examined a series of compounds of the type (55) for anti-tumour activity, and found the most effective derivative to be when X= -N=N-. The simple urethane (56) was also found to inhibit the Walker carcinoma[108].

By combining the concept of enzymic potentiation and the idea of incorporating 'biologically significant' compounds into alkylating agents, Benn et al.[106] synthesised the nitrogen mustards (57) and (58). Enzymic fission of the carbamate linkages would give rise to the cytotoxic aromatic mustard (49) plus glycine in the case of (57) and



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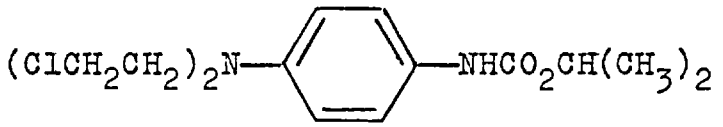

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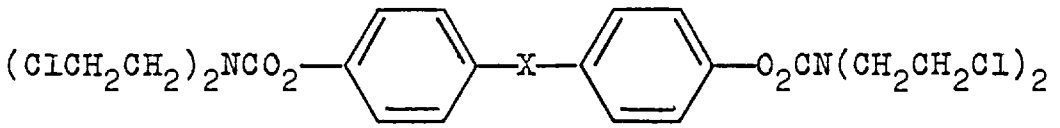

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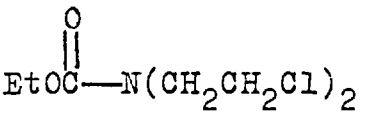

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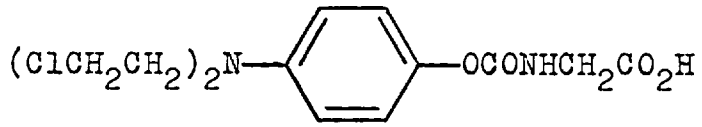

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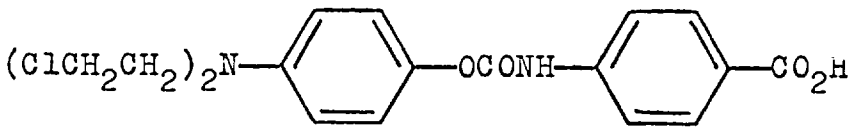


(58)

p-aminobenzoic acid in the case of (58). Further enhancement might be expected due to the solubilizing effect of the carboxyl group in both cases. The derivative (58, I.C. 140) has in fact shown great promise as a chemotherapeutic agent. Even without prior potentiation with a model urethane, I.C. 140 causes a high proportion of complete regressions of the Walker 256 carcinoma, in doses which are one tenth of the lethal dose[109].

The experimental work described in this thesis may be considered as an extension of the studies carried out by Benn *et al.*[106], and several new compounds containing urethane linkages will be described.

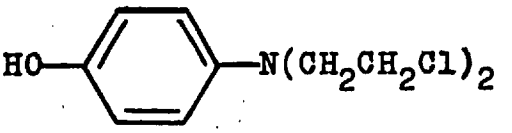
CHAPTER 5

Carbamates derived from p-(NN-di-2-chloroethylamino)phenol and some essential amino acids

The p-mustard phenol (49) is a known inhibitor of the Walker rat carcinoma, but its high toxicity limits its clinical usefulness. As has been stated in the preceeding chapter, urethane derivatives of (49) have proved useful chemotherapeutic agents, since Walker tumours have been shown to develop resistance to urethane[104]. The conversion of (49) into a carbamate has, therefore, a two-fold advantage. The toxicity of the mustard is greatly reduced, due to the decrease in electron density of the nitrogen atom, and, furthermore, the carbamate linkage can be enzymatically hydrolysed to the parent mustard phenol (49) by the prior administration of a model urethane.

It was considered important to develop the work initiated by Benn *et al.*[106], who incorporated the essential amino acid glycine as a urethane derivative (57) of the p-mustard phenol (49). Enhanced chemotherapeutic activity should be observed for such a compound from the point of view of both active transport mechanisms and the solubilizing effect of the carboxyl group.

p-(NN-Di-2-chloroethylamino)phenol (49) was synthesised starting from p-(NN-di-2-hydroxyethylamino)phenyl



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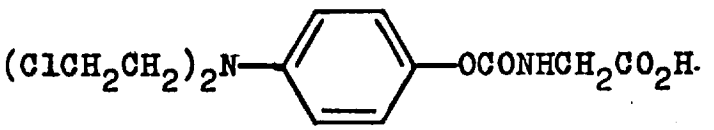
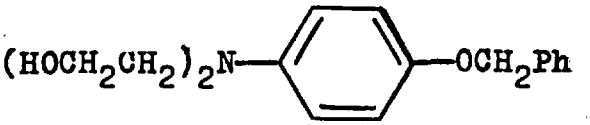


(57)

benzyl ether (59) by a modification of the procedure employed by Benn et al.[106]. The diol (59) was treated with phosphorus pentachloride to afford the dichloro-derivative (60). In order to convert the benzyl ether (60) into the hydrochloride of the desired mustard phenol (49), Benn et al.[106] subjected the hydrochloride of (60) to catalytic hydrogenation. It was felt that the debenzylation reaction might be achieved more easily by refluxing the ether (60) with a mixture of concentrated hydrochloric acid and glacial acetic acid. Such was found to be the case, and treatment of the reaction product with hydrogen chloride gave a high yield of the hydrochloride of the p-mustard phenol (49).

The first amino acid chosen for the series was DL-phenylalanine. This was esterified with methanol and hydrogen chloride according to the method of Fischer and Suzuki[111]. Treatment of the ester hydrochloride with phosgene after the manner of Siefken[112] afforded the isocyanate (61) as a colourless liquid. The isocyanate (61) was subsequently condensed with the hydrochloride of p-mustard phenol (49) using triethylamine as a catalyst, the product being the desired carbamate (62). In order to obtain the free carboxylic acid (63), the methyl ester (62) was refluxed in a 1:1 mixture of glacial acetic acid



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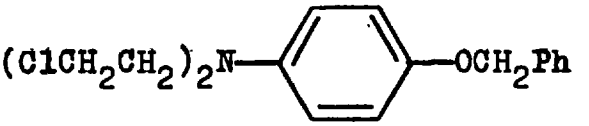

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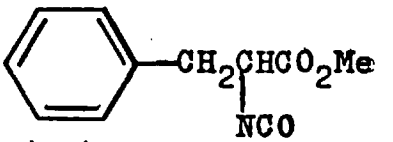

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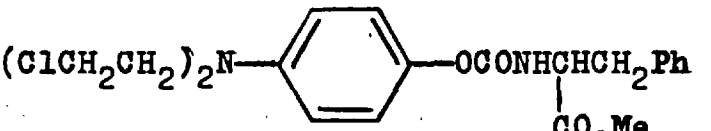

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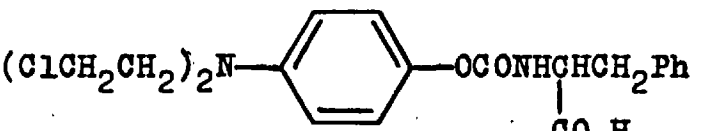
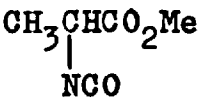


(63)

and concentrated hydrochloric acid. Creighton et al.[113] have found this medium to be very effective for the specific hydrolysis of urethanes containing methyl ester groups.

DL-Alanine was converted into its methyl ester hydrochloride under the same conditions that were employed for DL-phenylalanine. The reaction between the methyl ester hydrochloride and phosgene, however, afforded only a poor yield of the isocyanate (64). It was thought that the yield of (64) might be improved by adopting a synthetic route initiated by Iwakura et al.[114] who converted α-amino acids (65) into their corresponding 2-isocyanato-acyl chlorides (66) by the use of phosgene and hydrogen chloride. The derivatives (66) were subsequently esterified with an ethereal solution of ethanol to form compounds of the type (67), thus indicating that the acid chloride group is more reactive as an electrophile than the isocyanate group. The proposed mechanism for the sequence of reactions is illustrated in fig. 12.

Accordingly, DL-alanine was converted into its 2-isocyanatoacyl chloride (66, R=Me) in 64% yield. Treatment of this, however, with anhydrous methanol in ether gave rise to two products, the major one (51%) being the required isocyanate (64) which was characterised



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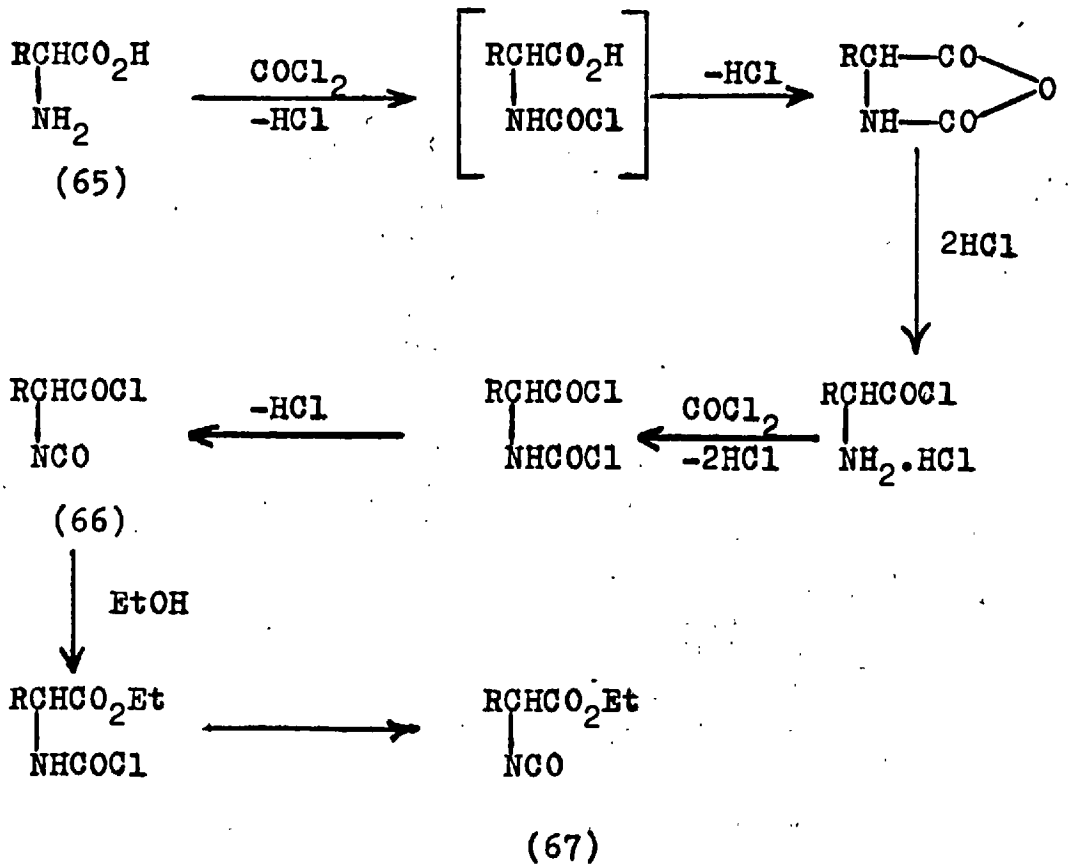
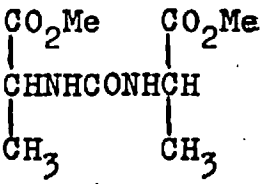


(65) (66) (67)

(fig. 12)

as the symmetrical urea (68). The isocyanate (64) was condensed with the hydrochloride of p-mustard phenol (49) to afford the desired urethane derivative (69).

DL-Methionine was esterified in the usual way using methanol and hydrochloric acid. It was not, however, found possible to isolate the ester hydrochloride as a crystalline solid. Treatment of the viscous ester hydrochloride with phosgene in toluene did not give rise to any of the desired isocyanate (70) but afforded only a charred residue, since effective stirring of the ester in toluene was not achieved. Again, the procedure of Iwakura et al.[114] was adopted. DL-Methionine was converted into its 2-isocyanatoacyl chloride (71) using the conditions that were applied to DL-alanine. It was observed, however, that the product (71) rapidly darkened on storage. Such rapid decomposition precluded the route as being synthetically viable and so it was abandoned. It was felt that the usual method of preparing isocyanates from the esters of amino acids should be possible in the case of DL-methionine provided that the methyl ester hydrochloride was neutralised to give the free base, which is soluble in toluene. The isolation of the free methyl ester of DL-methionine was carried out and after reacting this with a solution of phosgene in toluene,



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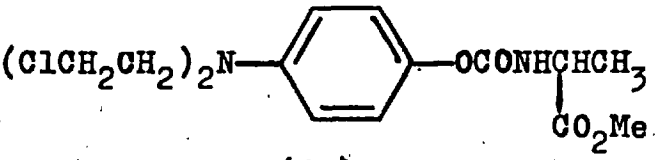

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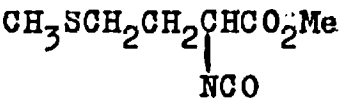

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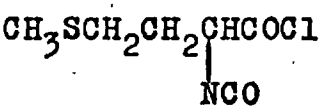
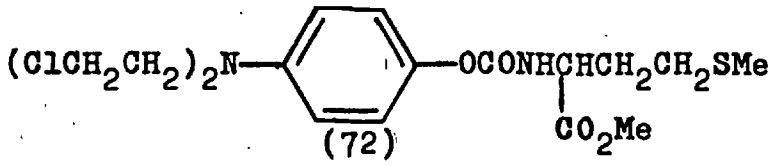


(71)

the isocyanate (70) was obtained in 65% yield. The hydrochloride of p-mustard phenol (49) and the isocyanate (70) were condensed to afford the carbamate (72) which was hydrolysed with a mixture of hydrochloric acid and glacial acetic acid to yield the carboxyl derivative (73).

Ethionine is a known inhibitor of methionine in biological systems, a phenomenon which is readily understood by considering the close structural similarity between the two compounds. On this basis alone it would seem worthwhile to incorporate the ethionine moiety into a urethane derived from the nitrogen mustard (49), since enzymic activation would liberate not only the cytotoxic mustard phenol (49) but also a molecule which is capable of interfering with methionine metabolism in neoplastic cells. Even more important, it has been reported by Burchenal et al.[115] that ethionine itself can inhibit the growth of the Jensen carcinoma in rats.

Accordingly, DL-ethionine was esterified in the usual way to give the methyl ester hydrochloride which was subsequently neutralised with ammonia solution. The methyl ester was reacted with phosgene to yield the isocyanate (74) as a pale-yellow liquid. A condensation between the isocyanate (74) and the hydrochloride of p-mustard phenol (49) yielded the urethane (75). The usual acid hydrolysis was then carried out to afford the free acid derivative (76).



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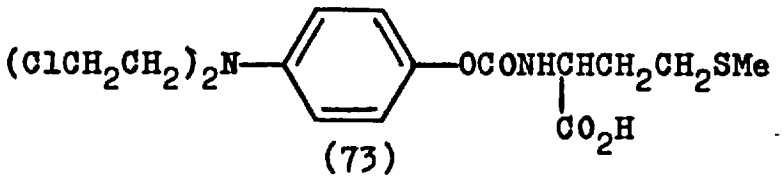

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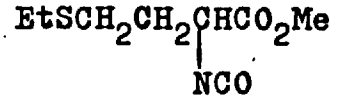

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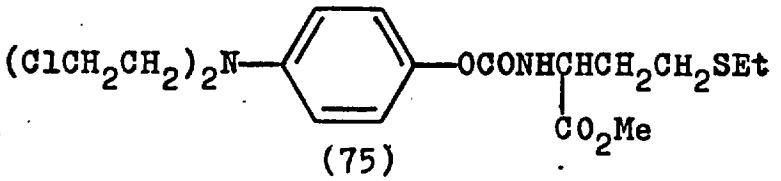

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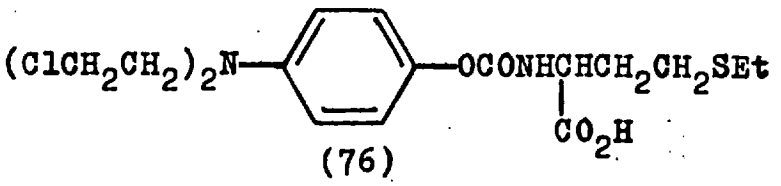


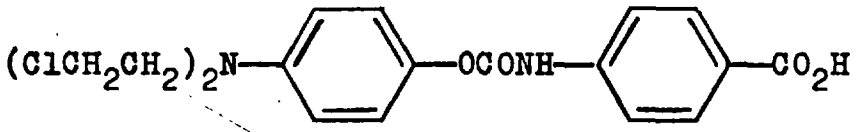
(76)

CHAPTER 6

SOME DERIVATIVES OF AROMATIC AMINO ACIDS

In the introduction it was stated that the nitrogen mustard (58), synthesised by Benn et al.[106] has proved an extremely effective agent for the regression of neoplastic tumours. Its chemotherapeutic index, when measured against the Walker 256 carcinoma in rats, was found to be 17. As there seems to be no reason why the most effective position for the carboxyl group should be para to the carbamate linkage, it was considered important to synthesise ortho and meta analogues. In this connection, it should be recalled that for the series of compounds (27), synthesised by Everett et al.[67], the most effective isomer, when n was equal to zero, was found to be the ortho derivative.

It has hitherto been assumed that agents possessing free carboxyl groups should show enhanced tumour inhibitory action, due to the solubilizing effect of the carboxyl group, but that this effect may be somewhat offset by the dissociation of carboxyl derivatives into carboxylate anions. Recent results of tests carried out on the urethanes (77) and (78), synthesised by Sridhar[116], have shown that the chemotherapeutic index of the ester (77)



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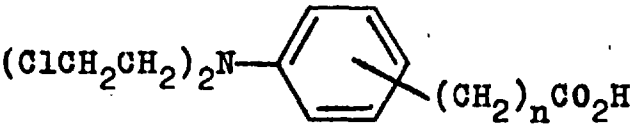

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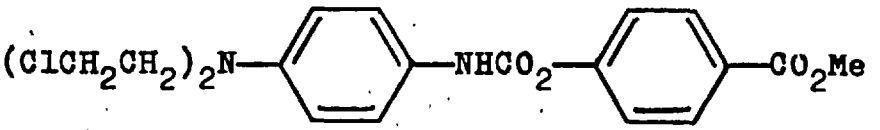

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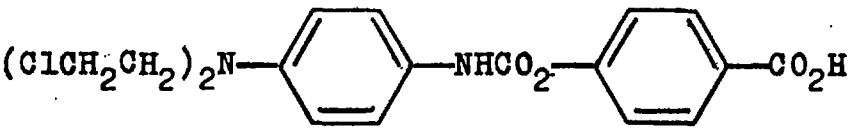
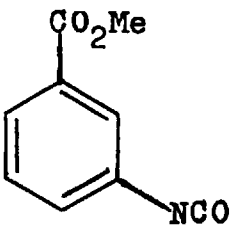


(78)

is three times higher than that of the free acid (78)[110]. Thus, it would appear that for these two compounds at least, the deactivation arising from ionization far outweighs any advantage to be gained from high solubility of the drug.

Methyl m-aminobenzoic acid was converted into its methyl ester hydrochloride in the usual way and treatment of this with phosgene, according to the method of Siefken[112], afforded the isocyanate (79). A condensation between the isocyanate (79) and the hydrochloride of p-mustard phenol (49) yielded the carbamate (80). By applying the normal acid hydrolysis, the urethane was converted into the free acid (81). It will be interesting to see whether or not the relative merits of the ester (80) and carboxyl derivative (81) follow the same pattern that was found for the compounds (77) and (78), which had chemotherapeutic indices of 131 and 46 respectively when measured against the Walker 256 carcinoma in rats.

To prepare ortho-analogues, methyl anthranilate was converted into the isocyanate (82) by the usual method. The isolation of the product, however, proved difficult on several occasions, since, on distillation, a high degree of polymerisation occurred. When the isocyanate (82) was reacted with the hydrochloride of p-mustard phenol (49) the desired urethane (83) was produced. An attempt to



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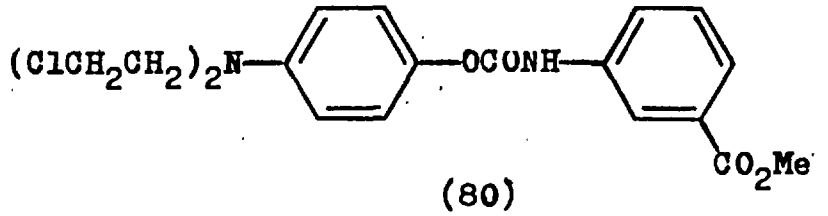

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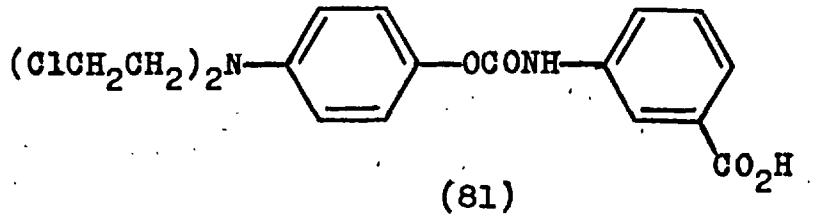

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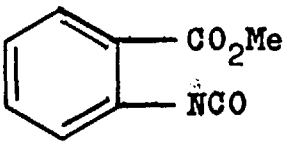

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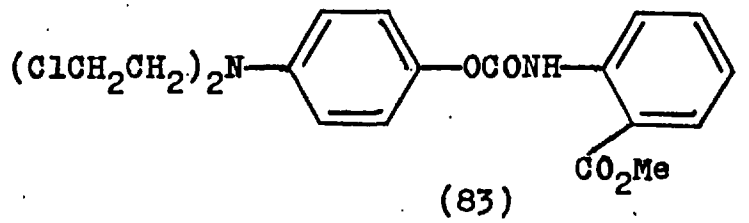
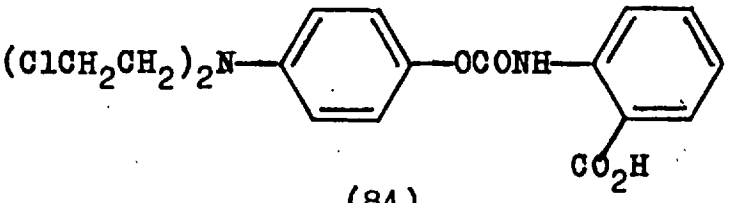


(83)

convert the ester (83) to the carboxyl derivative (84) by the usual method of acid hydrolysis did not succeed, and even after refluxing the ester for an hour in the acid mixture, the isolated product was found to be unreacted starting material. The result is not unexpected since it is well established that the hydrolysis of sterically hindered esters by the normal $A_{AC}2$ mechanism can be extremely difficult. It was thought that an $A_{AC}1$ type of hydrolysis might be applicable in this case, as the steric strain is considerably reduced by the formation of acylium ions in the rate-determining step. The methyl ester of mesitoic acid (85), for example, can be hydrolysed quite simply by dissolving in concentrated sulphuric acid and diluting the solution with iced water[117]. Treatment of the ester (83) with ice-cold 100% sulphuric acid and subsequent dilution with iced water yielded a solid which was slightly soluble in water, but insoluble in acetone. It was concluded that considerable sulphonation had occurred. The nitrogen mustard (86) synthesised by Sridhar[116] had a chemotherapeutic index of ca.100 when measured against the Walker 256 carcinoma in rats. It will be interesting to see whether the extremely high level of activity is shared by the compound (83), the only difference between them being the reversal of the carbamate linkage.



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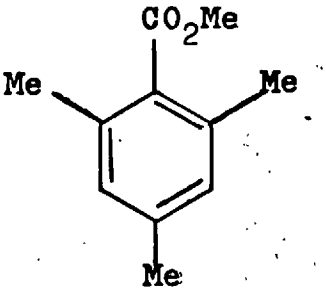

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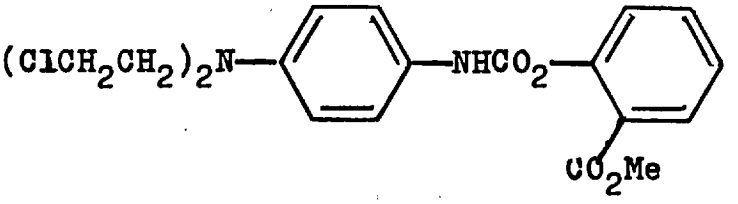


(86)

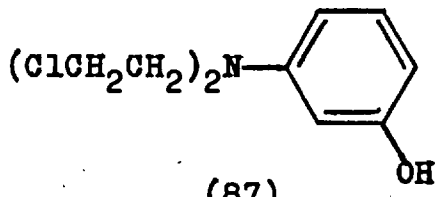
CHAPTER 7

CARBAMATES DERIVED FROM m-(NN-DI-2-CHLOROETHYLAMINO)PHENOL

m-(NN-Di-2-chloroethylamino)phenol(87), or m-mustard phenol as it will be called for convenience, has not previously been prepared, and represents, therefore, a new nitrogen mustard system. Clearly, the activating effect of the hydroxyl group in the p-mustard phenol (49) will not be apparent in the meta-analogue, since no effective transmission of electronic charge from the oxygen atom, through the aromatic ring, to the nitrogen atom, can occur. One would conclude, therefore, that the compound (87) will be less toxic than its para-isomer (49).

On the other hand, the deactivation of the parent mustard phenol (49) which results from the formation of urethane derivatives will not occur to the same extent in compounds of the meta-isomer (87) since only inductive forces will operate. It was important, therefore, to synthesise carbamate derivatives of (87) in order to ascertain whether or not these theoretical considerations are borne out in the results of biological tests.

Using the synthetic route initiated by Ross et al.[98] for the preparation of p-mustard phenol (49), m-aminophenol



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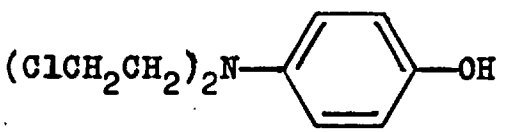
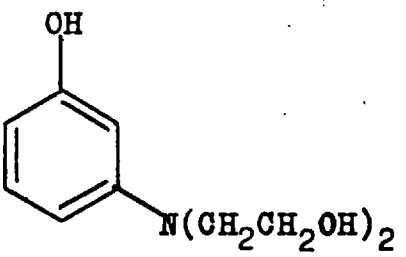


(49)

was treated with ethylene oxide in aqueous acetic acid to afford the tri-hydroxy derivative (88). In order to protect the phenolic group, the triol (88) was treated with ethanolic potassium hydroxide and benzyl bromide. The desired benzyl derivative (89) could not, however, be obtained pure, or even as a crystalline solid. The use of sodium hydride to generate the phenolate anion of (88) prior to the addition of benzyl bromide did not give rise to any of the benzyloxy-compound (89).

It was decided, therefore, to prepare m-aminophenyl benzyl ether (90) in a single stage reaction according to the method of Cortes and Walls[118]. The sodium salt of m-aminophenol was prepared and treated, in DMF, with benzyl chloride and in this manner the required benzyl ether (90) was obtained albeit in a low yield. A reaction between (90) and ethylene oxide in aqueous acetic acid afforded the di-hydroxy-derivative (89). Because the preparation of (88) involved the direct benzylation of m-aminophenol, a reaction which gave only a small yield of product, it was decided to establish an alternative route starting from m-nitrophenol.

A reaction between m-nitrophenol, ethanolic sodium ethoxide, and benzyl chloride, according to the method of Sova et al.[119] gave, in high yield, m-nitrophenyl benzyl ether (91).



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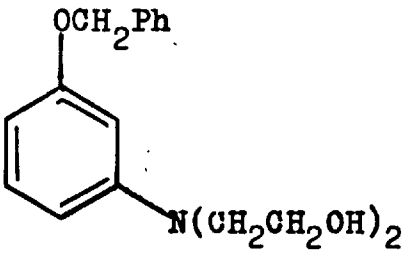

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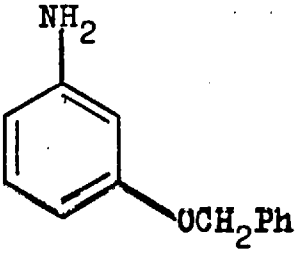

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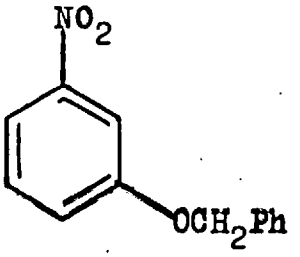


(91)

$$2\ ArNO_2 + 3\ NH_2NH_2 \xrightarrow{Pd/C} 2\ ArNH_2 + 3\ N_2 + 4H_2O$$

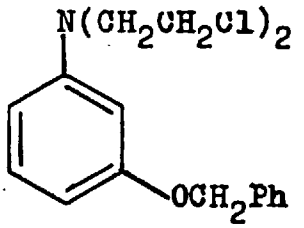
(fig.13)

In the original paper, potassium was used to generate ethoxide ions but it was found that the use of sodium afforded higher yields than that quoted in the literature[119]. The reduction of (91) to the amino-compound (90) using Raney nickel[119] was successful on only one occasion, the period of hydrogenation being five days. An alternative method, initiated by Pietra[120] for reducing nitro compounds in non-acidic media, involves the use of hydrazine and palladised charcoal. Adopting a modification of the procedure described by Bavin[121], m-nitrophenyl benzyl ether (91) was dissolved in ethanol to which was added excess hydrazine hydrate plus a catalytic amount of palladised charcoal. In this manner a high yield of the desired amino-compound (90) was isolated. The general equation for the reduction is shown in fig. 13. The reaction time appears to be important as does the presence of other potentially reducible functions, for when the period of reflux was greatly extended, the only product which could be isolated in the organic phase, was m-aminophenol. Presumably the excess hydrazine effected a hydrogenolysis of the benzyloxy group. The amino compound (90) was subsequently converted as before into the diol (89) in good yield.

In the preparation of p-mustard phenol (49), the chlorinating agent used by Benn et al.[106] was phosphorus pentachloride. However, the reaction between the amino-

compound (90) and phosphorus pentachloride resulted not in the desired dichloro-derivative (92) but in a viscous oil, the n.m.r. spectrum, i.r. spectrum and chlorine analysis of which suggest the chloro-phosphorus compound (93). Phosphorus oxychloride in chloroform was then used for the chlorination. However, after chromatographing the reaction product, four fractions were obtained all of which showed hydroxyl absorption in their i.r. spectra. Likewise, thionyl chloride gave none of the desired dichloro-derivative, but instead yielded only a charred residue after the removal of the solvent. Finally, phosphorus oxychloride in anhydrous benzene was found to be an effective reagent for the chlorination, giving the product (92) in reasonable yield.

In order to remove the protecting benzyl group from (92) a hydrogenolysis was carried out using palladised charcoal on catalyst. An n.m.r. spectrum of the product showed the presence of the benzyloxy function and so the acid hydrolysis, employed in the preparation of p-mustard phenol (49) (see chapter 5), was used. In this way the desired m-mustard phenol (87) was produced . A solid hydrochloride of (87) could not be obtained and instead the m-mustard phenol was characterised by converting it into the potentially cytotoxic urethanes described below.



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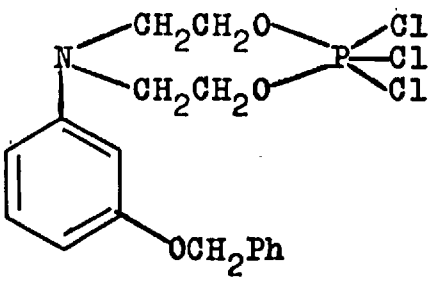
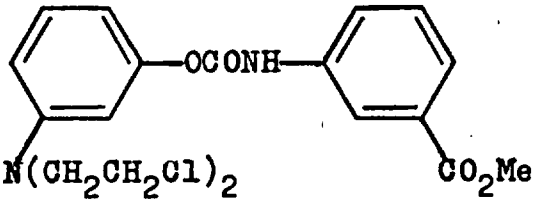


(93)

It has already been mentioned that the biological results on compounds synthesised by Sridhar[116] (see p. 72) indicate that carbamates containing methyl ester groups can be more effective tumour inhibitors than their carboxyl analogues. In this context, some of the compounds described in this chapter and following chapters have not been hydrolysed to their free acids.

The m-mustard phenol (87) was condensed with methyl-m-isocyanatobenzoate (79) to form the carbamate (94) which was subsequently hydrolysed in acid to yield the free carboxyl derivative (95). A similar condensation between methyl p-isocyanatobenzoate (96) and m-mustard phenol (87) afforded the desired urethane (97). The isocyanate (74) derived from the methionine antagonist, ethionine, was also reacted with m-mustard phenol (87), yielding the DL-ethionine derivative (98).



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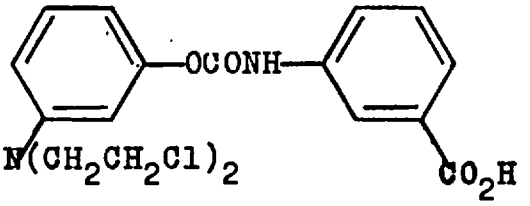

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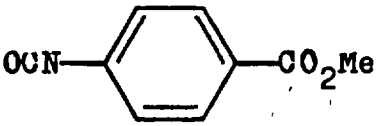

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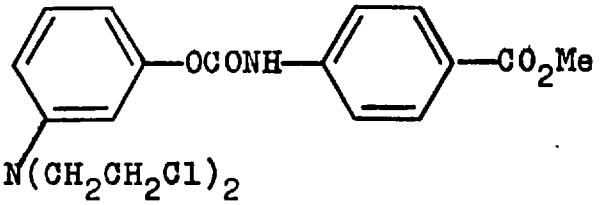

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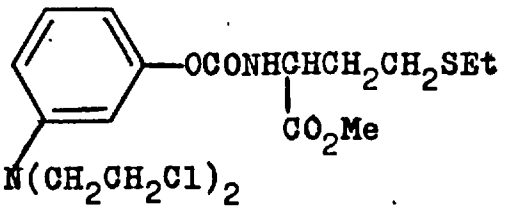
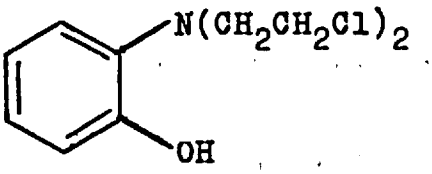


(98)

CHAPTER 8

CARBAMATES DERIVED FROM o-(NN-DI-2-CHLOROETHYLAMINO)PHENOL

The electronic differences that exist between p-mustard phenol (49) and m-mustard phenol (87) have been discussed in chapter 7. o-(NN-Di-2-chloroethylamino)-phenol (99), or o-mustard phenol, will, however, be electronically very similar to p-mustard phenol (49). The major difference between the two will, of course, be steric. If, for example, there is some steric inhibition of resonance in carbamate derivatives of (99), then perhaps the formation of such derivatives will not produce the same deactivating effect on the parent mustard phenol as it does in the case of derivatives of (49). Again, the enzymic hydrolysis *in vivo* of urethanes derived from (99) may not proceed with the same facility that attends the potentiation of p-mustard phenol (49) derivatives. The increased stability of o-mustard phenol (99) derivatives could be of immense advantage, since the diffusing entity is more likely to remain intact until it reaches the neoplastic cells, where the concentration of potentiating enzyme is far higher than in the surrounding tissue cells. A greater degree of specificity may, therefore, be achieved with agents derived from o-mustard phenol (99).



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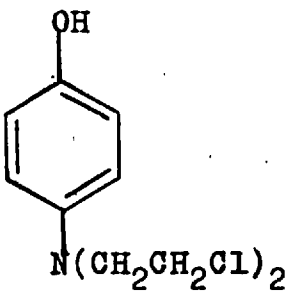
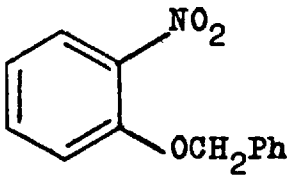


(49)

Adopting the route that was found to be successful for the preparation of m-mustard phenol (87), o-nitrophenol was treated with ethanolic ethoxide and benzyl chloride, after the manner of Sova et al.[119], to produce a high yield of o-nitrophenylbenzyl ether (100). The reduction of (100) to the amino compound (101) was effected by the use of hydrazine and palladised charcoal as for the preparation of the m-isomer (90). The amine (101) was reacted with ethylene oxide in the usual way to afford an uncrystallisable brown oil. A vacuum distillation of the oil was attempted, but this only resulted in the decomposition of the product.

To overcome this problem an attempt was made to benzylate o-aminophenol by the method of Cortes and Walls who synthesised m-aminophenyl benzyl ether (90)[118]. A distillation of the reaction product afforded three fractions only one of which exhibited an NH_2 doublet in its i.r. spectrum. The yield of the fraction was, however, very small.

It was decided then, to return to the synthetic route initiated by Ross et al.[98] for the preparation of p-mustard phenol (49). Treatment of o-aminophenol with



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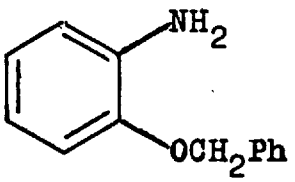
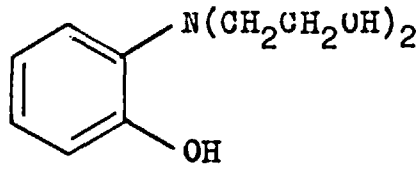


101)

liquid ethylene oxide in the normal manner yielded a product which on distillation afforded the triol (102) as a highly deliquescent, viscous, oil which was characterised as the crystalline tris-p-nitrobenzoate (103). When the triol (102) was reacted with ethanolic potassium hydroxide and benzyl bromide, the benzyl ether (104) was isolated as an oil which was characterised as the crystalline bis-p-nitrobenzoate (105).

In order to chlorinate the diol (104), phosphorus pentachloride in chloroform was used, after the procedure of Benn et al.[106] who prepared p-mustard phenol (49). In this way, the dichloro-compound (106) was afforded as a pale-yellow oil after chromatography, and was characterised as the picrate (107). The method of removing the protecting benzyl group from the p-isomer (60), using concentrated hydrochloric acid and glacial acetic acid, was tried on the ortho-compound (106). Presumably because of steric hindrance, it was not found possible to remove the benzyl group even after prolonged refluxing in the acid mixture.

The hydrogenolysis procedure of Benn et al.[106] was, however, found to be successful. A methanolic solution of the benzyl ether (106) was treated with hydrogen, using 10% palladised charcoal as a catalyst.



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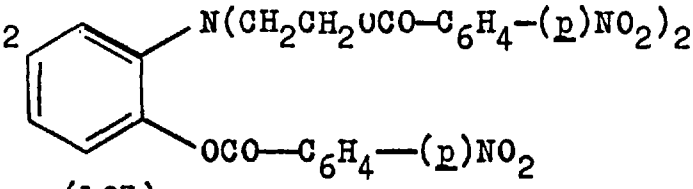

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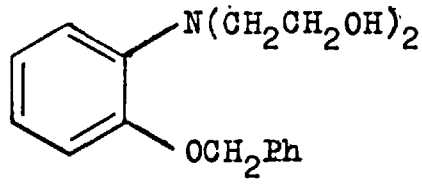

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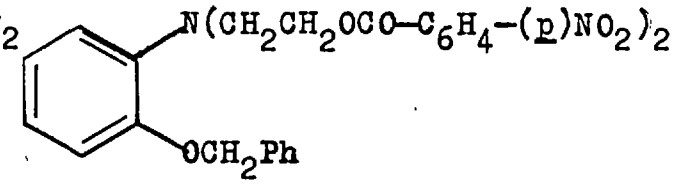

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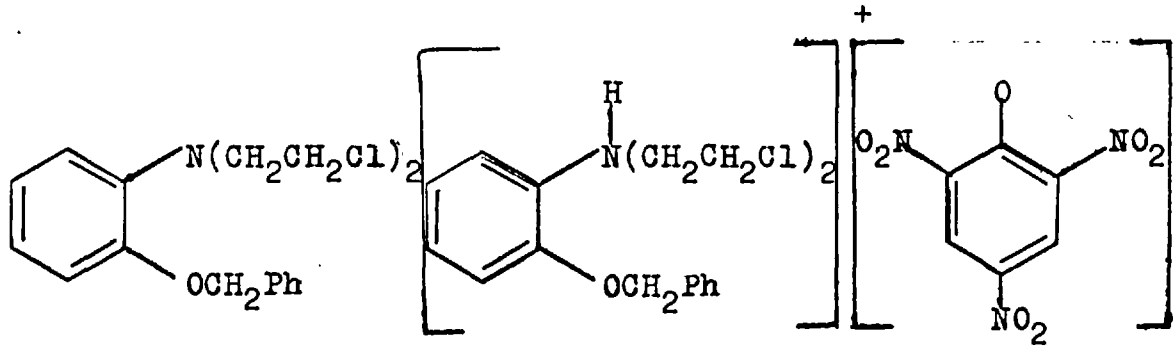
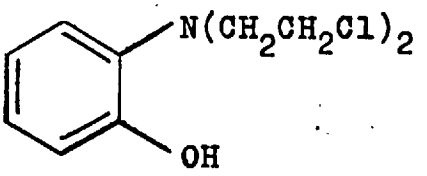


(106)

(107)

A chromatographic purification of the product yielded the o-mustard phenol (99) as a colourless oil, from which a crystalline hydrochloride was subsequently prepared.

A condensation between the hydrochloride of o-mustard phenol (99) and methyl p-isocyanatobenzoate (96) yielded the desired urethane (108) which was hydrolysed in the usual manner to give the free acid (109). The carbamate (109) is the ortho-analogue of I.C. 140 (58), a compound of known chemotherapeutic usefulness. Biological tests on the tumour inhibitory properties of (109) should help to decide the validity of those theoretical considerations outlined at the beginning of this chapter.



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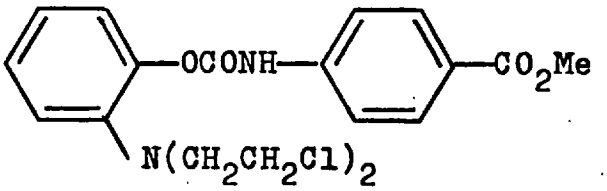

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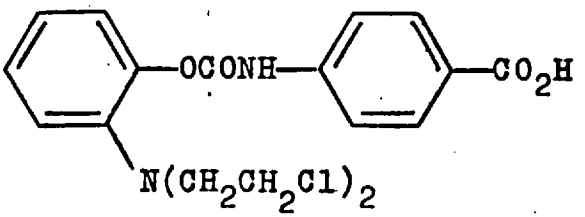

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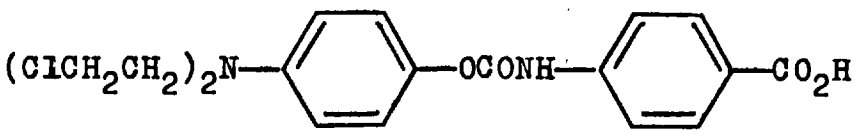


(58)

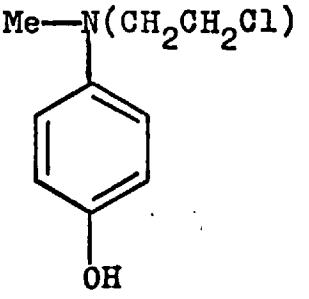
CHAPTER 9

SOME CARBAMATES DERIVED FROM

p-(N-METHYL-N-2-CHLOROETHYLAMINO)PHENOL

The early idea that monofunctional nitrogen mustards could not affect tumour inhibitions was discounted by the results of Biesele *et al.*[40] and Loveless and Ross[41]. Moreover, the work of Chakrabarti and Friedmann[85] has shown that some monofunctional agents have even greater cytotoxicity than difunctional derivatives. With this in mind it was considered important to synthesise some monofunctional carbamates. The particular nitrogen mustard chosen for this series was p-(N-methyl-N-2-chloroethylamino)phenol (110) or the "one-armed" mustard phenol, as it will be referred to for convenience. The choice was made on the basis that since a large variety of compounds have been synthesised from p-mustard phenol (49) by many workers in this field, it would be very useful to be able to synthesise the corresponding monofunctional agents in order to compare their effectiveness.

Attempts to react p-N-methylaminophenol (111) with ethylene oxide were unsuccessful due to the extremely rapid oxidation of the amine (111). When the more stable



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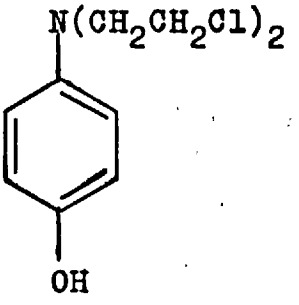
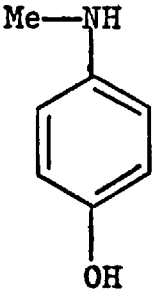


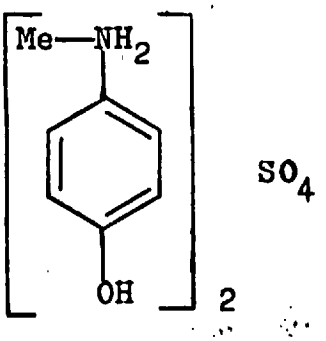
(49)



(111)

sulphate(Metol)(112) was treated, in aqueous acetic acid and sodium acetate, with ethylene oxide, the only product isolated from the organic phase, after working up, was the mono-acetate of ethylene glycol. The same product was formed when sodium hydroxide was substituted for sodium acetate. It was decided therefore to abandon the idea of reacting the amine (111) or its salt (112) directly with ethylene oxide, and instead to protect the phenolic group by benzylation so that a subsequent hydroxyethylation could be effected. Before this could be achieved, however, it was necessary to protect the secondary amino group in order to prevent N-benzylation.

Accordingly, then, Metol(112) was treated with acetic anhydride and sodium acetate, following the procedure of Robinson[122] to afford the N-acetyl derivative(113) in high yield. The subsequent reaction between the N-acetyl derivative(113) and a mixture of ethanolic sodium ethoxide and benzyl chloride gave the benzyloxy-derivative(114). To remove the protecting acetyl group, the compound(114) was refluxed in an ethanolic solution of potassium hydroxide, thus affording p-(N-methyl)aminophenyl benzyl ether(115) as a pale yellow liquid. In the original preparation, Robinson[122] did not purify the intermediate acetylated derivative(114), but it was found that by so



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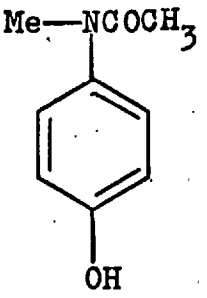
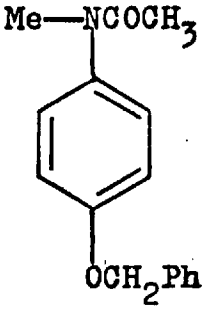


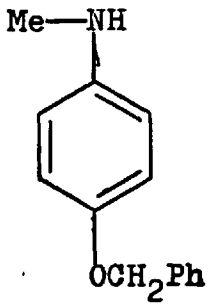
(113)



(114)

doing, the yield of the final product(115) was far higher than that quoted in the literature[122].

When the amine(115) was treated with ethylene oxide in aqueous acetic acid only a low yield of the hydroxyethyl-derivative(116) was obtained. In some attempts, no product at all could be isolated from the organic phase after working-up. This result could not be explained except by supposing that the large excess of ethylene oxide might have produced a water-soluble polymer. The experiment was repeated in a non-aqueous medium using chloroform and glacial acetic acid. In this way a crystalline product was obtained in high yield, but it was later shown not to be the required hydroxyethyl-derivative (116). The exact structure of the product has not been elucidated, and the problem of obtaining the compound(116) in reasonable quantities has not yet been resolved. The small amount actually obtained, however, was reacted with phosphoryl chloride in anhydrous benzene to give the desired chloro-derivative(117) as a white crystalline solid. In order to remove the protecting benzyl group from (117) a hydrogenolysis was carried out in methanol, using 5% palladised charcoal as catalyst. In this manner, the "one armed" mustard phenol(110) was obtained as an oil, treatment of which with gaseous hydrogen chloride, gave



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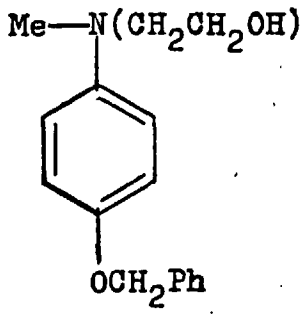

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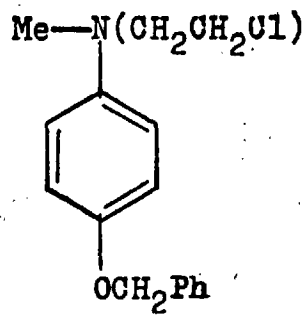
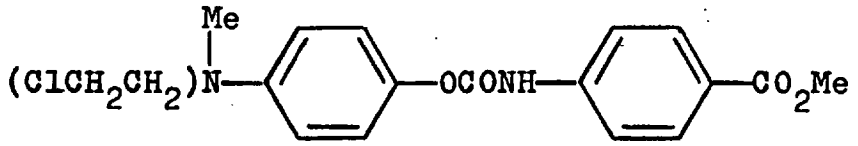


(117)

a stable, crystalline, hydrochloride.

A condensation was then effected between the hydrochloride of the "one armed" mustard phenol(110) and methyl p-isocyanatobenzoate(96), to produce the carbamate(118) which was subsequently subjected to acid hydrolysis in the usual manner, thus affording the free carboxyl derivative(119). Being the monofunctional analogue of I.C. 140 (58), which is a valuable tumour inhibitor, the results of biological tests on (119) should prove very interesting.

When methyl o-isocyanatobenzoate(82) was condensed with the hydrochloride of the "one armed" mustard phenol(110), the isomeric ortho-derivative(120) was produced. In view of the probable resistance of this compound towards selective hydrolysis of the methyl ester group (ortho-effect), no attempt was made to obtain the corresponding acid.



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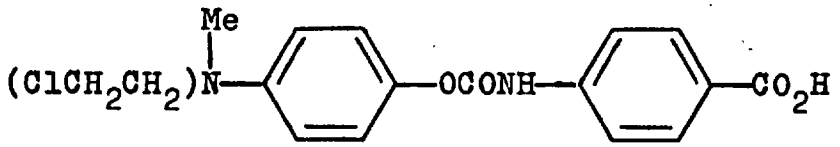

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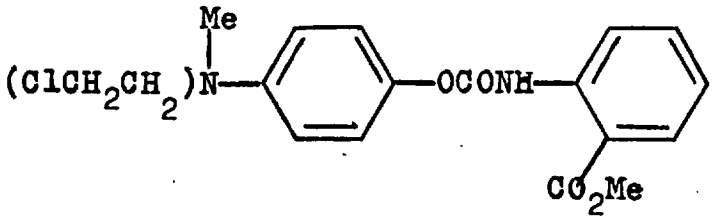


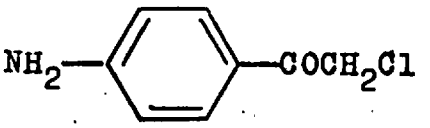
(120)

CHAPTER 10

SOME CARBAMATES DERIVED FROM p-AMINOPHENACYL CHLORIDE

The ∝-halo-ketones, of which p-aminophenacyl chloride(121) is an example, belong to the class of compounds known as lachrymators. The lachrymatory power of such compounds is associated with the positive nature of the halogen atom, due to the adjacent carbonyl group, and of course their volatility. It was pointed out on p. 11 that ∝-halo-ketones can function as biological alkylating agents, principally by reacting *in vivo* with molecules containing thiol groups. They are, therefore, effective inhibitors of enzymes which depend on the presence of thiol groups for their activity[123].

Moreover, Dixon[124] has shown that phenacyl chloride reacts with cysteine and thiol-containing proteins, the nature of the reaction being a rapid and irreversible alkylation of the sulphhydryl groups. Since these early results, other sites of attack on biological molecules have been discovered. Scholmann and Shaw[125] have recently shown that the compound(122) inhibits chymotrypsin at pH 7.2, the site of alkylation being a histidine residue, and probably the imidazole ring in the residue.



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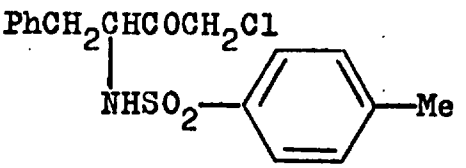
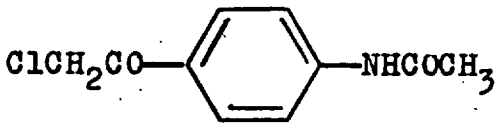


(122)

The importance of urethane linkages in biological alkylating agents has been stressed throughout the present discussion. It seemed, therefore, worthwhile to synthesise a series of carbamates derived from p-aminophenacyl chloride (121), with a view to such derivatives being enzymically hydrolysed *in vivo*. The enzyme-inhibiting lachrymator(121) which could be liberated from such a hydrolysis, could perhaps effect tumour regressions by irreversibly interfering with the metabolism of enzymes in neoplastic cells provided that a high enough degree of specificity was exhibited. The general synthetic method for the carbamates was the condensation of the amine(121) with a chloroformate derived from a hydroxy-ester.

p-Aminophenacyl chloride(121) was prepared according to the procedure of Kunkell[126]. Acetanilide was treated with chloroacetyl chloride and powdered aluminium chloride in carbon disulphide to afford p-(chloroacetyl)acetanilide (123) which was subsequently hydrolysed in 16% hydrochloric acid, yielding pale-brown crystals of (121).

The first hydroxy-ester to be studied in this series was methyl o-hydroxybenzoate. This was converted into the corresponding chloroformate(124) by reaction with phosgene in benzene, following the method of Einhorn and



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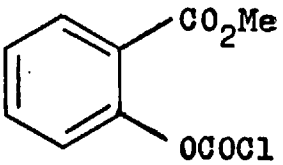
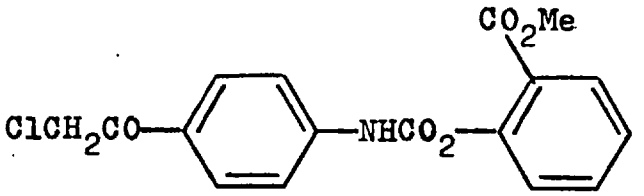


(124)

Bagh[127]. When the chloroformate(124) was condensed with p-aminophenacyl chloride, in the presence of pyridine, the required carbamate (125) was isolated.

An attempt to convert (125) into the carboxyl derivative(126) by the normal method of acid hydrolysis did not succeed even after refluxing the reaction mixture for one hour. The resistance of sterically hindered esters to hydrolysis is, of course, not unusual. However, the major product of the reaction was not the starting material but p-aminophenacyl chloride, thus indicating the susceptibility of the carbamate linkage, in the sterically hindered ester(125), to acid hydrolysis. It should be recalled that when the nitrogen mustard(83) was subjected to acid hydrolysis, no reaction took place at all. A possible explanation for the difference in reactivity of these two compounds is as follows: since the rate-determining step in the hydrolysis of carbamates is the attack of a water molecule on the carbonyl carbon atom of the conjugate acid, then any effect which increases the electrophilicity of the carbonyl group should favour the hydrolysis. The deactivating effect of the nitrogen atom in (126) is considerably suppressed due to the presence of the powerfully electron-withdrawing p-chloroacetyl group. In the mustard (83), on the other



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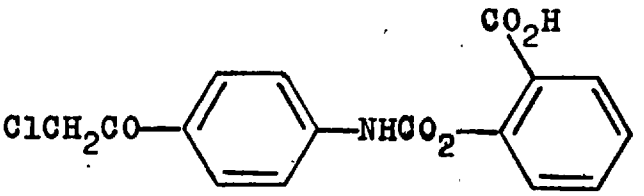

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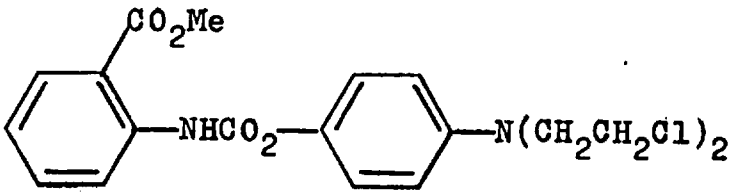
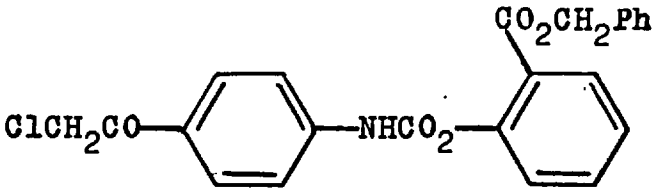


(83)

hand, the nitrogen atom still has sufficient basicity to decrease the positive nature of the carbonyl carbon atom, and thus to render (83) inactive to acid hydrolysis under the conditions used.

It was decided to try to overcome the problem of synthesising the carboxylic acid(126) by having a benzyl-ester grouping instead of a methyl-ester grouping, since it is known that benzyl esters undergo ready hydrogenolysis to give the free acid. Accordingly, the condensation between p-aminophenacyl chloride(121) and o-benzyloxycarbonylphenyl chloroformate was effected to give the urethane(127). When the ester(127) was subjected to catalytic hydrogenation, no reaction took place, and the starting material was recovered unchanged. Thus, steric hindrance would seem to have inhibited the hydrogenation reaction also.

m-Hydroxybenzoic acid was esterified with ethanol and sulphuric acid and the methyl ester was treated with phosgene in benzene to afford the chloroformate(128). When p-aminophenacyl chloride(121) was condensed with the chloroformate(128), the desired urethane(129) was isolated. The usual acid hydrolysis was readily effected now that the ester group was in the meta-position, thus converting the ester(129) into the free acid (130).



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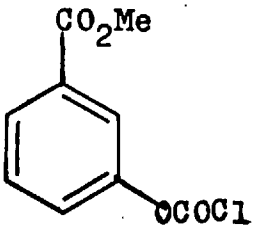

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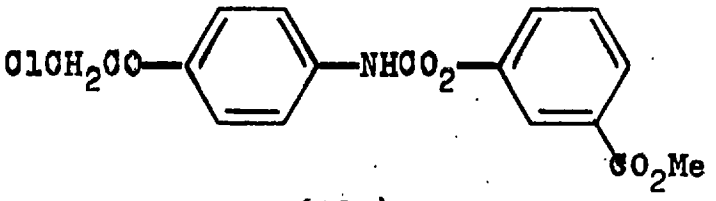

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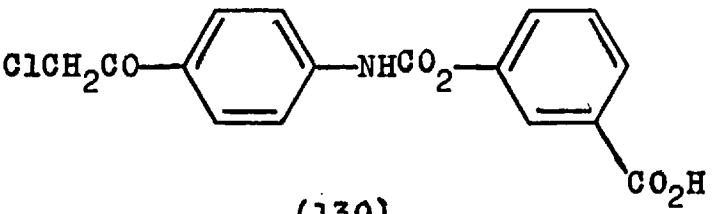


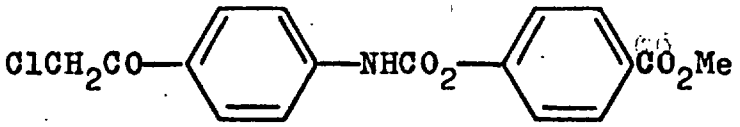
(130)

Using the method of Einhorn *et al.*[128], methyl *p*-hydroxybenzoate was converted into the corresponding chloroformate(131). The normal condensation was carried out between *p*-aminophenacyl chloride (121) and the chloroformate (131). Evidence that the product of the reaction was the desired carbamate (132) was furnished by its i.r. spectrum. However, when a small sample was purified by recrystallisation from methanol, the resulting compound was found to be the methyl carbamate (133), arising, presumably, from an ester exchange reaction during the recrystallisation. When the crude urethane (132) was hydrolysed in the normal way, the free carboxyl derivative (134) was readily obtained.

Potentially cytotoxic derivatives of the *m*- mustard amine (135) have been little studied, and have formed part of the work carried out in this laboratory by Sridhar[116]. Using a sample of (135)*, a condensation was effected with the chloroformate (136) derived from ethyl lactate. In this way, the urethane (137) was isolated. It is an ester analogous to the isopropyl urethane (138) prepared by Sridhar[116] as the *meta*-isomer of the carbamate (54), a compound which was extensively studied[105] from the point of view of enzymic potentiation.

* This compound was kindly donated by R. Sridhar.

(131)



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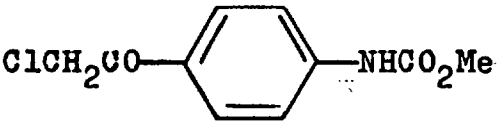

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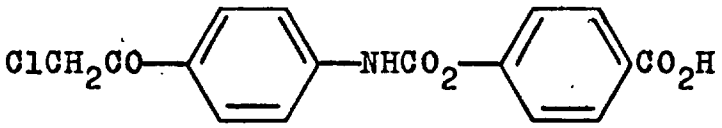

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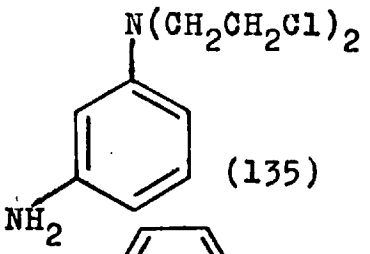

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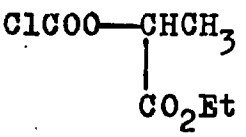

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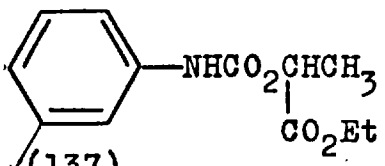

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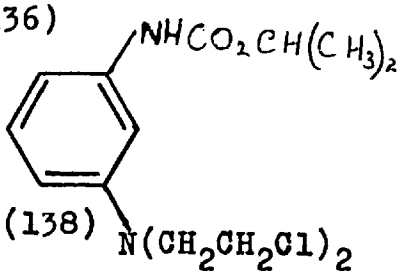

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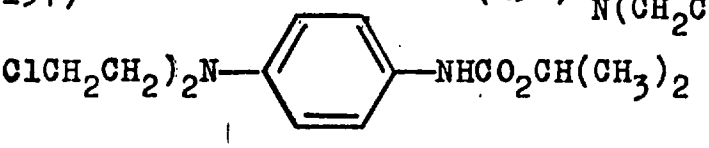
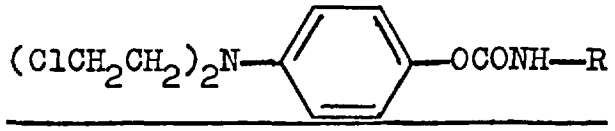


(54)

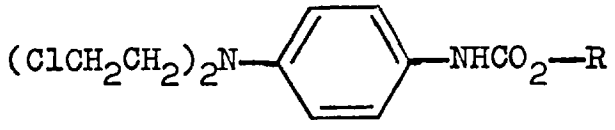
BIOLOGICAL RESULTS

Certain of the compounds synthesised were sent to the Department of Biochemical Pharmacology of the State University of New York at Buffalo, for biological tests. These tests were conducted under the auspices of Professor Hebborn who has kindly provided the results shown overleaf. For comparative purposes, the results obtained from some earlier compounds are also included.

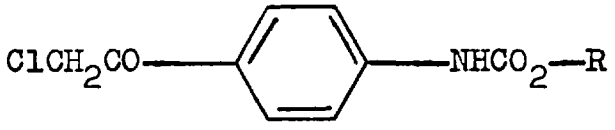
It would seem that agents derived from essential amino acids (I.C. 217 and I.C. 219) are not as effective as those derived from aromatic amino acids. In this context, the meta carboxy urethane, I.C. 233, has a promisingly high chemotherapeutic index, which is twice that of the para-isomer (the established anticarcinogen I.C. 140). When results are available on the urethanes derived from the ortho and para mustard phenols it may be possible to correlate biological activity with the substitution pattern. Another point of considerable interest emerges when one compares the indices of the methionine derivative (I.C. 219) and the ethionine analogue (I.C. 230). It is clear that the arguments advanced in the discussion for the synthesis of the latter are well substantiated. I.C. 230 is, in fact, the most effective amino acid derivative yet tested at Buffalo.



Code No.	R	Acute LD_{50} mg./kg. mouse	Acute LD_{50} mg./kg. rat	Walker ED_{90} mg./kg.	LD_{50}/ED_{90}
I.C.140	$C_6H_4CO_2H(p)$*	187	56	3.3	17.0
I.C.217	$CH(CO_2H)CH_2C_6H_5$	60	68	31	2.2
I.C.219	$CH(CO_2H)CH_2CH_2SCH_3$	150	56	35	1.6
I.C.230	$CH(CO_2H)CH_2CH_2SEt$		70	4.8	14.5
I.C.233	$C_6H_4CO_2H(m)$		75	2.4	31



I.C.234	$C_6H_4CO_2Me(m)$**		400	5.3	78
I.C.213	$C_6H_4CO_2H(p)$**	43	58	1.2	46.0
I.C.220	$C_6H_4CO_2Me(p)$**	135	110	0.84	131



I.C.254	$C_6H_4CO_2Me(o)$		200	>200	<1.0

* This compound was prepared by Owen *et al.*[106]

** This compound was prepared by Sridhar[116]

EXPERIMENTAL

Numbers shown in parentheses throughout this section refer to the page numbers of the original laboratory note-books.

The drying of solutions of compounds in organic solvents was carried out using magnesium sulphate, unless otherwise specified.

Infrared spectra were measured on a [illegible] Unicam S.P.200 spectrometer, and proton magnetic resonance spectra were measured by Mrs. A.I. Boston using a Varian A-60 instrument.

Melting points were determined on a Kofler micro-stage apparatus, and are uncorrected.

CHAPTER 11

CARBAMATES DERIVED FROM p-(NN-DI-2-CHLOROETHYLAMINO)PHENOL AND SOME NATURALLY OCCURRING AMINO ACIDS.

Benzyl p-(NN-Di-2-chloroethylamino)phenyl Ether[106] (10).

Benzyl p-(NN-di-2-hydroxyethylamino)phenyl ether (9 g.) in chloroform (60 ml.) was treated with phosphorus pentachloride (14.7 g.). After the reaction had subsided, the solution was heated under reflux (90 min.), cooled, and poured into water. The chloroform layer was separated, washed with aqueous sodium carbonate, dried and concentrated to an oil. The oil was passed in benzene through a column of alumina, and concentration of the eluates gave colourless crystals of the title compound (3.8 g.), m.p. 106.5-107° (lit.[106] m.p. 105-106°).

p-(NN-Di-2-chloroethylamino)phenol Hydrochloride[106] (12).

The above dichloro-ether (30 g.) was dissolved in a 1:1 mixture of concentrated hydrochloric acid and glacial acetic acid (300 ml.), and the solution was heated under reflux (1 hr.). After cooling, the solution was neutralised with solid sodium bicarbonate and a saturated solution of sodium bicarbonate. The product was extracted with chloroform (750 ml.) which was washed and dried. Hydrogen chloride was passed into the solution, giving a pink syrupy mixture

which, after being warmed on a steam-bath, deposited a white solid which was filtered off, washed with light petroleum, and dried to give the title compound, the i.r. spectrum of which was identical to that of an authentic sample (21 g., 93%), m.p. 180-181° (lit.[106] m.p. 170-173°).

DL-Phenylalanine methyl ester hydrochloride (17).

The compound was prepared from DL-phenylalanine by the action of methanol saturated with hydrogen chloride according to the method of Fischer and Suzuki[111] .
The product had m.p. 157-158° (lit. m.p. 158°).

Methyl α-isocyanato-β-phenylpropionate (20).

The above methyl ester hydrochloride of DL-phenylalanine (3.9 g.) was suspended in dry toluene (150 ml.) which was heated to reflux temperature. A stream of phosgene was bubbled through the heated solution (2 hr.), after which the excess phosgene was removed by passing nitrogen through the solution. Removal of the solvent and distillation of the residue afforded the isocyanate as a colourless liquid (2.5 g., 67%), b.p. 148-150°/6 mm. (lit.[129] b.p. 152-153°/12 mm.).

N-(p-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-phenylalanine methyl ester (22).

p-(NN-Di-2-chloroethylamino)phenol hydrochloride (700 mg.) was shaken with benzene (18 ml.) and saturated sodium bicarbonate solution. The solution of the free base was separated, washed, and dried. After filtering, the solution was heated under reflux with methyl α-isocyanato-β-phenylpropionate (500 mg.) for 5 hrs., two drops of pyridine being added as catalyst. After removing the solvent, a green oil was obtained. This was dissolved in hot ether to which was added light petroleum. On standing, a white solid was obtained, and this was recrystallised from benzene - petroleum (40-60°) to give white crystals of the title compound in low yield, m.p. 100-101.5°, ν max. (nujol) 3420, 1735 $cm.^{-1}$

(Found: C, 57.26; H, 5.51; N, 6.10. $C_{21}H_{24}N_2Cl_2O_4$ requires C, 57.38; H, 5.51; N, 6.38%).

N-(p-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-phenylalanine (60).

The above methyl ester (1 g.) was added to a 1:1 mixture of concentrated hydrochloric acid and glacial acetic acid (20 ml.). The solution was gently heated under reflux (5 min.) and then cooled in ice. Water was added and the

acids were neutralised by the addition of ION sodium hydroxide solution. The product was extracted with chloroform, washed with water, dried, and concentrated to give an oil. The oil readily crystallised out on trituration with light petroleum and the resulting solid was recrystallised from benzene - petroleum (40-60°), affording white crystals of the title compound (500 mg.), m.p. 136-138°, ν max. (nujol) 3300, 1730, 1690 $cm.^{-1}$. (Found: C, 56.38; H, 5.26; N, 6.35; Cl, 16.63. $C_{20}H_{22}Cl_2N_2O_4$ requires C, 56.48; H, 5.21; N, 6.59; Cl, 16.67%).

DL-Alanine methyl ester hydrochloride (30).

DL-Alanine (15 g.) was added to methanol (150 ml.) and the stirred suspension was saturated with hydrogen chloride until all the solid had passed into solution. The solution was heated under reflux (ca. 85°, 18 hrs.) and then concentrated so that a sticky solid was obtained. This was washed with acetone and ether and recrystallised from aqueous acetone, affording white crystals of the title compound, m.p. 162° (plus some melting at 129°) (lit. m.p. 158°).

Methyl α-isocyanatopropionate.

(a) From DL-alanine methyl ester hydrochloride (32).

The ester hydrochloride (5 g.) was suspended in dry toluene (150 ml.) and the mixture was heated to 120-130°. Phosgene was bubbled through the mixture (6 hrs.) and again, 16 hours later (1½ hrs.). The solution was cooled and the excess phosgene was removed by nitrogen. After removing the solvent, the isocyanate was distilled to give the title compound as a colourless liquid (1.6 g., 35%), b.p. 70°/0.2 mm., n_D^{19} 1.4220.

(b) From DL-Alanine[114].

α-Isocyanatopropionyl chloride (34).

DL-Alanine (15 g.) was suspended in dry dioxan (225 ml.) and the suspension was stirred and heated to 50-55°. Phosgene was introduced until a clear solution was obtained (2 hrs.). The solution was cooled (5-10°) and hydrogen chloride was bubbled through (4 hrs.) after which time the temperature was again raised (50-55°) and phosgene was again introduced (3 hrs.). After removing the excess

phosgene with nitrogen, the solution was concentrated under reduced pressure in order to remove the dioxan. The resulting oil was dissolved in benzene which was subsequently removed under reduced pressure. The isocyanate was finally distilled under vacuum to give the title compound as a colourless liquid (12.6 g., 64%), b.p. 64°/12 mm. (lit.[114] b.p. 67-68°/47 mm.).

Esterification step (38).

A solution of the α-isocyanatopropionyl chloride prepared above (9.8 g.) in dry ether (45 ml.) was stirred at room-temperature, and to this was added, drop wise, a solution of anhydrous methanol (2.7 g.) in dry ether (8 ml.). After 1 day, the ether was removed, leaving the white solid, carbamoyl chloride. This was distilled under vacuum to give two fractions (a) (5.1 g., 51%), b.p. 46-48°/0.5 mm., n_D^{17}1.4220; (b) b.p. 78-80°/0.5 mm., n_D^{17}1.4390.

By comparing the refractive indices with that of the product obtained from the previous method, it was concluded that fraction (a) was the title product, ν max. 2260, 1730 cm.$^{-1}$.

(Found: C, 46.29; H, 5.53; N, 10.80. $C_5H_7NO_3$ requires C, 46.48; H, 5.47; N, 10.85%).

NN'-(Di-1-methoxycarbonylethyl)urea (40).

In order to characterise the isocyanate produced above, it was converted into its symmetrical urea. To a solution of the isocyanate in acetone was added a small quantity of water. The mixture was stirred for several hours and then concentrated, leaving a white solid. The urea was crystallised from aqueous methanol to give white crystals of the title compound, m.p. 163-164°.

(Found: C, 46.88; H, 7.06; N, 12.04.

$C_9H_{16}N_2O_5$ requires C, 46.52; H, 6.95; N, 12.07%).

N-(p-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-alanine methyl ester (289)

p-(NN-Di-2-chloroethylamino)phenol hydrochloride (270 mg.) and dry triethylamine (110 mg.) were shaken together in dry benzene (10 ml.) until the liberation of the free base was complete. The isocyanate prepared above (129 mg.) was added and the whole was heated under reflux overnight. The mixture was filtered to remove triethylamine hydrochloride and the filtrate was washed with water, dried, and concentrated to give an oil which readily crystallised on trituration with petroleum. The solid was recrystallised from benzene-petroleum (40-60°) to yield white crystals of the title product (110 mg.), m.p.64-65°, ν max. (nujol) 3350, 1740, 1700 cm.$^{-1}$.

(Found: C, 49.30; H, 5.33; N, 7.66; Cl, 19.85. $C_{15}H_{20}N_2O_4Cl_2$ requires C, 49.63; H, 5.55; N, 7.72; Cl, 19.53%).

α-Isocyanato-γ-(methylthio)butyric acid methyl ester

(a) Attempted preparation via DL-methionine (48, 50).

The method according to Iwakura, Uno, and Kank[114] was adopted here. DL-methionine (7 g.) in dry dioxan (105 ml.) was heated to 50-60°, and phosgene was bubbled into the stirred suspension (30 min.). The resulting solution was cooled in ice, and dry hydrogen chloride was bubbled through (7 hrs.), by which time a white precipitate had formed. The mixture was reheated (50-55°), and phosgene was again passed through until the suspension had become clear (1 hr.). The solvent was removed and the resulting liquid was distilled to give α-isocyanato-γ-(methylthio)butyryl chloride as a pale yellow liquid, b.p. 60°/0.5 mm., n_D^{19} 1.5339, ν max. 2240, 1770 (v.small), 1645 cm.$^{-1}$. The product rapidly darkened on storage.

(Found: C, 46.15; H, 4.49; N, 8.63.

$C_6H_8ClNO_2S$ requires C, 37.19; H, 4.16; N, 7.23%).

It was concluded that decomposition was too rapid to afford a correct analytical result, or to make this a useful synthetic route.

(b) Via DL-methionine methyl ester (52).

DL-Methionine was esterified with methanol and hydrogen chloride according to the method of Fischer and Suzuki[111], and the ester hydrochloride was isolated as a yellow oil which could not be crystallised. The free base was liberated using sodium carbonate, and was extracted with ethyl acetate, and finally distilled, b.p. 75°/0.2 mm.

Dry toluene (50 ml.) was cooled in ice and saturated with phosgene (ca. 1 hr.). DL-Methionine methyl ester (1.7 g.) in dry toluene (10 ml.) was added drop-wise to the stirred solution (1 hr.), while a constant stream of phosgene was bubbled through the mixture. A white precipitate was formed, but this disappeared after heating the mixture under reflux. The toluene was removed under reduced pressure and the isocyanate was distilled to yield the title compound as a colourless liquid (1.3 g., 65%), b.p. 104°/0.53 mm., ν max. 2230, 1730 cm.$^{-1}$.

N-(p-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-methionine methyl ester (56).

p-(NN-Di-2-chloroethylamino)phenol hydrochloride (670 mg.) and dry triethylamine (350 mg.) were stirred together in dry benzene (20 ml.) after which methyl-α-isocyanato-γ-(methylthio)butyrate (470 mg.) was added. The mixture was heated under reflux (6 hrs.), cooled, and the triethylamine hydrochloride was filtered off. The filtrate was washed with 2N hydrochloric acid, water, dried, and concentrated to give a pale-yellow oil. The urethane was dissolved in a small quantity of ether to which was added light petroleum (40-60°). After standing for a day, a white solid was obtained and this was recrystallised from benzene to yield white crystals of the title compound, m.p. 75-76°.
(Found: C, 48.42; H, 5.70; N, 6.26; Cl, 16.82. $C_{17}H_{24}Cl_2N_2O_4S$ requires C, 48.20; H, 5.72; N, 6.60; Cl, 16.76%).

N-(p-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-methionine (62).

The methyl ester prepared above (500 mg.) was added to a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid (4 ml.), and the whole was heated under reflux (5 min.). After cooling the solution, ice-cold water (4 ml.) was added, as well as ION sodium hydroxide solution, until a pH of 4 was reached. The free acid was then extracted with chloroform which was washed with water, dried and concentrated to afford a white solid. This was recrystallised from benzene to give white crystals of the title compound, m.p. 125-126°, ν max. (nujol) 3330, 1745, 1700 $cm.^{-1}$.
(Found: C, 47.20; H, 5.36; N, 6.74; Cl, 17.42. $C_{16}H_{18}N_2Cl_2O_4S$ requires C, 46.94; H, 5.42; N, 6.85; Cl, 17.33%).

DL-Ethionine methyl ester (64).

DL-Ethionine was esterified with methanol and hydrogen chloride according to the method of Fischer[111], to yield the methyl ester hydrochloride as a yellow uncrystallisable oil. This was shaken with ammonia solution and the free ester was extracted with chloroform and subsequently distilled to give a 63% yield of

the title compound as a colourless liquid, b.p. 104-105°/ 0.2 mm., N_D^{23}1.4840 (lit.[130] b.p. 97-98°/3mm.).

Methyl ∝-isocyanato-γ-(ethylthio)butyrate (66).

Ice-cooled toluene (300 ml.) was saturated with phosgene (2 hrs.) and to the stirred solution was added drop-wise a solution of the methyl ester prepared above (6.8 g.) in toluene (50 ml.). The mixture was heated under reflux after which the solvent was removed, and the isocyanate was distilled to give the title product as a pale-yellow liquid (6.5g., 83%), b.p. 108-110°/1.5 mm., n_D^{20}1.4806.
(Found: C, 46.81, H, 6.22., N, 6.69.
$C_8H_{13}NO_3S$ requires C, 47.28; H, 6.45; N, 6.89%).

N-(p-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-ethionine methyl ester (68).

p-(NN-Di-2-chloroethylamino)phenol hydrochloride (2.7 g.) was treated in dry benzene (80 ml.) with dry triethylamine (1.3 g.) until the free base was liberated. To the solution was added the isocyanate prepared above (2.0 g.) and the mixture was heated under reflux (5 hrs.).

The triethylamine hydrochloride was filtered off and the filtrate was worked up in the usual way to afford a yellow oil which, after standing for two weeks under petroleum, yielded the solid urethane. This was recrystallised from benzene-petroleum (60-80°) to give white crystals of the title product (2.8 g., 65%), m.p. 85.5-87.5°, ν max. (nujol) 3320, 1730, 1710 cm.$^{-1}$.

(Found: C, 49.41; H, 6.05; N, 6.60; Cl, 15.99. $C_{18}H_{26}N_2O_4Cl_2S$ requires C, 49.50; H, 6.00; N, 6.41; Cl, 16.22%).

N-(p-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-ethionine (72).

The methyl ester prepared above (2.5 g.) was added to a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid (25 ml.) and the solution was heated under reflux (5 min.). The mixture was cooled, diluted with ice-cold water, and neutralised to pH4 by the addition of ION sodium hydroxide. The product was extracted

with chloroform (ca. 150 ml.) and, after working up in the usual way, the free acid was isolated as a solid. This was recrystallised from ethyl acetate-petroleum (60-80°), affording white crystals of the title compound (1.0 g., 41%), m.p. 137.5-139.5°. (Found: C, 48.04; H, 5.76; N, 6.44; Cl, 16.75. $C_{17}H_{24}N_2O_4Cl_2S$ requires C, 48.22; H, 5.71; N, 6.62; Cl, 16.75%).

CHAPTER 12

SOME DERIVATIVES OF AROMATIC AMINO ACIDS.

Methyl m-aminobenzoate (97, 383)

m-Aminobenzoic acid was esterified with hydrogen chloride and methanol according to the method of Fischer [111]. The methyl ester hydrochloride was isolated, m.p. 202° (lit.[131] m.p. 200-202°), and this was neutralised with ammonia solution to give the free base. After working-up and purifying, the title product was obtained in the form of light-brown crystals, m.p. 34° (lit.[131] m.p. 36-38°).

Methyl m-isocyanatobenzoate (99).

Dry benzene (250 ml.) was saturated with phosgene at room temperature, and to the stirred solution was added a solution of methyl m-aminobenzoate (9 g.) in benzene (50 ml.), slowly, dropwise. A brown precipitate was formed, and after stirring this (½ hr.), the excess phosgene was removed by nitrogen. The mixture was then heated under reflux and the benzene was removed under reduced pressure. The resulting oil was distilled to give the title product as a viscous, colourless liquid (8.3 g., 79%), b.p. 103-104°/3.0 mm. (lit.[132] b.p.

125-128°/10 mm.). After standing overnight, the liquid crystallised out to give a white solid, m.p. 35-36°.

p-(NN-Di-2-chloroethylamino)phenyl N'-m-methoxycarbonylphenylcarbamate (101).

p-(NN-Di-2-chloroethylamino)phenol hydrochloride (2.7 g.) and dry triethylamine (1.3 g.) were stirred in dry benzene (50 ml.). The isocyanate prepared above (1.93 g.) was added and the mixture was heated under reflux (5½ hr.). The solution was filtered, and the residue on the filter was seen to contain more than triethylamine hydrochloride. This was therefore washed several times with water in order to remove all the base hydrochloride. The melting points of the remaining solid and that obtained from the benzene solution (which was worked-up in the usual way) were identical (ca.167°) and so the two were combined and recrystallised from ethyl acetate-carbon tetrachloride-petroleum (60-80°) to give white crystals of the title compound (2.5 g.), m.p. 167-168°, ν max. (chloroform) 3420, 1740, 1715 cm.$^{-1}$.
(Found: C. 55.59; H, 5.11; N, 6.67; Cl, 16.89. $C_{19}H_{20}N_2Cl_2O_4$ requires C, 55.49; H, 4.90; N, 6.81; Cl, 17.24%).

p-(NN-Di-2-chloroethylamino)phenyl N'-m-carboxyphenylcarbamate (85).

The methyl ester prepared above (250 mg.) was dissolved in a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid (5 ml.), and the solution was heated under reflux (15 min.). The solution was cooled in ice, poured into excess cold water, and the resulting white precipitate was filtered off. The free acid was recrystallised from petrol (60-80°)-benzene-ethyl acetate to yield white crystals of the title compound, m.p. 196-197°, ν max. (nujol) 3300 $cm.^{-1}$, 1705 $cm.^{-1}$, 1675 $cm.^{-1}$. (Found: C, 54.20, H, 4.31; N, 7.14; Cl, 17.53. $C_{18}H_{18}N_2Cl_2O_4$ requires C, 54.29, H, 4.53; N, 7.03, Cl, 17.80%).

Methyl o-isocyanatobenzoate (74, 103).

Methyl anthranilate (7 g.) in dry benzene (5 ml.) was added drop-wise (30 min.) to a saturated solution of phosgene in benzene (20 ml.). Phosgene was passed through the reaction mixture throughout the addition and for a further 30 min. A white precipitate was formed, but this disappeared after heating the mixture

under reflux. The solvent was removed and the isocyanate was distilled under vacuum. This proved difficult since polymerisation occurred and the product was very viscous. The title compound was, however, isolated as a white crystalline solid, b.p. 90-110°/1.5-3.0 mm. (lit.[133] b.p. 125°/10 mm.), m.p. 39°.

p-(NN-Di-2-chloroethylamino)phenyl N'-o-methoxycarbonylphenylcarbamate (76, 105).

p-(NN-Di-2-chloroethylamino)phenol hydrochloride (2.7 g.) was stirred in dry benzene (80 ml.) and dry triethylamine (1.4 g.) was added. After liberating the free base, the isocyanate prepared above (1.93 g.) was added and the mixture was heated under reflux (4½ hr.). The reaction mixture was worked-up in the same manner as that adopted for similar urethanes, and an orange-yellow, crystalline solid was obtained. This was recrystallised from benzene-petrol (60-80°) to afford white crystals of the title compound (2.8 g., 63%) m.p. 136-138°, ν max. 3300 cm.$^{-1}$, 1725 cm.$^{-1}$, 1680 cm.$^{-1}$.

(Found: C, 55.64; H, 4.88; N, 6.93; Cl, 17.02. $C_{19}H_{20}N_2Cl_2O_4$ requires C, 55.49; H, 4.90; N, 6.81; Cl, 17.24%).

CHAPTER 13

SOME CARBAMATES DERIVED FROM m-(NN-DI-2-CHLOROETHYLAMINO)PHENOL

(a) Via m-aminophenol

m-(NN-Di-2-hydroxyethylamino)phenol (92, 93, 116).

m-Aminophenol (20 g.) was added to a mixture of water (30 ml.) and glacial acetic acid (20 ml.). The suspension was stirred and cooled in ice, after which liquid ethylene oxide (30 ml.) was added. After 1 hr., more ethylene oxide (15 ml.) and water (30 ml.) were added, and the clear solution was left stirring for a further 2 hrs., after which it was kept at 0° overnight. The acid was then neutralised with sodium bicarbonate and the product was extracted with ethyl acetate. After drying and removing the solvent, a pale yellow oil was left which, after trituration with petroleum, afforded a white solid. Recrystallisation from ethyl acetate afforded the title compound (28.8 g., 80%), m.p. 72-73° ν max. (nujol) 3000-3500 $cm.^{-1}$ (broad).

(Found: C, 60.75; H, 7.51; N, 6.95.

$C_{10}H_{15}NO_3$ requires C, 60.89; H, 7.67; N, 7.10%).

m-(NN-Di-2-hydroxyethylamino)phenyl benzyl ether (107)

The triol prepared above (24 g.) was dissolved in ethanol (150 ml.) to which was added solid potassium hydroxide (6.82 g.). The mixture was stirred and warmed, after which a solution of benzyl bromide (20.81 g.) in ethanol (25 ml.) was added drop-wise. The mixture was heated under reflux (4 hrs.) and was then found to be non-basic. The inorganic salt was filtered off and the filtrate was concentrated to give a dark-red oil which could not be crystallised. A chromatographic purification was attempted using silica gel and ethyl acetate as the eluting agent. In this manner, three components were obtained, but it proved impossible to crystallise any of these.

m-aminophenyl benzyl ether (150, 154)

The method due to Cortes and Walls[118] was adopted here. m-Aminophenol (27.5 g.) was dissolved in methanol (250 ml.) and a solution of sodium hydroxide (20 g.) in water (50 ml.) was added. The mixture was evaporated to dryness and heated (ca.150°) under vacuum for 1 hr. The sodium salt was dissolved in D.M.F. (350 ml.) to which was added drop-wise a solution of benzyl chloride (31.5 g.) in D.M.F. (50 ml.). The mixture was stirred overnight, and the inorganic salt was filtered off. A large volume of benzene was added to

the brown filtrate which was then shaken with 10% sodium hydroxide solution. The benzene layer was removed, dried, and concentrated to give a yellow oil which subsequently crystallised out to yield pale yellow crystals.
A recrystallisation with benzene–petroleum (80-100°) afforded white crystals of the title product (19.1 g., 38%), m.p. 61-62.5° (lit.[118] m.p. 64°).

m-(NN-Di-2-hydroxyethylamino)phenyl benzyl ether (156, 158, 184, 385).

The amino-ether prepared above (2 g.) was stirred in a 1:1 mixture of glacial acetic acid and water (10 ml.), the whole being cooled in an ice-bath. To the suspension was added liquid ethylene oxide (5 ml.) and 2 hrs. later, a further 5 ml. was added. The solution was left stirring for 9 hrs. after which time the acid was neutralised with sodium bicarbonate solution and the product was extracted with chloroform which was washed and dried. After removing the solvent, a pale-yellow oil was obtained. Trituration with petroleum gave a white solid which was recrystallised from benzene-petroleum (80-100°) to afford white needles of the title product (2 g., 69%), m.p. 58-59°.
(Found: C, 70.80; H, 7.14; N, 4.67.
$C_{17}H_{21}NO_3$ requires C, 71.04; H, 7.37; N, 4.87%).

(b) Via m-nitrophenol

m-Nitrophenyl benzyl ether[119] (140, 347, 434).

To a solution of sodium (7.7 g.) in absolute ethanol (150 ml.) was added a solution of m-nitrophenol (47 g.) in absolute ethanol (120 ml.). Benzyl chloride (42 g.) was added to the red mixture and the whole was stirred and heated under reflux (16 hrs.). After filtering off the sodium chloride, the product began to crystallise out. Water was added to complete the precipitation of the product which was subsequently washed with 5% sodium hydroxide solution (300 ml.). The benzyl ether was recrystallised from chloroform-petroleum (40-60°) to afford yellow crystals of the title compound (52,5 g., 68%), m.p. 55° (lit.[119] m.p. 57°).

m-Aminophenyl benzyl ether

(i) Using Raney nickel[119] (144, 146, 200)

The nitro-ether prepared above (35 g.) was dissolved in absolute ethanol (700 ml.) to which freshly-prepared Raney nickel (7 g.) was added. The mixture was placed in an autoclave and was treated with hydrogen, at an initial pressure of 50 atmospheres, for 5 days, at room temperature. The solution was removed, filtered through

charcoal, and finally concentrated, to leave an oil which crystallised out to give a yellow solid. The product was then dissolved in ether and filtered to remove some insoluble material, the ether being subsequently evaporated under reduced pressure. The resulting solid was recrystallised from benzene-petroleum (40-60°) to yield the title compound (23.5 g., 77%), m.p. 62° (lit.[119] m.p. 64°).

(ii) Using hydrazine[121] (201).

m-Nitrophenyl benzyl ether (58 g.) was dissolved in ethanol (600 ml.) and excess hydrazine hydrate (30 g.) was added. The mixture was warmed on a water-bath after which 10% palladised charcoal (200 mg.) was slowly added. After the initial vigorous reaction had subsided, a further 100 mg. of catalyst was added and the mixture was heated under reflux for several hours. The catalyst was then removed by filtration over a mixture of celite and charcoal and the filtrate was concentrated. The organic residue was shaken with water to remove excess hydrazine, and extracted with ether. The ether was in turn shaken with dilute hydrochloric acid to separate the amine from any unchanged m-nitrophenyl benzyl ether. The aqueous portion was neutralised and the product was again extracted

with ether which was subsequently washed, dried, and removed under reduced pressure, leaving yellow crystals of the title product m.p. 63° (lit.[119] m.p. 64°).

The product was treated with ethylene oxide as before to afford m-(NN-di-2-hydroxyethylamino)phenyl benzyl ether in 77% yield).

m-(NN-Di-2-chloroethylamino)phenyl benzyl ether

(a) Attempted preparation using phosphorous pentachloride (160, 162, 172).

A solution of m-(NN-di-2-hydroxyethylamino)phenyl benzyl ether (4.1 g.) in chloroform (15 ml.) was added drop-wise to a stirred suspension of phosphorus pentachloride (5 g.) in chloroform (15 ml.). After the initial vigorous reaction, the mixture was heated under reflux (3 hrs.) and then neutralised and extracted with chloroform. The chloroform was dried and removed to leave a yellow oil which was passed in benzene through a column of alumina. Concentration of the colourless eluates provided a pale-yellow oil which could not be crystallised. An i.r. spectrum showed no hydroxyl absorption. An n.m.r. spectrum (deuteriochloroform) showed a multiplet at 2.5-3.8 τ (9H), a singlet at 4.9 τ (2H) and a multiplet at 6.4 τ (8H).

(Found: Cl, 25.23. $C_{17}H_{19}NOCl_2$ requires Cl, 21.87%).

The i.r. and n.m.r. spectra support the idea that the

product is the chloro-phosphorus compound((93), p. 84) $C_{17}H_{19}NO_3PCl_3$ requires Cl, 25.18%.

(b) Using phosphoryl chloride (178, 188, 194, 198, 468, 496)

m-(NN-Di-2-hydroxyethylamino)phenyl benzyl ether (1.086 g.) was suspended in dry benzene (10 ml.) and to the stirred suspension was added phosphoryl chloride (574 mg.). the mixture was heated under reflux overnight, after which a small amount of insoluble material was observed. The solution was filtered and the filtrate was washed with sodium bicarbonate solution, water, and dried. After concentrating the solution, an oil was obtained and this was purified on a silica plate (Rf. 0.9 in benzene). The resulting oil could not be crystalised and was therefore distilled to yield the title product as a pale-yellow, viscous oil, b.p. ca. $230^{\circ}/5x10^{-4}$mm. The i.r. spectrum showed no hydroxyl absorption.
(Found: C, 63.28; H. 6.06; N, 4.43; Cl, 21.54.
$C_{17}H_{19}NOCl_2$ requires C, 62.96; H, 5.91; N, 4.32; Cl,21.86%).

m-(NN-Di-2-chloroethylamino)phenol

(a) Attempted preparation by hydrogenation (492)

The benzyl ether prepared above was dissolved in ether through which was bubbled hydrogen chloride. After removing the ether, a pink oil was left and this formed a sticky solid after being left under vacuum.
The hydrochloride (2 g.) was dissolved in ethanol (20 ml.) to which 5% palladised charcoal (100 mg.) was added. The solution was treated with hydrogen at atmospheric pressure (4 days) after which the catalyst was removed by filtration, and the filtrate was concentrated to leave a light-green amorphous solid, ν max. (nujol) 2400-2800, 3100-3500 (weak) cm^{-1}. The hydrochloride was neutralised and worked-up to leave the free base as an oil. An n.m.r. spectrum showed that the benzyl group was still present.

(b) Successful preparation by hydrolysis (209, 213, 470, 472, 522)

The benzyl ether prepared above (1 g.) was dissolved in a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid (10 ml.), and the whole was heated under reflux (3½ hrs.) after which the solution was poured onto

ice, neutralised with sodium bicarbonate, and extracted with chloroform. The chloroform solution was shaken with 10% sodium hydroxide solution (2 x 20 ml.) and the aqueous portion was neutralised with dilute hydrochloric acid. The product was again extracted with chloroform which was worked up in the usual way to afford the phenol as a viscous oil which could not be crystallised. The i.r. spectrum showed a broad band in the hydroxyl region and the n.m.r. (deuteriochloroform) showed a multiplet at 2.7-3.9 τ (4H), a broad singlet at 5.0 τ (1H, removed by D_2O), and a singlet at 6.4 τ (8H). The product was not characterised, but used directly to form derivatives as described below.

m-(NN-Di-2-chloroethylamino)phenyl N'-m-methoxycarbonylphenyl-carbamate (530)

m-(NN-Di-2-chloroethylamino)phenol (2.91 g.) and methyl m-isocyanatobenzoate (2.21 g.) were dissolved in dry benzene (50 ml.) to which was added dry pyridine (100 mg.) as a catalyst. The mixture was refluxed overnight after which the benzene was removed by evaporation to leave a red oil. The oil crystallised on prolonged trituration with light petroleum to yield a pink solid, which was dissolved in benzene and filtered to remove some insoluble material.

Petroleum (40-60°) was added to precipitate the product which was recrystallised several times from benzene-petroleum (40-60°) to afford white crystals of the title product (2 g.), m.p. 146-148°, ν max. (nujol) 3300, 1725, 1700 cm.$^{-1}$.

(Found: C, 55.51; H, 5.05; N, 6.65; Cl, 16.70. $C_{19}H_{20}N_2Cl_2O_4$ requires C, 55.49; H, 4.90; N, 6.81; Cl, 17.24%).

m-(NN-Di-2-chloroethylamino)phenyl N'-m-carboxyphenylcarbamate (534)

The methyl ester prepared above (500 mg.) was added to a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid (10 ml.) and the whole was heated under reflux (1 hr.). The solution was cooled and poured into iced water whereupon a white precipitate of the free acid was formed. The solid was filtered, washed and dried, and recrystallised from chloroform-petroleum (60-80°) to yield white crystals of the title compound (200 mg.) m.p. 161-163°, ν max. (nujol) 3280, 1710, 1670 cm.$^{-1}$.

(Found: C, 54.59; H, 4.63; N, 7.01, Cl, 17.88. $C_{18}H_{18}N_2O_4Cl_2$ requires C, 54.29; H, 4.53; N, 7.03; Cl, 17.80%).

m-(NN-Di-2-chloroethylamino)phenyl N'-p-methoxycarbonyl-phenylcarbamate (554)

m-(NN-Di-2-chloroethylamino)phenol (2.33 g.) and methyl p-isocyanatobenzoate (1.77 g.) were dissolved in anhydrous benzene (40 ml.) to which was added dry pyridine (200 mg.) as a catalyst. The mixture was heated under reflux overnight after which the solvent was removed by evaporation. The resulting dark-red oil was triturated with light petroleum but could not be crystallised. The oil was passed in chloroform through a column containing silica gel and evaporation of the eluates afforded a red oil which was crystallised with difficulty by trituration with petroleum. A recrystallisation from ethanol-petroleum (40-60°) yielded the desired carbamate in poor yield, m.p. 132-134°.
(Found: C, 55.77; H, 5.17; N, 6.80.
$C_{19}H_{20}N_2Cl_2O_4$ requires C, 55.49; H, 4.90; N, 6.81%).

N-(m-NN-Di-2-chloroethylamino)phenoxycarbonyl-DL-ethionine methyl ester (538)

m-(NN-Di-2-chloroethylamino)phenol (1.76 g.) and methyl α-isocyanato-γ-(ethylthio)butyrate (1.62 g.) were added to dry benzene (40 ml.) to which was then added dry pyridine (100 mg.) as catalyst. The mixture was refluxed overnight

and then worked-up in the usual way to yield a yellow oil which could not be crystallised. The oil was dissolved in chloroform and chromatographed using silica gel and chloroform as the eluting agent. Concentration of the eluates provided a pale-yellow oil which was crystallised after prolonged trituration with light petroleum. The product was recrystallised from chloroform-petroleum (60-80°) to afford white crystals of the title compound in poor yield, m.p. 82-84°, ν max. (nujol) 3300, 1725, 1700 cm.$^{-1}$.

(Found: C, 49.60; H, 5.95; N, 6.35; Cl, 7.60. $C_{18}H_{26}N_2O_4Cl_2S$ requires C, 49.43; H, 5.99; N, 6.40; Cl, 7.33%)

CHAPTER 14

SOME CARBAMATES DERIVED FROM o-(NN-DI-2-CHLOROETHYLAMINO)PHENOL

o-Nitrophenyl benzyl ether[119] (168).

Potassium metal (11.7 g.) was dissolved in absolute ethanol (150 ml.) and the solution of potassium ethoxide was added to a yellow solution of o-nitrophenol (41.7 g.) in absolute ethanol (120 ml.), whereupon an orange precipitate was immediately formed. Benzyl chloride (42.2 g.) was added to the mixture which was then heated under reflux (20 hr.). After cooling, the inorganic salt was filtered off and the filtrate was concentrated to an oil. The oil was washed with 5% potassium hydroxide solution, the aqueous portion being subsequently shaken with ether. The oily products and ethereal extracts were combined, dried, and concentrated to a yellow oil which was distilled under vacuum to yield the title product as a yellow liquid (55 g. 82%, lit.[119] yield 78%), b.p. $100^{\circ}/10^{-2}$mm. (lit.[119] b.p. 181-182°/3 mm.).

o-Aminophenyl benzyl ether from the nitro compound (180).

The nitro compound prepared above (45 g.) was dissolved in ethanol (450 ml.) to which was added hydrazine hydrate in excess (23 g.). Palladised charcoal (150 mg.) was

added as catalyst and after the initial reaction had subsided, a further 100 mg. of catalyst was added. The mixture was heated under reflux for several hours and then worked-up in a manner analagous to that employed in the preparation of m-aminophenyl benzyl ether. The amine was isolated as an oil which was distilled under vacuum to afford the title compound as a pale-yellow liquid (28.8 g., 74%), b.p. 110-120°/0.1 mm. (lit.[119] b.p. 157°/3 mm.).

Reaction of o-aminophenyl benzyl ether with ethylene oxide (430).

The amine prepared above (20 g.) was added to a 1:1 mixture of glacial acetic acid and water (100 ml.) and the whole was stirred in an ice-bath until a temperature of 2° had been reached. Liquid ethylene oxide (50 ml.) was added and the mixture was stirred in ice for several hours after which it was left stirring overnight. The acid was neutralised with sodium bicarbonate and the product was extracted with ethyl acetate; the extract was washed and dried. After removing the solvent, a brown oil was obtained, which failed to crystallise. A distillation of the product was attempted but only resulted in decomposition.

o-Aminophenyl benzyl ether from o-aminophenol (164).

The method of Cortes and Walls[118], was adopted here. o-Aminophenol (27.5g.) was dissolved in methanol (250 ml.) to which was added a solution of sodium hydroxide (20 g.) in water (50 ml.). The dark-red mixture was evaporated and dried under vacuum (100°) for several hours. The solid sodium salt was dissolved in D.M.F. (250 ml.) which was warmed to effect complete dissolution. The solution was stirred, and to it was added, drop-wise, benzyl chloride (37.5 g.). After the addition the mixture was left stirring at 70° overnight. The inorganic salt was filtered off and benzene (650 ml.) was added to the filtrate, which was then shaken with 10% sodium hydroxide solution (500 ml.). The benzene layer was washed, dried, and concentrated to a dark red liquid. Distillation under vacuum afforded three fractions: (a) b.p. 40°/0.3 mm., (b) b.p. 80°/0.3 mm., (c) b.p. 148°/0.3 mm. (lit.[119] b.p. 157°/3 mm.). Only fraction (c) contained in its i.r. spectrum a doublet at 3400 $cm.^{-1}$ corresponding to the amino group, but the yield was very small.

o-(NN-Di-2-hydroxyethylamino)phenol (221, 341, 389, 516).

o-Aminophenol (21 g.) was added to a mixture of water (140 ml.) and glacial acetic acid (70 ml.) and the whole was stirred in an ice-bath until the temperature had fallen to 0°. Liquid ethylene oxide (105 ml.) was added and the mixture was stirred in ice for 6 hr. after which it was left stirring overnight. After working-up in the usual way, a reddish-brown oil was obtained. The oil was triturated with petroleum and left at 0° overnight, after which a sticky solid was obtained (18 g. crude) which could not be crystallised. A small sample was distilled under vacuum to afford the product as a pale yellow, viscous oil, which was found to be highly deliquescent, b.p. ca.150°/10^{-4}mm.
(Found: C, 59.82, H, 7.23; N, 6.57.
$C_{10}H_{15}NO_3$ requires C, 60.89; H, 7.67; N, 7.10%).
The triol (250 mg.) was treated with p-nitrobenzoyl chloride (800 mg.) in pyridine (5 ml.) and characterised as its tris-p-nitrobenzoate which was recrystallised from glacial acetic acid to yield yellow crystals, m.p. 179°.
(Found: C, 57.54; H, 3.93; N, 8.54.
$C_{31}H_{24}N_4O_{12}$ requires C, 57.75; H, 3.75; N, 8.69%).

o-(NN-Di-2-hydroxyethylamino)phenyl benzyl ether (345, 393, 444, 448, 520).

The triol prepared above (2.0 g.) was dissolved in ethanol (25 ml.) to which was added potassium hydroxide (0.57 g.). To the mixture was added benzyl bromide (1.71 g.), and the whole was heated under reflux (3 hr.) after which the mixture was poured into cold water. The oily product did not crystallise and was extracted with ether, which was subsequently washed and dried. After removing the solvent, a brown uncrystallisable oil was left and this was chromatographed on an alumina column using ethyl acetate as the eluting agent. Concentration of the eluates afforded the title product as an oil. A portion was treated with p-nitrobenzoyl chloride in pyridine and thus characterised as a bis-p-nitrobenzoate which was recrystallised from chloroform-petroleum (60-80°) to yield yellow crystals, m.p. 99-101°.

(Found: C, 63.32; H, 5.00; N, 6.98.
$C_{31}H_{27}N_3O_9$ requires C, 63.56; H, 4.65; N, 7.17%).

o-(NN-Di-2-chloroethylamino)phenyl benzyl ether (450, 526).

The crude diol prepared above (21 g.) was dissolved in chloroform (140 ml.) to which phosphorus pentachloride (34.3 g.) was added. The mixture was heated under reflux overnight during which time considerable bumping occurred so that a large proportion of the product was lost. After working-up in the usual way, a brown oil was obtained. The oil was passed in ethyl acetate through a column of alumina. Concentration of the eluates afforded the dichloro-compound as a pale-yellow oil. This was characterised by treatment of a portion with an ethanolic solution of picric acid to give the picrate which was recrystallised from ethanol to yield yellow crystals, m.p̂. 115-118°.
(Found: C, 50.07; H, 4.23; N, 10.02; Cl, 12.31. $C_{23}H_{22}N_4Cl_2O_8$ requires C, 49.94; H, 4.01; N, 10.13; Cl, 12.81%).

o-(NN-Di-2-chloroethylamino)phenol hydrochloride (548).

The mustard benzyl ether prepared above (10 gm.) was dissolved in methanol (100 ml.) to which was added 10% palladised charcoal (250 mg.), and a few drops of concentrated hydrochloric acid. The mixture was

treated with hydrogen at atmospheric pressure overnight, after which the solution was filtered and evaporated. The resulting red oil was passed in benzene through a column of silica gel. The colourless eluates were evaporated to a small volume, and hydrogen chloride was bubbled through the solution. A colourless oil was precipitated and after decanting off the solvent and triturating with petroelum, the hydrochloride was formed as a white solid. A recrystallisation from ethanol-petroleum (40-60°) afforded colourless, orthorhombic crystals of the title compound (4 g., 50%), m.p. 102-105°.

(Found: C, 44.59; H, 5.40; N, 5.18; Cl, 39.12. $C_{10}H_{14}NOCl_3$ requires C, 44.38; H, 5.21; N, 5.18; Cl, 39.32%).

o-(NN-Di-2-chloroethylamino)phenyl N'-p-methoxycarbonylphenylcarbamate (550).

o-(NN-Di-2-chloroethylamino)phenol hydrochloride (270 mg.) and anhydrous triethylamine (120 mg.) were shaken together in dry benzene (10 ml.) until the free base had been liberated. p-Methoxycarbonylphenyl isocyanate (179 mg.) was added and the mixture was heated under reflux overnight. The triethylamine hydrochloride was removed by filtration and the filtrate was worked-up in the usual manner to yield a pale-brown oil, which was dissolved in chloroform and subjected to column chromatography (silica gel). Concentration of the colourless eluates afforded a white solid which was recrystallised from chloroform-petroleum (40-60°) to give white crystals of the title compound (120 mg.), m.p. 157-158°, ν max. (nujol) 3350, 1715 (broad) $cm.^{-1}$. (Found: C, 55.77; H, 4.79; N, 6.66; Cl, 17.15. $C_{19}H_{20}N_2Cl_2O_4$ requires C, 55.49; H, 4.90; N, 6.81; Cl, 17.24%).

o-(NN-Di-2-chloroethylamino)phenyl N'-p-carboxyphenyl-carbamate (552)

The methyl ester prepared above (100 mg.) was added to concentrated hydrochloric acid (2 ml.) and glacial acetic acid (4 ml.). The mixture was heated under reflux (1 hr.), cooled, and poured into cold water. The free acid formed a white precipitate which was filtered off and recrystallised from ethanol-water to give white crystals of the acid, m.p. 193-193.5°, ν max. (nujol) 3300, 1710, 1680 cm^{-1}.
(Found: C, 54.51: H, 4.78; N, 7.02; Cl, 17.52. $C_{18}H_{18}N_2Cl_2O_4$ requires C, 54.29; H, 4.53; N, 7.03; Cl, 17.80%).

CHAPTER 15

SOME CARBAMATES DERIVED FROM p-(N-METHYL-N-2-CHLOROETHYLAMINO)PHENOL

p-(N-Methyl-N-acetyl)aminophenol[122] (305,307,317,327,476)

Metol (34 g.) was dissolved in water (450 ml.) at 45°, and acetic anhydride (46 ml.) was added in one portion with stirring. A solution of crystalline sodium acetate (60 g.) in water (180 ml.) was added to the mixture and a white solid began to crystallise out. The reaction mixture was left in ice (½ hr.) after which the acetyl derivative was filtered off and recrystallised from aqueous ethanol to give white crystals of the title product (24.3 g., 74%), m.p. 245-246° (lit.[122] m.p. 246-247°).

p-(N-Methyl-N-acetylamino)phenyl benzyl ether[122] (309,319).

The phenol prepared above (24.3 g.) was dissolved in a solution of sodium (3.46 g.) in absolute ethanol (150 ml.). The solution was heated under reflux while benzyl chloride (19.2 g.) was added drop-wise. After 5 hours, ca. 120 ml. of solvent was removed by evaporation and the residue was poured into cold water (400 ml.). The benzyl ether was filtered off and recrystallised from aqueous ethanol to yield white crystals of the title product, m.p. 121-122° (lit.[122] m.p. 122°).

p-(N-Methyl)aminophenyl benzyl ether[122] (321).

The acetyl derivative prepared above was dissolved in a warm solution of potassium hydroxide (42 g.) in 90% ethanol (400 ml.). The mixture was heated under reflux (16 hr.) after which 360 ml. of the solvent was removed by evaporation. The resulting yellow emulsion was extracted with ether (3 x 60 ml.) and the extracts were dried and concentrated to give a yellow oil. The oil was distilled to afford the title compound as a pale yellow liquid (25 g., 78.5% from p-(N-methyl-N-acetyl)aminophenol, lit.[122] yield 54%), b.p. 140-160°/ca. 2 x 10^{-3}mm. (lit.[122] b.p. 204-209°/11-12 mm.), n_D^{22}1.6026 (lit.[122] n_D^{20}1.6043).

p-(N-Methyl-N-2-hydroxyethylamino) phenyl benzyl ether.

(a) Attempted preparation (488).

The amine prepared above (5 g.) was added to chloroform (50 ml.) and glacial acetic acid (10 ml.). The mixture was stirred in an ice-bath and liquid ethylene oxide (20 ml.) was added. The whole was stirred in ice for several hours and then left stirring overnight. Both the chloroform and acetic acid were then removed under vacuum to leave a brown oil. Prolonged trituration with petroleum gave a pink solid which tended to revert to an oil on exposure to the atmosphere. The solid was dissolved in warm ethanol to which ether was added until incipient cloudiness was observed. A white crystalline product was formed and this after recrystallisation from chloroform had m.p. 74°, (5 g.). The i.r. spectrum showed a strong hydroxyl absorption and a strong, broad band in the 1540-1600 $cm.^{-1}$ region.

(Found: C, 61.27; H, 7.46; N, 3.43.

$C_{16}H_{19}NO_2$ requires C, 74.69; H, 7.44; N, 5.45%).

The structure of this compound has not been elucidated.

(b) Successful preparation (323, 325, 331, 482, 484, 518).

The amine prepared previously (1 g.) was added to a solution of glacial acetic acid (10 ml.) and water (20 ml.), and the stirred suspension was cooled in an ice-bath. Liquid ethylene oxide (10 ml.) was added and the mixture was stirred in ice for several hours after which it was left stirring overnight. The solution was neutralised with sodium bicarbonate and extracted with ethyl acetate. The solvent was washed, dried, and concentrated to give a brown solid in poor yield. The product was recrystallised from benzene-petroleum (40-60°) to afford light-brown plates of the title compound, m.p. 67-68°, ν max. (nujol) 3300 cm.$^{-1}$ (broad). (Found: C, 74.84; H, 7.35; N, 5.31. $C_{16}H_{19}NO_2$ requires C, 74.69; H, 7.44; N, 5.45%).

p-(N-Methyl-N-2-chloroethylamino)phenyl benzyl ether (502).

The hydroxy compound prepared above (1.54 g.) was dissolved in dry benzene (20 ml.) to which was added phosphoryl chloride (320 mg.), and the mixture was heated under reflux overnight. The yellow benzene solution was decanted off from a brown viscous residue which was found to be soluble in sodium bicarbonate solution. The benzene solution

was washed with sodium bicarbonate, water, dried and finally concentrated to give a reddish-brown oil which crystallised on standing. The solid was recrystallised from aqueous methanol, and finally from petroleum (40-60°) yielding white crystals of the title compound (1.0 g.), m.p. 121.5-122.5°.
(Found: C, 70.00; H, 6.32; N, 4.84; Cl, 12.86. $C_{16}H_{18}NOCl$ requires C, 69.68; H, 6.58; N, 5.08; Cl, 12.85%).

p-(N-Methyl-N-2-chloroethylamino)phenol hydrochloride (538).

p-(N-Methyl-N-2-chloroethylamino)phenyl benzyl ether (1 g.) was dissolved in methanol (20 ml.) and palladised charcoal (100 mg., 5%) was added to the solution, which was subsequently hydrogenated at room temperature overnight. The reaction mixture was then filtered and evaporated under reduced pressure. The resulting oil was dissolved in benzene and the colourless solution was decanted off from a small amount of brown insoluble material. Hydrogen chloride gas was bubbled through the solution, whereupon a colourless oil separated out. After standing overnight the hydrochloride crystallised out and was recrystallised from ethanol-petroleum (40-60°) to yield

white crystals of the title compound (400 mg. 50%), m.p. 135-140°.
(Found: C, 48.40; H, 6.13; N, 6.48; Cl, 31.85. $C_9H_{13}NOCl_2$ requires C, 48.66; H, 5.90; N, 6.31; Cl, 31.93%).

p-(N-Methyl-N-2-chloroethylamino)phenyl N'-p-methoxycarbonylphenylcarbamate (540).

p-(N-Methyl-N-2-chloroethylamino)phenol hydrochloride (110 g.) and pyridine (50 mg.) were stirred in dry benzene (5 ml.) for 30 minutes in order to liberate the free base. To the mixture was added p-methoxycarbonylphenyl isocyanate (88 mg.) and the whole was stirred overnight at room temperature. The pyridine hydrochloride was filtered off and the filtrate was heated under reflux (3 hrs.). The solvent was then removed under reduced pressure, leaving a white solid. The product was recrystallised from chloroform-petroleum (40-60°) to yield white crystals of the title compound (150 mg., 83%), m.p. 174-175°, ν max. (nujol) 3300, 1710, 1700 $cm.^{-1}$.
(Found: C, 59.66; H, 5.44; N, 7.79; Cl, 9.84. $C_{18}H_{19}N_2O_4Cl$ requires C, 59.60; H, 5.28; N, 7.72; Cl 9.77%).

p-(N-Methyl-N-2-chloroethylamino)phenyl N'-p-carboxyphenylcarbamate (544).

The methyl ester prepared above (100 mg.) was added to a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid (5 ml.) and the whole was heated under reflux (1 hr.). The mixture was then cooled and poured over ice and the solid precipitate was filtered off and dried. A recrystallisation from ethanol-water afforded white orthorhombic crystals of the title product, m.p. 201-202°, ν max. (nujol) 3250, 1740, 1670 cm.$^{-1}$.
(Found: C, 58.45; H, 5.05; N, 8.15,; Cl, 10.02. $C_{17}H_{17}N_2O_4Cl$ requires C, 58.53; H, 4.91; N, 8.03; Cl, 10.16%).

p-(N-Methyl-N-2-chloroethylamino)phenyl N'-o-methoxycarbonylphenylcarbamate (546).

p-(N-Methyl-N-2-chloroethylamino)phenol hydrochloride (74 mg. and o-methoxycarbonylphenyl isocyanate (57 mg.) was added to dry benzene (5 ml.). Dry triethylamine (50 mg.) was added and the mixture was stirred until the free phenol was liberated, after which the whole was heated

under reflux (18 hrs.). After cooling, the mixture was filtered to remove triethylamine hydrochloride and the benzene was removed under reduced pressure to yield an oil, which crystallised out on trituration with light-petroleum. Recrystallisation from benzene-petroleum (40-60°) and chloroform-petroleum (40-60°) afforded white crystals of the title compound (60 mg.), m.p. 100-102°, ν max. (nujol) 3250, 1730, 1680 $cm.^{-1}$.
(Found: C, 59.86; H, 5.25; N, 7.53; Cl, 9.97. $C_{18}H_{19}N_2O_4Cl$ requires C, 59.60; H, 5.28; N,7.72; Cl, 9.77%).

CHAPTER 16

SOME CARBAMATES DERIVED FROM p-AMINOPHENACYL CHLORIDE

p-Chloroacetyl-acetanilide[126] (235, 363)

To a mixture of dry carbon disulphide (70 g.), acetanilide (10 g.) and chloroacetyl chloride (15 g.) was added, slowly, powdered aluminium chloride (35 g.). After the initial vigorous reaction, the mixture was heated under reflux (60°, 14 hrs.). The solvent was then decanted off leaving a red, viscous oil which was subsequently poured into iced water. The yellow precipitate which formed was filtered off and washed with a small amount of ethanol. A recrystallisation from chloroform-ethanol afforded light-brown crystals of the title product (11.4 g., 73%), m.p. 210° (lit.[126] m.p. 212°).

p-Aminophenacyl chloride[126] (239, 367)

The acetyl derivative prepared above (11.4 g.) was added to a solution of 16% hydrochloric acid (580 ml.) and the mixture was heated at 100° (90 min.), after which time the solid had passed into solution. The brown solution was then cooled and neutralised with sodium bicarbonate, giving a light brown precipitate of the free amine. The solid was recrystallised from ethanol-benzene to yield light-brown crystals of the title compound (6.4 g., 67%), m.p. 144° (lit.[126] m.p. 147°).

o-Methoxycarbonylphenyl chloroformate (251)

Methyl o-hydroxybenzoate was converted into the corresponding chloroformate according to the method of Einhorn and Bagh[127]. To an ice-cooled, 20% solution of phosgene in benzene (160 ml.), was added, drop-wise, with stirring, a solution of methyl salicylate (40 g.) and dry quinoline (34 g.) in benzene (80 ml.). The mixture was stirred overnight and then poured into water to dissolve the quinoline hydrochloride. The benzene layer was washed with sodium bicarbonate solution, water, dried and concentrated to leave the liquid chloroformate. A distillation afforded the title product as a colourless liquid (48.9 g., 87%), b.p. ca. 90°/0.5 mm., ν max. 1780, 1720 $cm.^{-1}$. (lit.[127] b.p. 142°)

o-Methoxycarbonylphenyl N-(p-chloroacetyl)phenylcarbamate (257)

The chloroformate prepared above (3.2 g.) was dissolved in dry benzene (25 ml.) and to the solution was added p-aminophenacyl chloride (2.5. g.) suspended in dry benzene (75 ml.), together with dry pyridine (1.19 g.). The mixture was stirred at room-temperature (16 hrs.) after which time water was added to dissolve the pyridine hydrochloride. The benzene layer was worked up in the usual way and was observed to contain a solid. This

was filtered off and retained. The benzene solution was concentrated to leave a pale-yellow solid. The i.r. spectrum of both solids were found to be identical and so they were combined. A recrystallisation from chloroform-carbon tetrachloride afforded white crystals of the title compound (2.7 g., 52.5%), m.p. 162°, $\nu_{max.}$ (nujol) 3280, 1730, 1710, 1675 cm.$^{-1}$. (Found: C, 58.55; H, 4.13; N, 3.82; Cl, 10.46. $C_{17}H_{14}NO_5Cl$ requires C, 58.70; H, 4.06; N, 4.03; Cl, 10.20%).

Attempted hydrolysis of o-methoxycarbonylphenyl N-(p-chloroacetyl)phenylcarbamate (295)

The methyl ester prepared above (1.7 g.) was heated under reflux (60 min.) with a mixture of concentrated hydrochloric acid (20 ml.) and glacial acetic acid (30 ml.). The solution was then poured onto ice and adjusted to pH4 by the addition of solid sodium carbonate. After working-up in the usual way, a yellow solid was obtained (1 g.). An i.r. spectrum showed one NH-stretching band at 3300 cm.$^{-1}$, plus a doublet at 3400 cm.$^{-1}$, indicating the presence of free amine. A t.l.c. confirmed the presence of both starting material and p-aminophenacyl chloride (major component).

o-Benzyloxycarbonylphenyl N-(p-chloroacetyl)phenylcarbamate (369, 373)

p-Aminophenacyl chloride (1.69 g.) and pyridine (0.79 g.) were added to chloroform (75 ml.). To the mixture was added o-benzyloxycarbonylphenyl chloroformate* (3.1 g., 5% excess to overcome slight impurity) and the whole was stirred for 2 days at 45°. The mixture was then poured into water and worked-up in the usual way, and after evaporating off the solvent, a pale yellow solid was obtained. This was recrystallised from benzene to afford white crystals of the title compound (2.5 g.), m.p. 152-154°, ν max. (nujol) 3300, 1745, 1710, 1680 cm.$^{-1}$.
(Found: C, 64.11; H, 4.56; N, 3.14; Cl, 8.42.
$C_{23}H_{18}NO_5Cl$ requires C, 64.16; H, 4.41; N, 3.40; Cl, 8.61%).

*This compound was kindly donated by R. Sridhar.

Methyl-m-hydroxybenzoate (379)

m-Hydroxybenzoic acid (28 g.), anhydrous methanol (81 ml.), and concentrated sulphuric acid (8 ml.) were heated together under reflux overnight, after which most of the methanol was removed under reduced pressure. The residue was poured into water(250 ml.) and, on standing, the solid ester separated out. The product was washed with saturated sodium bicarbonate solution, water, and finally recrystallised from benzene-

petroleum (40-60°) to give white crystals of the title product (22 g., 72%), m.p. 69° (lit.[134] m.p. 69°).

m-Methoxycarbonylphenyl chloroformate (381)

The hydroxy-ester prepared above (19 g.) was dissolved in warm quinoline (16.5 g.) and the solution was added drop-wise to an ice-cooled saturated solution of phosgene in benzene (150 ml.). The mixture was left stirring overnight and the excess phosgene was removed with nitrogen. The product mixture was washed with water, 2N hydrochloric acid, water, sodium bicarbonate solution, water, and finally dried, decolourising charcoal being added during the drying operation. Removal of the solvent afforded the title product as a pale-yellow pungent-smelling oil which partially solidified on cooling (20.3 g., 76%), ν max. (nujol) 1770, 1715 $cm.^{-1}$ (Sridhar[116] quotes ν max. (liquid film) 1770, 1712 $cm.^{-1}$.

m-Methoxycarbonylphenyl N-(p-chloroacetyl)phenylcarbamate (401)

The chloroformate prepared above (4.28 g.) and p-aminophenacyl chloride (2.54 g.) were added to chloroform (75 mls.). Pyridine (1.58 g.) was added, and the mixture was stirred at room temperature (24 hrs.). Some solid residue was observed

and this was filtered off. It was found that the solid was not water-soluble and could not therefore be pyridine hydrochloride. The compound was washed with 2N hydrochloric acid, water, sodium bicarbonate solution, water, and ether.

The chloroform solution was similarly worked-up and afforded, after removal of the solvent, a yellow solid. The i.r. spectra of both solids were found to be identical and so the compounds were combined and recrystallised from ethyl acetate to yield white crystals of the title product (3 g., 57%), m.p. 200-201°, ν max. (nujol) 3330, 1755, 1715, 1680 $cm.^{-1}$. (Found: C, 58.65; H, 4.30; N, 3.70; Cl, 10.40. $C_{17}H_{14}NO_5Cl$ requires C, 58.70; H, 4.06; N, 4.03; Cl, 10.20%).

m-Carboxyphenyl N-(p-chloroacetyl)phenylcarbamate (405, 558)

The methyl ester prepared above (200 mg.) was added to a mixture of concentrated hydrochloric acid (2½ ml.) and glacial acetic acid (8 ml.) and the whole was heated under reflux (1 hr.) after which it was cooled and poured into water. The yellow precipitate which formed was filtered off and recrystallised from ethanol-water to afford the acid as a pale-yellow amorphous solid (90 mg.), m.p. 220-222°, ν max. (nujol) 3350, 1760, 1680 (broad) $cm.^{-1}$. (Found: C, 57.35; H, 3.80; N, 4.23; Cl, 10.35. $C_{16}H_{12}NO_5Cl$ requires C, 57.59; H, 3.63; N, 4.20, Cl, 10.63%).

p-Methoxycarbonylphenyl chloroformate (269)

Methyl p-hydroxybenzoate was treated with a solution of phosgene in benzene, in the presence of quinoline, according to the method of Einhorn et al.[128] After working up, the product was distilled under vacuum when a large proportion was lost due to its decomposition to the carbonate. The chloroformate was a white solid, m.p. 58° (lit.[128] m.p. 58°).

p-Methoxycarbonylphenyl N-(p-chloroacetyl)phenylcarbamate

The chloroformate prepared above (3.2 g.), p-aminophenacyl chloride (2.5 g.), and pyridine (1.2 g.) were stirred at room temperature in anhydrous benzene (75 ml.) for 4 days. The yellow suspension which had formed was filtered and the residue was retained. After working-up the filtrate in the usual way, a white solid was isolated and was found to be the carbonate derived from p-methoxycarbonylphenyl chloroformate. The yellow residue was washed with a large quantity of water to remove pyridine hydrochloride, and then dried to afford the crude carbamate as a yellow solid, m.p. 185°, ν max. 3300, 1740, 1685 (broad) $cm.^{-1}$. A sample was recrystallised from methanol-petroleum (40-60°) and then from chloroform to yield a white crystalline product, m.p. 221-223°, ν max. (nujol) 3300, 1715, 1670 $cm.^{-1}$.

(Found: C, 52.42; H, 4.54: N, 5.87; Cl, 15.86. $C_{17}H_{14}NO_5Cl$ requires C, 58.70; H, 4.06; N, 4.03; Cl, 10.40%).

It seems likely that an ester-exchange reaction occurred during the recrystallisation from methanol, to give methyl N-p-chloroacetylphenylcarbamate. $C_{10}H_{10}NO_3Cl$ requires C, 52.75; H, 4.43; N, 6.15; Cl, 15.62%). Consequently the crude carbamate, m.p. 185°, was used for the following experiment.

p-Carboxyphenyl N-(p-chloroacetyl)phenylcarbamate (432)

The crude p-methoxyphenyl N-(p-chloroacetyl)phenylcarbamate (500 mg.) was added to a 1:1 mixture of glacial acetic acid and concentrated hydrochloric acid (10 ml.) and the mixture was heated under reflux (1 hr.). After working up in the usual way, a yellow solid was isolated. A recrystallisation from acetic acid-water afforded the acid as a pale yellow amorphous powder, m.p. 191-194°, ν max. (nujol) 3300, 1745, 1705, 1670 $cm.^{-1}$.

(Found: C, 57.75; H, 3.85; N, 4.23; Cl, 10.48. $C_{16}H_{12}NO_5Cl$ requires C, 57.59; H, 3.63; N, 4.20, Cl, 10.63%).

1-Ethoxycarbonylethyl N-(m-NN-di-2-chloroethylamino)-phenylcarbamate (560)

NN-Di-(2-chloroethyl)-m-phenylenediamine* (464 mg.) and anhydrous triethylamine (200 mg.) were added to dry benzene and cooled in ice. 1-Ethoxycarbonylethyl chloroformate (360 mg.) was added and the mixture was stirred overnight. The base hydrochloride was filtered off and the filtrate was worked-up in the usual way to afford an oil which crystallised on trituration with light petroleum. A recrystallisation from petroleum (60-80°) yielded white crystals of the carbamate (300 mg.), m.p. 76-78°, ν max. (nujol) 3300, 1710 (broad) $cm.^{-1}$. (Found: C, 51.08; H, 5.90; N, 7.44; Cl, 18.75. $C_{16}H_{22}N_2O_4Cl_2$ requires C, 50.94; H, 5.88; N, 7.43; Cl, 18.79%).

* This compound was kindly donated by R. Sridhar.

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