A Thesis entitled

SYNTHESES OF SOME COMPOUNDS WITH POTENTIAL CYTOTOXIC ACTIVITY

presented by

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A B S T R A C T

In the introduction some of the more important aspects of cancer chemotherapy are reviewed, special emphasis being placed on the chemistry and design of drugs derived from nitrogen mustards and from α-halo-ketenes.

Some enol carbamates, representing a class of compound not described in the literature so far, have been synthesised from 2-chloro-2-phenylacetophenone, via the enol chloroformate. Such enol carbamates, derived from α-halo-ketones, are expected to undergo enzymic cleavage *in vivo*, to yield two cytotoxic units.

Syntheses are described of a range of compounds containing a carbamate bridge, linking the amino group of *N,N*-di-(2-chloroethyl)-*p*-phenylenediamine (or the *m*-isomer) to the hydroxyl function in the following types of compounds: (i) alcohols, (ii) phenols, (iii) hydroxy-aromatic acids and esters, (iv) hydroxy-amino-acids (serine, threonine, tyrosine) and their derivatives. Several model compounds analogous to these nitrogen mustard derivatives have been synthesised for possible use as "enzymic potentiators". Some other derivatives of *N,N*-di-(2-chloroethyl)-*p*-phenylenediamine (and of its *m*-analogue), containing an amido or an imido linkage, have been synthesised by acylation of the amino group with a variety of acyl chlorides.

T O M Y P A R E N T S

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CHEMOTHERAPY OF CANCER

Chemotherapy is concerned with the treatment of disease by the use of chemicals. An ideal chemotherapeutic agent would be capable of selectively eliminating or inhibiting the parasite species without causing any damage to the host species. There are several ways of expressing the efficiency of drugs, and for anti-tumour agents a common method is to quote the ratio of the median lethal dose (LD_{50}) to the minimum dose required to cause 90% complete tumour regression (ED_{90}); this is known as the chemotherapeutic index (C.I.). The larger the C.I. of a drug, the more useful it is.

The problem in cancer chemotherapy is one of achieving selective toxicity of a drug towards cancer cells, with minimum damage to normal cells. The striking similarity between normal and cancer cells makes this a difficult problem. Warburg[1] showed that all the known chemical constituents of normal tissues were also present in cancerous tissues. Fortunately, however, many quantitative chemical differences between normal and cancerous cells have been observed; e.g., pH, enzyme activities and contents, functionally available amounts of co-enzymes and co-factors, and rates of synthesis of proteins and nucleic acids, etc. These differences are small and it has been suggested by Danielli [2,3] that a high degree of selectivity is more likely to be achieved by using

a drug which depends, not on just one, but on two or more of the above differences for its anti-tumoural activity.

Several cancer-distinctive lipids and proteins have been isolated and some fifteen different examples of cancer-distinctive antigens have been described[4]. There is some hope that immunochemical studies in cancer would yield some information vital to the understanding of the disease.

The cytotoxic agents used in cancer chemotherapy can be divided into the following groups: alkaloids, hormones, antibiotics, compounds containing radioactive isotopes which give rise to cytotoxic radiations, biological alkylating agents and other miscellaneous types.

Another feature which adds to the complications of cancer chemotherapy is the ability on the part of tumours to develop drug resistance. In certain cases the host animal may even develop drug dependence. Boyland [5] has pointed out that it is very unlikely that a tumour is capable of developing resistance to two drugs simultaneously. A combination therapy with two different types of drug (say an anti-metabolite and an alkylating agent) would be far better than the sequential use of two types of drug, and the possibility of drug resistance would be reduced. Brewer et al.[6] have recently described a system of triple therapy employing an alkylating agent, an anti-metabolite and an

antibiotic. Drug resistance is not likely to occur with drugs which depend on a radioactive isotope for their cytotoxic action.

Amoroso [7] was the first to recognise the anti-tumoural activity of colchicine, a very toxic alkaloid. A search for less toxic colchicine compounds resulted in the isolation of "demecolin" from colchicum autumnale [8]. It is far less toxic than colchicine and has been used effectively against myeloid leukaemia. The most prominent among the alkaloids of use in cancer chemotherapy are the alkaloids of vinca rosea, viz., vinblastine ("velban") and vincristine ("oncovin") which are known to be neurotoxic [9]. However, it is fortunate that children under the age of 10 years are resistant to the neurotoxic action of this new class of anti-cancer agents. The vinca alkaloids are very safe and active against leukaemia in children [10].

Hormone therapy has been used extensivley in certain special types of cancer. The use of synthetic or natural oestrogens in the control of cancer of the prostate, the use of androgens against cancer of the female sex organs and the treatment of Hodgkin's disease using cortisone are well known examples of hormone therapy. Hormone therapy sometimes gives rise to unpleasant side effects.

Several antibiotics such as actinomycin, chromomycin, cinerubin, daunomycin, mithramycin, mitomycin, narangomycin,

olivomycin and ussamycin are known to exhibit anti-tumoural activity. Mithramycin and actinomycin probably act through their ability to bind themselves on to DNA, but not to RNA, through guanine units [11]. Gause [12] has pointed out that mithramycin, chromomycin and olivomycin are capable of forming a complex with DNA in the presence of Mg^{2+} ions and thus inhibit RNA synthesis in bacterial and animal cells. Actinomycin has been used as a safe chemotherapeutic agent in Wilm's tumour and choriocarcinoma of the uterus in spite of its high toxicity [13].

The chemotherapeutic version of X-ray or radiation therapy involves the use of a radioactive chemical which can be administered orally or parenterally and is thus distributed throughout the body. By careful drug design it should be possible to achieve accumulation of the radioactive drug at a desired site. Compounds containing ^{32}P (half-life 14.3 days; yields S and a β- particle on decay. The average energy of the β-particles corresponds to a range of about 2 to 4 mm. in tissue), ^{131}I (half-life 8 days) and ^{82}Br (half-life 34 hours) have been used with no outstanding success. Mitchell[14] has stated that tritium appears to be the most promising radioactive isotope for incorporation into a suitable carrier molecule because it emits only soft β-particles of average energy corresponding to a range of about 1μ in

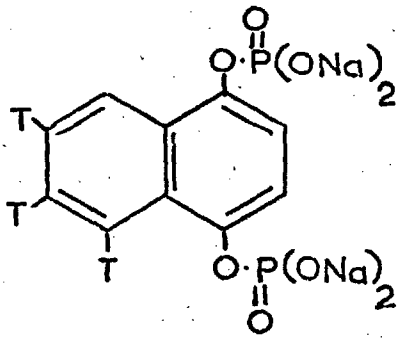
tissue and has a very high half-life (12.4 years) compared with the values of biological half-life which are much less (13 days for some tumours to 6 days for many normal tissues, including bone-marrow). Mitchell[15] found that "synkavit" (1) accumulates to some extent in malignant cells of certain human and animal tumours.

Recently, Ball et al.[16] have reported the synthesis of N,N-di-(2-chloroethyl)[3,5-$^3H_2$] aniline of high specific activity. Derivatives of this should hold a fair degree of promise as anti-cancer agents.

Anti-metabolites or metabolite analogues have found extensive application in the field of cancer chemotherapy. Certain metabolites (substrates and co-enzymes) which are present only in small amounts in a cell can be antagonised by substances called metabolite analogues. These generally contain a region which is similar in dimensions and electron-distribution to the corresponding region of the metabolite which makes contact with the enzyme protein. Thus metabolite analogues probably act by their ability to block certain important metabolic processes for which the parent metabolite is indispensable. Purine, pyrimidine, folic acid and amino-acid antagonists are common examples of drugs used in chemotherapy of cancer. An excellent review has been given by Timmis [17].

As their name implies, biological alkylating agents are chemicals capable of functioning as alkylating agents under physiological conditions. They are cytotoxic by virtue of their ability to alkylate biologically important molecules such as proteins, hormones, vitamins, enzymes and nucleic acids. Benzyl halides, halomethyl ethers, 2-chlorovinyl sulphides, epoxides, ethylene-imines, nitrogen mustards, α-halo-ketones, β-lactones, and esters of sulphuric, sulphonic and phosphoric acids are some of the common types of biological alkylating agent. Of these, the nitrogen mustards have been studied most extensively with cancer chemotherapy in view. The chemistry and fundamental design of compounds for selective toxicity form the basis of a review on biological alkylating agents by Ross [18].

Much knowledge about the chemical and physiological properties of nitrogen mustards has resulted from research on vesicant war gases during World War II. The peace-time interest in nitrogen mustards stems from the early recognition by Prelog [19] and Ward [20] that the water soluble hydrochlorides of methyl-di-(2-chloroethyl)amine ("HN2"), (2) and tri-(2-chloroethyl) amine ("HN3"), (3) were non-vesicant but cytotoxic and leucopenic (i.e., they produced a drop in white blood cell count) in their action. Their leucopenic action warranted their use in treatment of leukaemia, a disease



(1)

$CH_3 \cdot N(CH_2 \cdot CH_2Cl)_2$

(2)

$N(CH_2 \cdot CH_2Cl)_3$

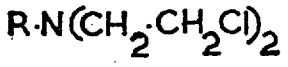
(3)

characterised by a high white blood cell count.

Rhoads[21] was among the first to point out that the nitrogen mustards exert their cytotoxic action on all rapidly proliferating tissues without distinguishing between normal and neoplastic tissues. Boyland[22] and Warwick[23] have pointed out that the nitrogen mustards resemble X-rays in their ability to cause mutagenic changes, chromosomal damage and antibody suppression, and have referred to them as radiomimetic agents. The above limitations (coupled with their non-selectivity vis â vis the cancer cell) present a formidable problem in drug design, and most of the work on nitrogen mustards has been directed towards modifying "HN2" (2) with a view to overcoming the limitations and disadvantages mentioned below. Many derivatives (4), of (2) with a variety of groups R, have been examined for anti-tumour activity.

Immunological barrier is the chief obstacle in organ transplantation in animals and humans.[24, 25, 26, 27] The immunosuppresive action of the nitrogen mustards, though a disadvantage in cancer chemotherapy, could very well prove useful in organ transplant surgery.

The isolation of di-(guanin-7-yl) - derivatives from the reaction of mustards on DNA by Lawley and Brookes[28, 29, 30] has shown that the mustards derive their biological activity from their ability to effect



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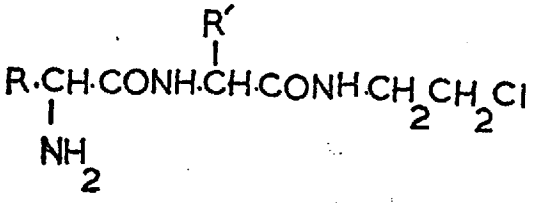


(5)

a cross-linkage between guanine moieties in opposite strands of the nucleic acid double helix. This cross-linking prevents the separation of the two DNA strands necessary for the replication process. Nitrogen mustards do not inhibit a bacteriophage containing a single-stranded DNA. Nitrogen mustards inactivate DNA-containing viruses more readily than RNA viruses. Although they inhibit mitosis, they are poor inhibitors of protein function. As a rule, "two-armed" mustards (i.e., those having two chloroethyl groups attached to the nitrogen) are more cytotoxic than "one-armed" mustards. However, Chakrabarti and Friedman[31] have observed that certain "one-armed" derivatives based on amino-acids are more cytotoxic than "two-armed" derivatives. Bearing this in mind, they have synthesised some "one-armed" mustards (5), derived from various peptides.

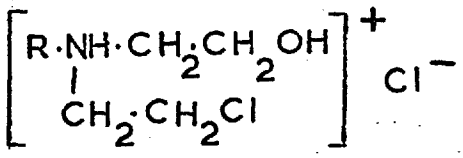
REACTIONS OF NITROGEN MUSTARDS

Hydrolysis

Aliphatic nitrogen mustards (4, R=alkyl) undergo rapid ionisation in water, by a unimolecular process, with the liberation of one equivalent of halide ion followed by a slower liberation of one equivalent of hydrogen ion. The hydrolysis ceases after one equivalent of hydrogen halide has been liberated, due to the formation of the hydrochloride (6). However, it is possible to complete the hydrolysis of (6) in suitably buffered solution.

The initial unimolecular step has been interpreted as the formation of a cyclic ethyleneimmonium ion (7), the existence of which was shown by Golumbic *et al.* [32], and Fruton and Bergmann [33], who were able to isolate derivatives such as picryl sulphonate salts from aqueous solutions.

Further proof for the formation of the cyclic ion was furnished by Ross [34], who showed that the formation of the rearranged product (8) resulting from the hydrolysis of (9) could be explained only by the formation of cyclic ethyleneimmonium ion followed by an S_N2 ring opening. Nucleophilic attack on the cyclic ethyleneimmonium ions derived from simple aliphatic nitrogen mustards is usually bimolecular, the rate depending on the nature and concentration of attacking nucleophiles. Ring opening



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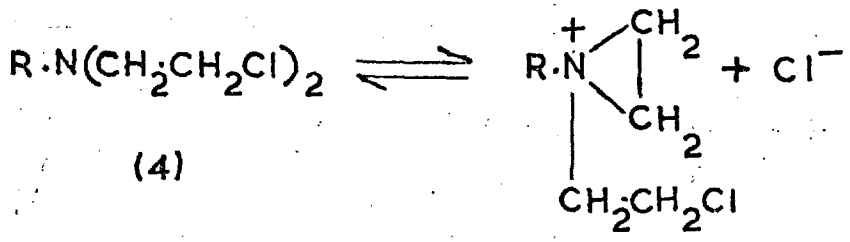
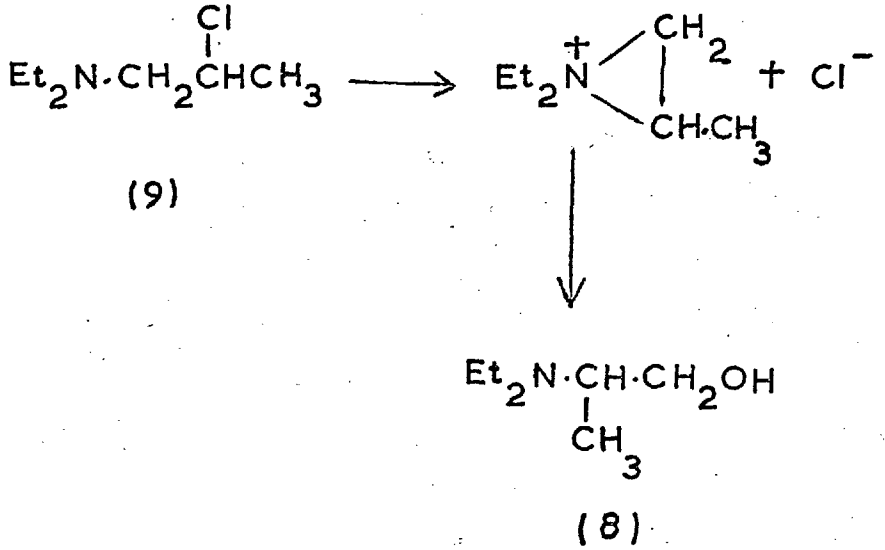


(4) (7)



(9) (8)

occurs in two ways in the case of the cyclic ion derived from 2-chloro-n-propylamine [34,35] (see page 20).

The nitrogen atom in aromatic nitrogen mustards (4, R = aryl) is very much less basic than that of aliphatic nitrogen mustards and hence the formation of an ethyleneimmonium ion from aromatic nitrogen mustards is less likely. Originally, the hydrolyis of aromatic nitrogen mustards was considered to occur by a simple S_N1 process, involving the formation of a carbonium ion (13) from the halide in the rate determining step. This mechanism was put forward by Ross [36] on the basis of the following observations:-

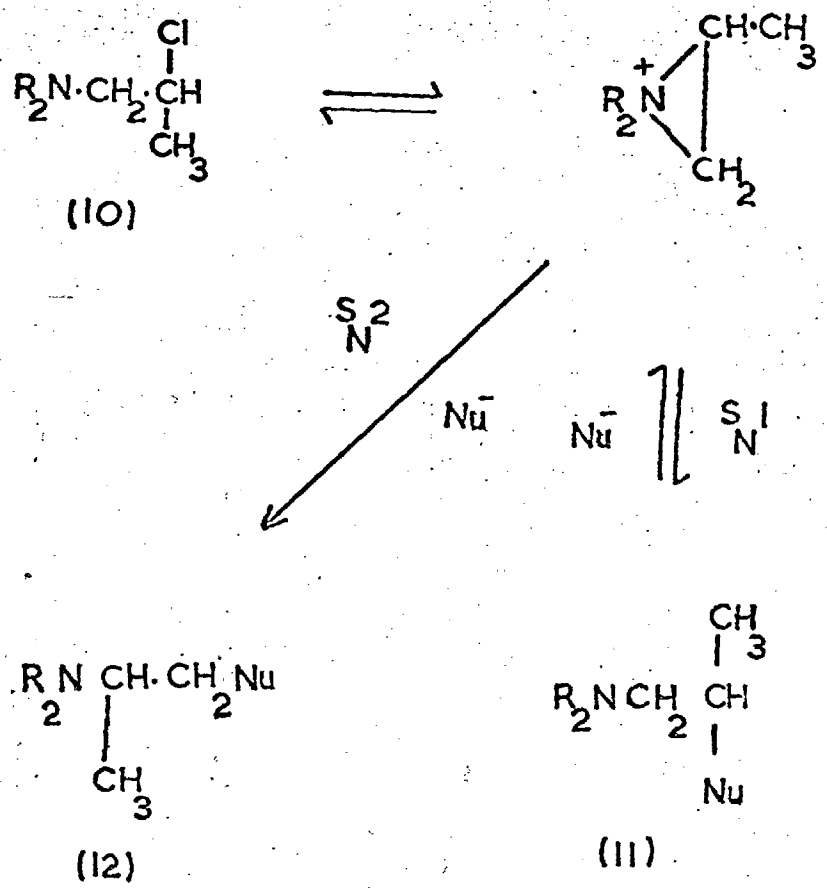
(i) The reaction obeys first-order kinetics.

(ii) Added halide ion retards the reaction.

(iii) The halide and hydrogen ion are liberated simultaneously.

(iv) There is an approximate correlation between the basicity of the nitrogen atom and the ease of hydrolysis.

This mechanistic difference in the hydrolysis of aliphatic and aromatic nitrogen mustards, if true, implies that the biological alkylating action of aliphatic nitrogen mustards is not as efficient as that of their aromatic analogues, where the rate of alkylation is independent of the concentration of nucleophilic centres


Cl
R2N·CH2·CH
CH3
CH·CH3
+
R2N
CH2
SN2
Nu⁻
Nu⁻
SN1
R2N CH·CH2Nu
CH3
CH3
R2NCH2 CH
Nu

(10)

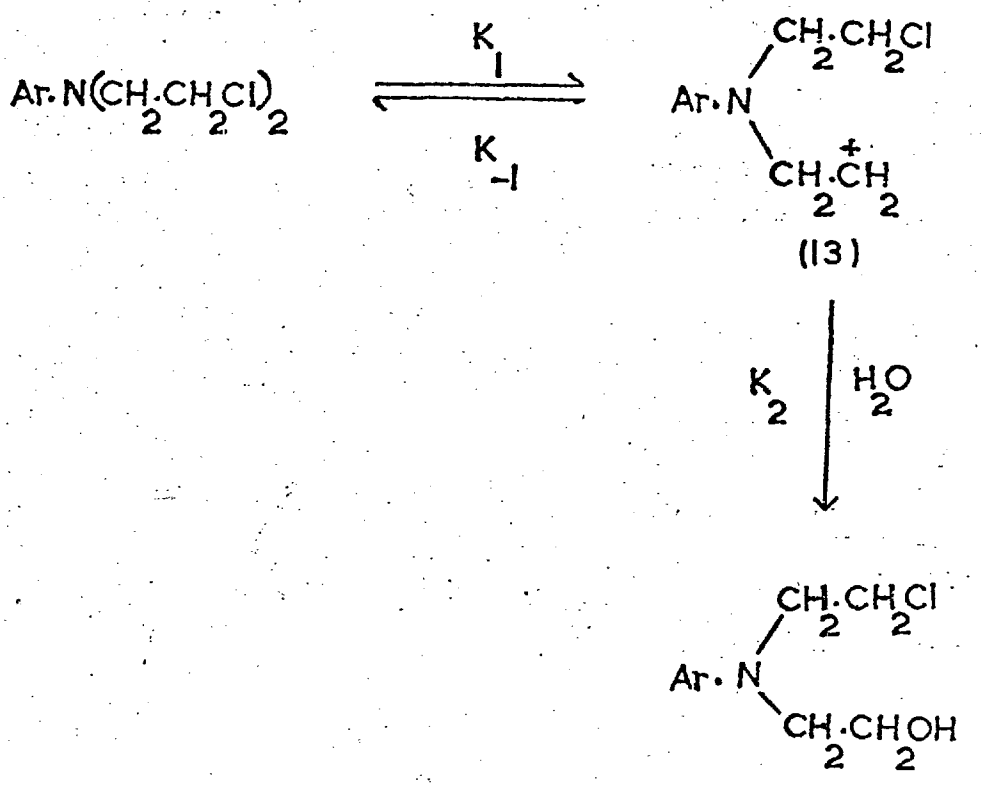
(12)

(11)

in the system.

This simple view of the mechanism of hydrolysis of the aromatic nitrogen mustards has more recently been the subject of some dispute [37,38,39,40] and it has been shown that the addition of nucleophiles other than the halide ion accelerates the hydrolysis of aromatic nitrogen mustards. This is not to be expected of a simple S_N1 type process. Triggle [39] has stated that a study of the solvolyses of N,N-di-2-chloroethylaniline and 3-phenyl-n-propyl chloride points to a mechanism involving the participation of the nitrogen atom in the nucleophilic displacement reaction. He has also pointed out that the observation of first-order kinetics in the liberation of chloride ion can be explained by either path A or path B. Common ion effect is a characteristic of an S_N1 process only when simple substitution reactions are under consideration. However, common ion effect can be expected whenever a discrete reactive intermediate is produced in a substitution process and this again fails to distinguish between paths A and B. Triggle criticises Ross's [18] assumption that electron donating substituents on the aromatic ring of nitrogen mustards will facilitate the ionisation by path A on the basis that it assumes efficient transmission of an inductive effect over more than 2 carbon atoms which is highly improbable [39].

PATH A



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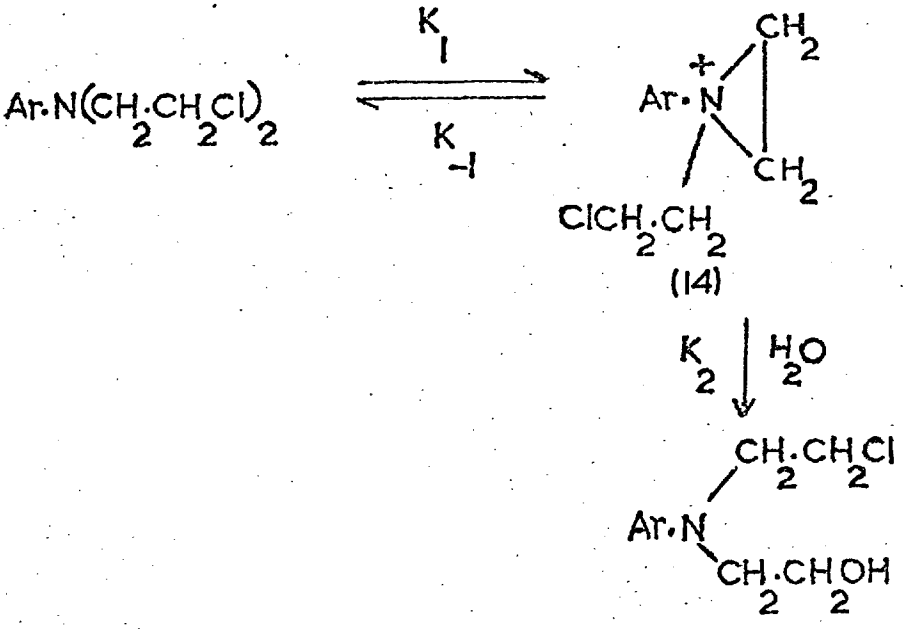
PATH B

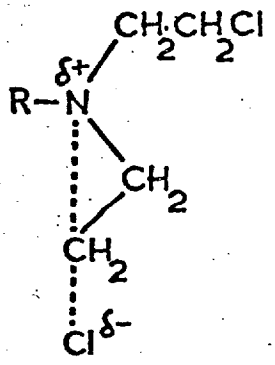


(14)

Triggle argues that it is far simpler to account for the approximate correlation that exists between the basicity of the nitrogen atom and the rate of hydrolysis on the basis of path B. The correlation between the nucleophilicity and the basicity of the attacking nucleophile in a given series of nucleophilic displacement reactions is not uncommon [41]. Understandably the difference between the basicities of N,N-di-(2-chloroethyl)methylamine (pK_a = 2.2) and N,N-di-(2-chloroethyl)aniline (pK_a = 6.1) would suggest a large difference in rates of cyclisation. The simultaneous liberation of the chloride and hydrogen ion in equivalent quantities would be expected for path B if $K_2 \rangle K_1$. The slow liberation of halide ion from aromatic nitrogen mustards and the highly unstable nature of the ethyleneimmonium ion (14) involved in path B are indeed indicative of a situation where $K_2 \rangle K_1$.

Therefore, it appears that the nitrogen atom must participate as a neighbouring group even though complete transference of charge may not occur leading to the formation of the ethyleneimmonium ion (14), the transition state may involve something approximating to it, viz., (15).

However, whatever the exact mechanism may be, the fact remains that the reactivity of aromatic nitrogen mustards is such that it lends itself to control and



(15)

$$R{\cdot}N\left(CH_2{\cdot}CH_2{\cdot}Hal\right)_2$$

(16)

modification through introduction of suitable substituents on the aromatic ring. Such modification of reactivity is not possible to the same extent with aliphatic nitrogen mustards.

An approximate method of assessing the cytotoxicity of a nitrogen mustard has been developed by Ross [42]. This involves volumetric estimation of the hydrochloric acid liberated on boiling for 30 minutes a 50% aqueous-acetone solution containing 20 m.mole per litre of the nitrogen mustard. If the "hydrolysis rate" under these conditions is 20% or more, then the mustard is usually sufficiently reactive to exhibit inhibitory action against Walker 256 rat carcinoma, but of course mustard derivatives having quite small "hydrolysis rates" may show pronounced biological activity because they become activated *in vivo* [43]; this possibility will be discussed below.

The nature of the group R and the nature of β-halogens in the nitrogen mustard (16), exert a profound influence on the "hydrolysis rate" and hence on the cytotoxicity. If R is an electron withdrawing group, the rate of hydrolysis will be increased in the case of aliphatic nitrogen mustards and decreased for aromatic nitrogen mustards. The situation is reversed in the case

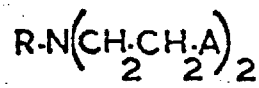
where R is an electron donating group.

Bromo-mustards hydrolyse at a faster rate than the corresponding chloro-mustards. The corresponding iodo-compounds, however, hydrolyse at a slower rate than the corresponding bromo-mustards. This could be attributed to a duality of mechanism in operation in the hydrolysis of iodo-mustards. This rationalisation is based on the order of reactivity, $Cl < Br < I$, in the case of 3-halogeno-propylamines which are known to undergo hydrolysis by a bimolecular process. The iodo-compounds have not proved to be of much use as cytotoxic agents.

Reaction with anions

When an aliphatic nitrogen mustard is made to react with acyl anions (A^-) in water, a substituted aminoethyl ester (17) is formed . These esters are very easily hydrolysed to the corresponding diol (18).

Fruton et al.[44] found that methyl and ethyl-di-(2-chloroethyl)amine yield only the diol on reaction with a slightly alkaline solution of sodium acetate, although the formation of the aminoethyl acetate could be shown by the fact that acetate ion competes for the mustard with alanine ion at pH 7.5 - 8.0. Thus it has been demonstrated that aliphatic mustards react with



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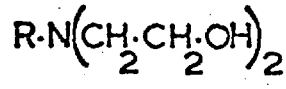

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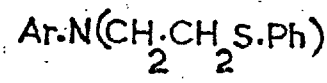

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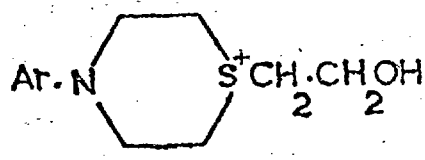


(20)

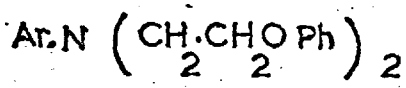
phosphates, and anions derived from various amino-acids and peptides.

In contrast to the aliphatic nitrogen mustards, the aromatic nitrogen mustards yield stable, substituted aminoethyl esters on reaction with aqueous solutions containing anions [45].

Reaction of nitrogen mustards with ionised thiols leads to the formation of compounds of the type (19) and (20);[46,47] while ethers such as (21) result from the action on phenoxides [48]. In the presence of suitable condensing agents, nitrogen mustards can be made to react with compounds containing an active methylene group to obtain 1,4-substituted piperidines[48]. The halogen atoms in nitrogen mustards can be exchanged for anions such as iodide, thiocyanate and thiosulphate [45,49].

Reaction with amines and thioethers.

Piperazines are formed by the action of nitrogen mustards on primary amines [50,51]. Compounds of types (22) and (23) result from the reaction of a secondary amine such as N-methylaniline with nitrogen mustards [51]. The double quaternary salt (24) is obtained by the action on N,N-dimethylaniline [51].



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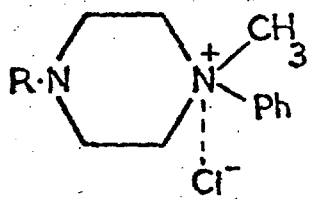

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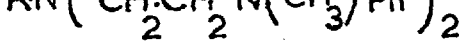

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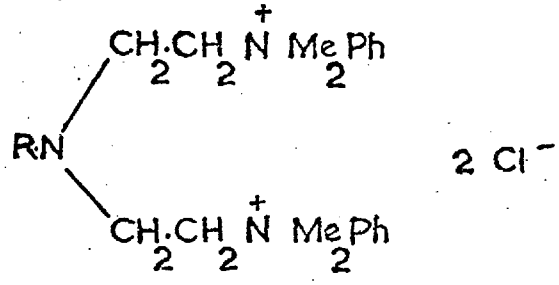


(24)

Fruton et al [52] have shown that the amino groups in amino-acids were attacked by nitrogen mustards only when pH conditions favoured the existence of the unionised form of the amino-acid. Ross [47] obtained sulphonium salts by the action of thioethers (such as methyl phenyl sulphide) on aromatic nitrogen mustards.

Reactions in biological systems.

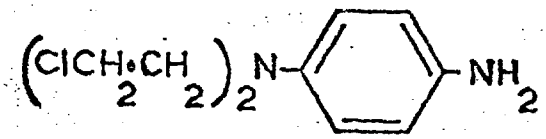
The pH of most biological systems is about 7.5. At this pH, the number of groups which exist in a reactive nucleophilic form and which are prone to attack by biological alkylating agents is greatly reduced. Thus in amino-acids, the carboxylic group can be readily alkylated, while only a small fraction of the amino group exists in a reactive uncharged form. Hydroxyl groups of sugars, sulphydryl and phenolic hydroxyl groups are not alkylated to any appreciable extent at pH 7.5. However, the phosphoric acid hydroxyl groups in hexose phosphoric acid are readily alkylated. The ring nitrogen atom of pyrimidines and purines are capable of undergoing alkylation at physiological pH.

DRUG DESIGN

The quest for effective chemotherapeutic agents in cancer, as in other diseases, has been largely empirical. Once a new class of drug has been found, the rational approach of structure modification is made, bearing in mind the exploitable differences which exist between normal and neoplastic tissue. Although there are no qualitative differences between normal and neoplastic cells, slight quantitative differences do exist, e.g., enzyme activities, rates of protein and nucleic acid synthesis, adaptation and resistance to drugs, pH etc[1]. Many nitrogen mustards, which make use of one or more differences in increasing their anti-cancer activity, have been synthesised. The frequently applied principles of drug design are described below.

Enzymic activation.

When a certain enzyme is present at a higher level of activity in the tumour cells than in normal tissue, it can be utilised to liberate an active mustard from an inactive derivative. The following is a well known example illustrating the principle of enzymic activation. (N,N-Di-2chloroethyl)-p-phenylenediamine (25) is very toxic to the host whereas the acylated derivatives (26) and (27) are far less toxic. This is due to the electron attracting acyl groups, which deplete the electron density



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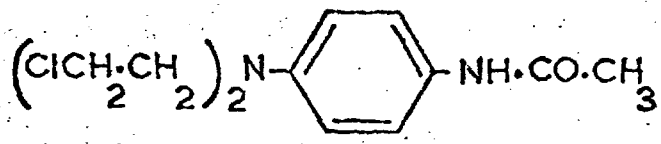

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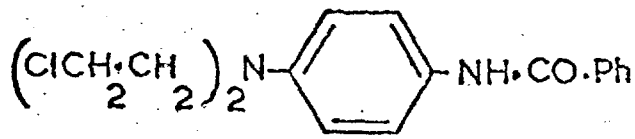

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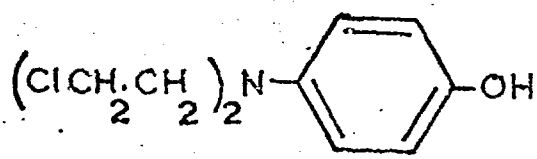
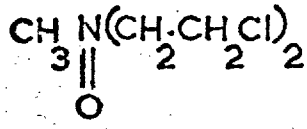


(28)

at the mustard grouping. Danielli and Hebborn [3] found that the acetyl derivative (26) is active against the tumour, whereas the benzoyl derivative (27) is not. This is attributed to the presence in the tumour of an enzyme, capable of deacetylating an acetamido- but not a benzamido-group, thus liberating the amine (25) at the tumour site. Danielli and Hebborn [3] have demonstrated the presence of enzymes capable of hydrolysing acetate and benzoate esters derived from the nitrogen mustard (28).

The aliphatic nitrogen mustard derivative "nitromin" [53] (29), an oxidation product of "HN2" (2), is known to cause complete regressions of the Yoshida sarcoma. The activity in this case is probably due to the presence of a reductase in a higher level of activity, capable of converting (29) into "HN2" (2), in the sarcoma. Ross [54, 55] prepared many azobenzene derivatives, such as (30), which were found to be active, presumably because of their conversion into (25) in vivo by the action of reductive enzymes. A variety of compounds such as halogeno-acetamides, phosphoramidates, ureas and thioureas derived from (25), have been designed to be activated by enzyme systems present in high levels of activity in the tumour. The synthesis has been achieved of several nitrogen mustard derivatives which depend on the enzymatic cleavage of the azomethine group for their cytotoxic



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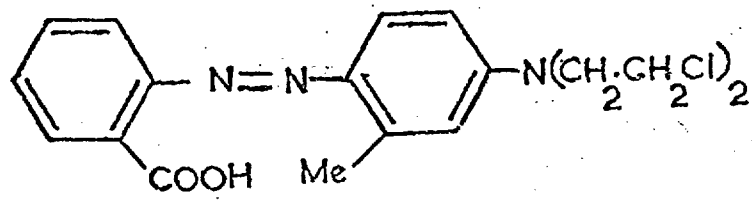


(30)

action [56,57,58].

Enzymic deactivation.

Certain enzymes are present in normal cells at a high level of activity but are not present to the same extent in the tumour. If these enzymes are capable of deactivating a drug administered in the active form, then selective cytotoxic action should result at the tumour site. Benn [59] attributed the high selectivity of bromo-mustard thiol M.SH to the enzymic detoxication in the normal cells due to the conversion into the insoluble disulphide M.S.S.M by the oxidative enzyme present at a higher level in normal tissue than in the tumour.

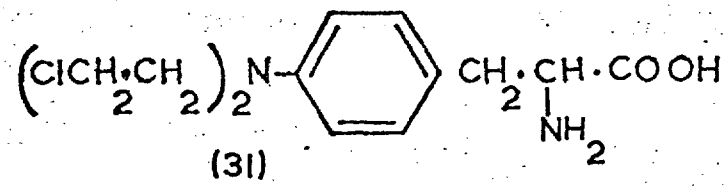
Active transport or facilitated diffusion.

Certain biologically important molecules like sugars and α-amino-acids pass through certain patches of cell membrane more easily than other molecules. A drug obtained by attaching a nitrogen mustard to a biologically important molecule required by the tumour, should be selective for the tumour. Faith in the principle of facilitated diffusion would be strengthened by the recent knowledge about the manner in which cancer cells can be more easily attacked through their membrane, which is significantly changed, than normal cells [60]. Many drugs have been synthesised with this principle in view.

A well known example is "melphalan" (31) derived from L-phenylalanine. The D- form of (31) known as "medphalan" was found to be less active and "merphalan" or "sarcolysine", the DL- form of (31) had an intermediate activity [61]. Recently Bergel et al [62] have synthesised derivatives of "melphalan" having synthetic poly(amino-acids) as carriers. Cytotoxic mustard derivatives carrying protein units have been found to be effective [63]. Several derivatives of amino-acids such as glycine,[64,65,66,67] alanine, [64,68] phenylalanine, [65,69] serine, [70,71] threonine, [70,71] tyrosine [72,65] and glutamic acid [73] have been synthesised with active transport in view.

Sugar derivatives such as (32) and (33) proved to be very toxic. [59,74,75] However, 1,6-di-(2-chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride, "BCM" (34), was found to possess anti-tumour activity of some use.[76]

Creighton [77] synthesised derivatives of di-O-isopropylidene-D-galactose having a sulphur mustard unit attached through the 6-position of (35), which on removal of the isopropylidene groups would yield the free sugar, which should be capable of acting via an active transport mechanism.



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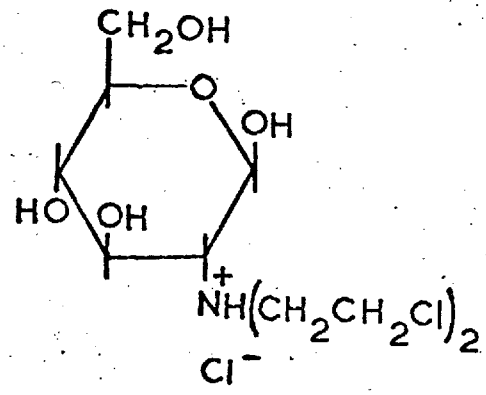

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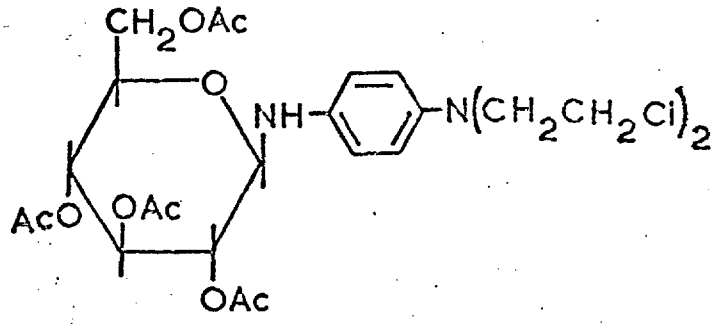

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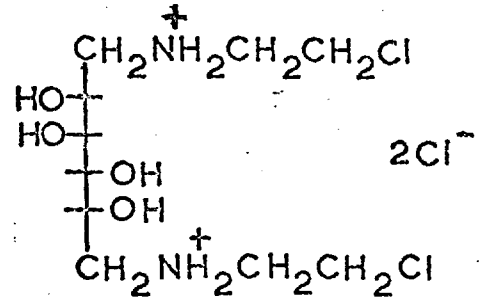

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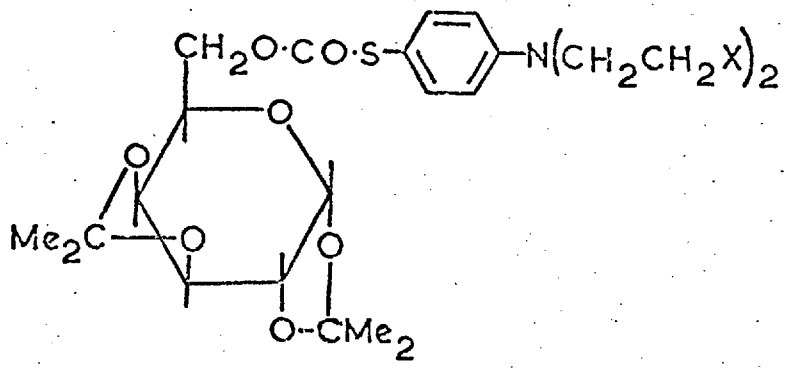

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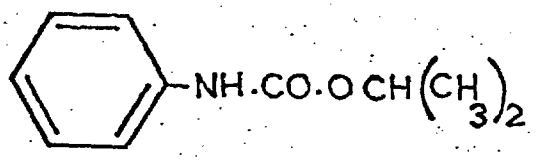
Adaptation and resistance

The ability on the part of the tumour (and to a lesser extent the host cells) to develop resistance to cytotoxic drugs, can be made use of in designing drugs. Such drug resistance generally arises by the evolution of detoxifying enzymes or by deletion of activating enzymes, and occasionally by the rapid elimination of the drug by a modified active transport mechanism. Thus the Walker rat carcinoma develops resistance to ethyl carbamate. It was shown by Danielli [78] that the tumour develops an enzyme capable of liberating aniline from the carbamate (36). This adaptive enzyme can be utilised to liberate the active mustard amine (25) from carbamates (37), in which a variety of groups R have been tried. An example of success based on this principle due to Danielli[78] is given below. Administration of the isopropyl urethane (36), to a rat bearing the Walker tumour resulted in a very small decrease in the rate of tumour growth, and after a time resistance to the drug began to develop. After full resistance had been induced, the urethane (38) was administered. This resulted in 80% complete regression of the tumour compared to 0% for (38) alone and 10% for (36) alone. This is presumably because the detoxifying enzyme for (36) acts as the activating enzyme for (38).



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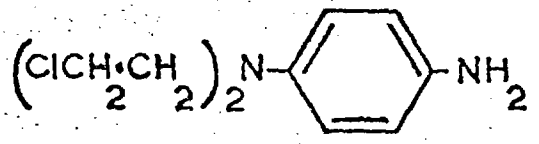

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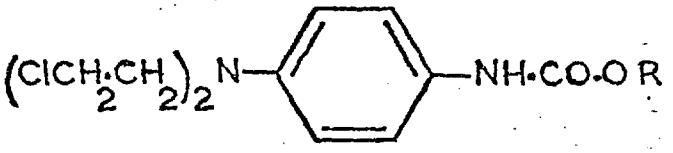

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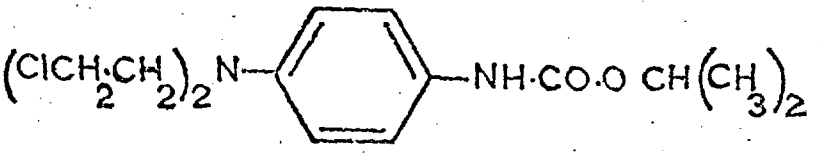


(38)

This technique of potentiation has been extended to other types of nitrogen mustards, and may well prove useful with other classes of cytotoxic agents.

Other factors in drug design.

In addition to the factors mentioned above, there are certain properties such as solubility, "hydrolysis rate", etc. which have to be considered in drug design.

α -HALO-KETONES AS BIOLOGICAL ALKYLATING AGENTS

The α-halo-ketones belong to a special class of biological alkylating agents referred to as lachrymators. Their lachrymatory powers are associated with the presence of a halogen atom which has acquired some positive character due to the adjacent carbonyl group. A quantitative relationship between the degree of positive character of halogen and the lachrymatory power was demonstrated by Ford-Moore [79]. Lachrymators act as enzyme inhibitors in the case of enzymes which depend on thiol groups for their activity [80,81]. In general, lachrymators containing an active halogen atom are more efficient than others. Lachrymators such as 2-chloro- and 2-bromo-acetophenone bring about rapid and irreversible alkylation of cysteine and of proteins [82].

$$Cy.SH + Br.CH_2.COPh \longrightarrow Cy.S.CH_2.CO.Ph + H\,Br.$$

"Thiol reactivity" of lachrymators is a fair index of their toxicity [83] but no correlation seems to exist between "amino reactivity" and toxicity. Insulin and ribonuclease are inactivated by 2-bromo-4'-nitroacetophenone, which alkylates the imidazole group of a histidine unit [84]. Yu-Kun Sun and Chan-hu Tsou [85] found that at pH 5, ethyl bromoacetate or ethyl iodoacetate inhibit papain

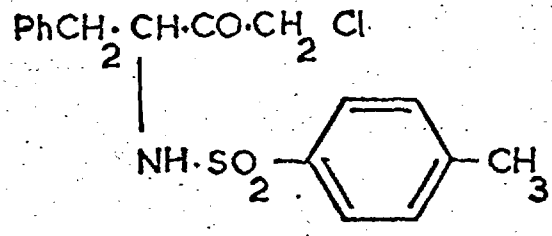
strongly even in the presence of excess cysteine. Moreover, the thiol content of the enzyme remained unchanged even after the inhibition of the enzyme was complete. Finally, the absence of S-carboxymethylcysteine in the hydrolysis product of the inactivated enzyme indicated that the histidine and not the cysteine unit in papain is attacked by the lachrymator. N-Tosylphenylalanylmethyl chloride (39) inhibits chymotrypsin at pH 7.2, due to its ability to alkylate the imidazole ring in a histidine residue.[86] Thus it appears that alkylation of histidine moieties in enzymes, including certain thiolic ones, may be responsible for inactivation of enzymes by lachrymators.

A few derivatives of 2-chloro-2-phenylacetophenone, desylchloride (40), will be described in this thesis, and it is therefore relevant to consider the mechanisms concerned in the nucleophilic reactions of phenacyl halides.

The reactions of α-halo-ketones with nucleophiles.

The halogen atom in desyl chloride is much more reactive than the halogen atom in the corresponding deoxygenated compound (41).

The alkylating action of desyl chloride probably involves the formation of a normal S_N2 substitution product (42) which may result in three ways:-



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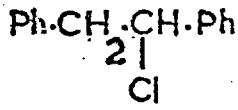

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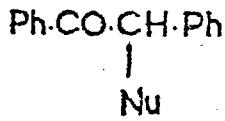
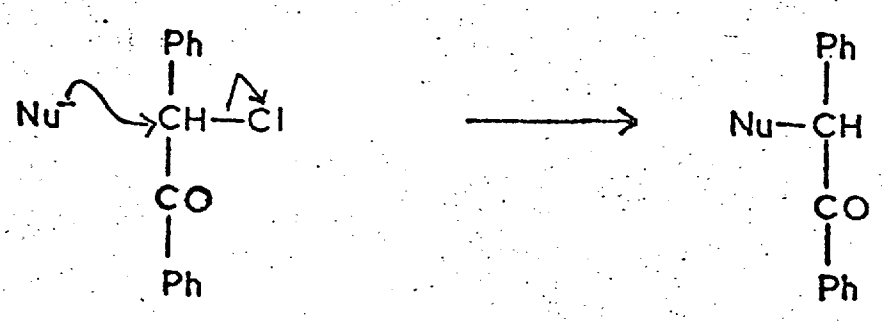


(42)

(i) from an S_N2 process, (ii) through the rearrangement of anion (43), and (iii) from the isomerisation of the epoxide (44).

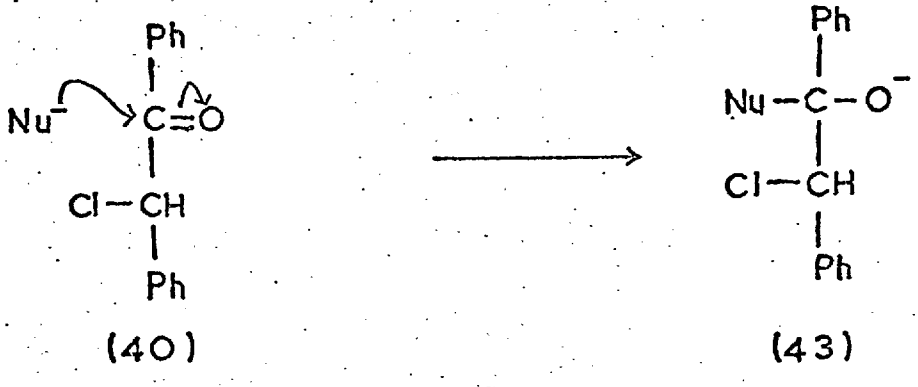
The reaction of α-halo-ketones with nucleophiles generally obeys second-order kinetics, and the observation by Baker [87], that the reaction of substituted 2-halo-acetophenones with pyridine in dry acetone is retarded by the introduction of electron-repelling groups into the ring and accelerated by electron-attracting groups, suggested a mechanism involving initial attack on the carbonyl function, i.e., mechanism (ii) or (iii). However, the earlier observation by Baker [88] that 2-bromo-acetophenone reacts some 60 times faster with pyridine than does 2-chloro-acetophenone indicates initial attack on the α-carbon atom, since the rates of addition of pyridine to the carbonyl groups of the two ketones would not be expected to differ appreciably. Pearson et al. [89] found that α-bromo-ketones are more reactive towards thiourea than towards pyridine, which is a weaker nucleophile but a stronger base than thiourea. This observation excludes the possibility of initial attack on the carbonyl group, for which the rate of reaction would be expected to be a function of the basicity rather than the nucleophilicity of the attacking reagent. Furthermore, it is known that the rates of S_N2 displacements of halides

MECHANISM (i)



(40) (42)

MECHANISM (ii)



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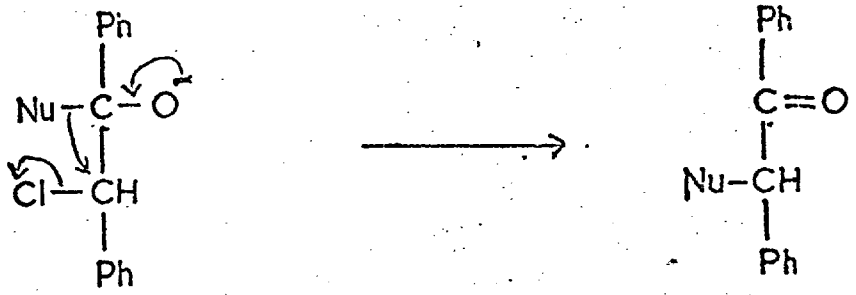
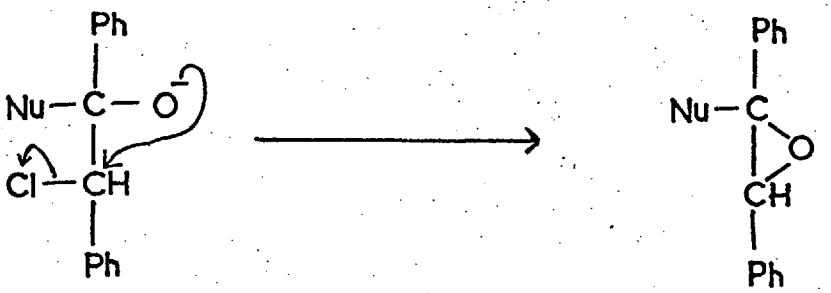


(43) (42)

MECHANISM (iii)



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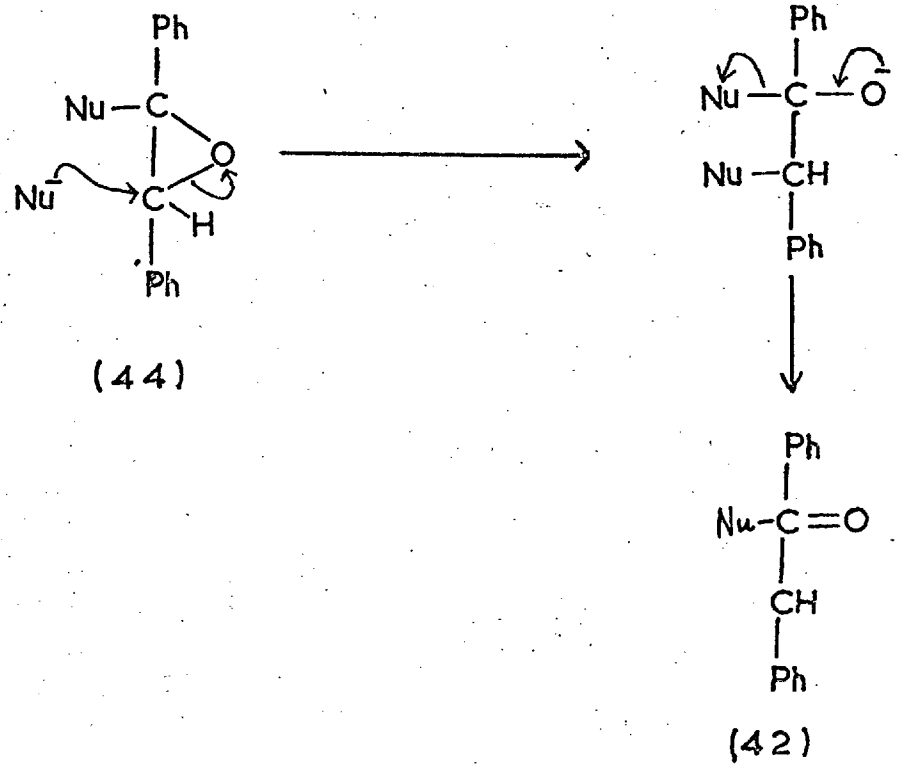


(44) (42)

from alkyl halides are a function of the nucleophilicity and not the basicity of the attacking reagent. The S_N2 displacement of the halogen atom in (40) is facilitated by the stabilisation of the transition state through overlap of the π-orbitals.[90] This explains the fact that α-chloro-acetophenone reacts with potassium iodide in acetone at 75° about 32,000 times as fast as n-butyl chloride does under the same conditions.[91]

Support for mechanism (iii), on the other hand, comes from the isolation of the intermediate epoxides of the type(44) under conditions favouring an S_N2 pathway.[92,93] Nevertheless, mechanism (iii) has been criticised by Pearson et al.[89], who showed that in a competition reaction between aniline and pyridine for 2-bromo-acetophenone, the ratio of the rate constants is equal to the ratio of the products. This result is compatible with mechanism (iii) only if the epoxide intermediate (44), supposedly involved in the substitution mechanism, undergoes ring closure and ring opening at the same relative rate; this is highly improbable. The most convincing evidence against the epoxide mechanism was obtained very recently by Lutz [91] who argued that the ratio of the rate of inversion to the rate of exchange for the exchange reaction between desyl chloride (40) and $^{36}Cl^-$ would be unity in the case where an S_N2 mechanism is operative.

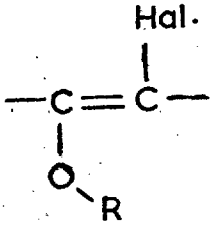
A value of infinity for the above ratio would be indicative of the epoxide mechanism, which involves a double inversion, i.e., a retention of configuration at the α-carbon atom. In the case where both mechanisms are operating the value of this ratio would lie between unity and infinity. The actual value obtained for this ratio from an experiment using $Li^{36}Cl$ and (+)-desyl chloride was 0.985. This shows that desyl chloride undergoes nucleophilic attack mainly by the S_N2 route.[91]

From the above it is clear that the α-halo-ketones are cytotoxic due to their ability to inactivate a wide variety of enzymes through alkylation of thiol and imidazole units, by a mechanism predominantly S_N2 in nature. The S_N2 character of the alkylating action implies that the rate of alkylation is dependent on the concentration of nucleophilic sites in the physiological system and it is also very likely that much of the cytotoxic halo-ketone may react before reaching the tumour site. A few non-lachrymatory derivatives of the α-halo-ketone (40), which are expected to undergo enzymic activation at the tumour site, will be described later in this thesis.

Since α-halo-ketones derive their lachrymatory powers from the activating influence of the carbonyl group on the halogen atom, the destruction of the carbonyl function

should render them non-toxic. This can be achieved by conversion into a stable derivative (45), of the unstable enolic form of the α-halo-ketone, in which the reactivity of the chlorine atom is very greatly reduced not only due to the loss of the activating carbonyl function, but also through attachment to an ethylenic carbon atom.

If the derivative (45) is so designed that the R-O bond is capable of undergoing easy cleavage by an enzyme present at a relatively high level of activity in tumour cells (or by an enzyme which can be induced in the tumour by the administration of a non-toxic potentiator), then a useful anti-cancer drug should result.[94]
The enol esters (45, R = CO.alkyl or CO.aryl), enol phosphates (45, R = phosphate, phosphate salt, or phosphate ester) and enol carbamates (45, R = $CO-\underset{|}{N}$-alkyl; R = $CO-\underset{|}{N}$-aryl) are likely to undergo enzymic activation in vivo. Cooper and Owen[94] have achieved the syntheses of enol acetates and enol phosphates derived from α-halo-ketones. Enol phosphates derived from α-halo-ketones can be expected to show a certain degree of selectivity in their action because of the substantial phosphatase activity associated with the high rates of metabolism in neoplastic cells, and it might be expected that enol carbamates may prove useful through their activation at the tumour site



(45)

by an enzyme (capable of cleaving carbamates), which could be induced by the prior administration of a relatively inactive carbamate towards which the tumour is known to develop resistance. There is, however, no reference in the literature to enol carbamates, and those described later in this thesis are probably the first examples of this new class of compound.

OTHER CLASSES OF ANTI-TUMOUR AGENTS

The following are a few isolated examples of tumour control by drugs which do not specifically belong to any class discussed so far.

Shiro Saito[95] observed that intraperitoneal administration of an iron-dextran complex or NaCo chlorophyllin to rats, led to the accumulation of the drug in a nodal tumour which had previously been implanted together with a barium ferrite ring under the skin of the rat. Following this observation, he found that an intraperitoneal injection of the iron complex of 7 -hydroxy-9-methoxymitomycin inhibited tumour growth to a greater extent when barium ferrite rings were used for magnetic drug-trapping at the tumour site.

Vester *at al.*[96] found that eleven of the nineteen protein fractions isolated from mistletoe (*v.album*) exhibited marked carcinostatic effects in extremely low doses. Further, the tumour-inhibitory action was independent of toxicity, i.e., some were strong carcinostatic agents and non-toxic, some were toxic but ineffective carcinostatic agents, some were toxic and effective carcinostatic agents and others had neither toxicity nor carcinostatic activity. A few of the fractions were specific for malignant HeLa cells.

Recently Sutemi Oka et al.[97] have obtained certain polysaccharides, from fractionation of bagasse polysaccharide, which were found to possess anti-tumour activity.

Locher[98] was the first to suggest the use of ^{10}B and other nuclides with high neutron-capture cross-section values as a means of selectively destroying cancer cells. Thus a non-toxic compound of boron (normal abundance of ^{10}B is about 19.6%), which has been so designed as to accumulate preferentially in the tumour, is administered; and subsequently the tumour is exposed to a high flux of thermal or slow-neutrons. Interaction of the slow-neutrons (by themselves quite harmless) with ^{10}B nucleii in the tumour results in the production of the nuclear reaction represented by the following equation:-

$$^{10}_{5}B + ^{1}_{0}n \longrightarrow (^{11}_{5}B) \longrightarrow ^{7}_{3}Li + ^{4}_{2}He + 2.4 \text{ MeV.}$$

The energy involved in the above reaction, which is shared by the fragments of the reaction, is dissipated to the tissue; and any cell absorbing such a high energy at the molecular level would be destroyed. Moreover, the helium and lithium particles produced in the above reaction have a range of only 9 microns in the tissue. This distance is comparable to the cross-section of a red blood cell, and hence the destructive action of the capture reaction

would be confined to just the one cell where capture reaction occurs or its immediate neighbours. It is rather fortuitous that the other main elements present in normal tissue have low values of neutron-capture cross-section. In spite of their low neutron-capture cross-sections, hydrogen and nitrogen atoms which are present in very high concentrations in all tissues do contribute to the total radiation dose through the following nuclear reactions:

$$ {}_{1}^{1}H + {}_{0}^{1}n \longrightarrow \left({}_{1}^{2}H \right) \longrightarrow {}_{1}^{2}H + \gamma $$

$$ {}_{7}^{14}N + {}_{0}^{1}n \longrightarrow \left({}_{7}^{15}N \right) \longrightarrow {}_{1}^{}p^{+} + {}_{6}^{14}C \longrightarrow $$

$$ {}_{7}^{14}N + \beta^{-} $$

However, the contribution due to the above reactions is not alarmingly high, and the main radiation dose arises due to the ^{10}B - capture reaction.

The main problem in ^{10}B - slow-neutron therapy is the design and synthesis of a non-toxic boron compound which is incorporated or accumulated mainly in the malignant cell and not the normal cell. An excellent review on boron compounds in cancer chemotherapy has been given by Soloway.[99]

Nitrous oxide and inert gases such as argon, krypton, xenon and neon have been found to show some tumour inhibiting action.[100]

There have been several scattered reports about tumour-inhibiting immunological preparations.[101,102]

SYNTHESES OF SOME ENOL CARBAMATES

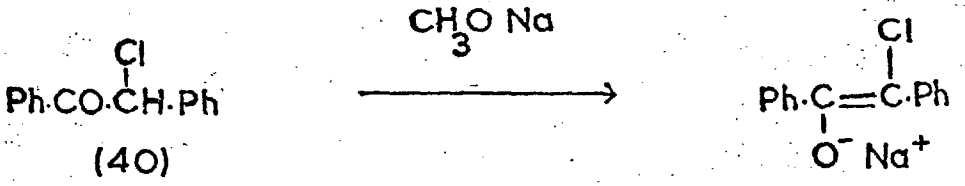
DERIVED FROM 2-CHLORO-2-PHENYLACETOPHENONE

It has been shown in the introduction that the enol acetates, phosphates and carbamates derived from α-halo-ketones are non-lachrymatory and are likely to undergo enzymic activation in vivo (page 49). It was also pointed out that enol esters (acetates and phosphates) derived from α-halo-ketones have been synthesised by Cooper and Owen,[94] but that no enol carbamates have been made. In view of the special interest attached to mustard carbamates, it was considered desirable to attempt the synthesis of enol-carbamates derived from α-halo-ketones.

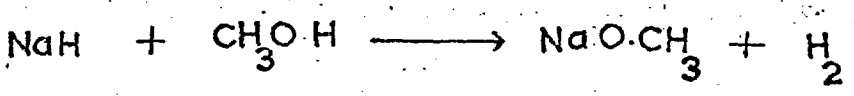
2-chloro-2-phenylacetophenone (desyl chloride) (40), was converted into the sodium enolate (46) by the action of sodium methoxide suspended in anhydrous ether or anhydrous benzene at temperatures ranging from -20 to 5°. The sodium methoxide itself was generated in situ by the action of methanol (1 mole) on sodium hydride (2 moles); the excess of sodium hydride being necessary to remove the methanol formed by the reaction of sodium methoxide on desyl chloride. This procedure of preparing the sodium enolate of desyl chloride has been recommended by Cooper and Owen.[94]

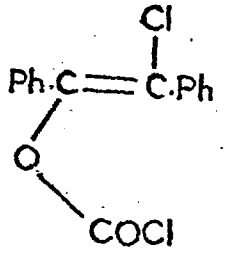
Treatment of the enolate (46) with phenyl isocyanate or diethylcarbamoyl chloride failed to yield the corresponding enol carbamates. Carbamates can, however, be prepared by an alternative method, namely the reaction of an amine with a chloroformate. The enolate (46) was therefore treated with excess of phosgene to give the enol chloroformate (47) albeit not in a very pure state. The crude chloroformate showed characteristic absorption in the infrared spectrum at about 1770 cm.$^{-1}$ (C = O stretch of the chloroformyl group). Very recently Stork and Hudrlik[103] have described a simple procedure for the preparation of metal enolates of ketones. This involves trapping the enolate, generated from the ketone (by the action of sodium hydride), as its trimethylsilyl ether by treatment with trimethylsilyl chloride. This ether on treatment with an organometallic compound such as methyl lithium yields the lithium enolate. The purity and the yield of the chloroformate (47) could probably be increased by adopting this new method of metal enolate formation.

Only one enol chloroformate has been reported in the literature so far, viz., the chloroformate (48) derived from the enol of acetone, which has been described as a pungent smelling liquid b.p. 95^{o}.[104] This chloroformate has not been characterised by conversion

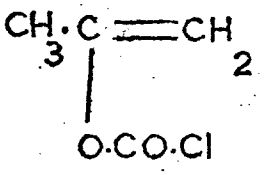

Cl
Ph·CO·CH·Ph
CH3O Na
Cl
Ph·C=C·Ph
O⁻ Na⁺

(40) (46)


NaH + CH3O H ⟶ Na·O·CH3 + H2


Cl
Ph·C=C·Ph
O
COCl

(47)

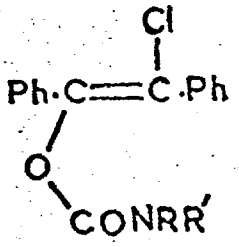

CH3·C=CH2
O·CO·Cl

(48)

into solid derivatives. The sole evidence for its structure comes from the determination of chlorine equivalent.

Treatment of the chloroformate (47) with various primary and secondary amines led to the formation of the corresponding enol carbamates (49a to 49h). All the enol carbamates (49a to 49h) were obtained as crystalline solids and petroleum ether (b.p. 80-100^{0} or b.p. 100-120^{0}) was found to be a good solvent for their recrystallisation.

The infrared and ultraviolet spectral properties of the enol carbamates are summarised in Tables I and II given on pages 61 and 62 respectively.

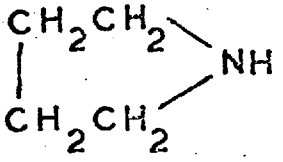


(49 a to h)

49 a, $R = H$; $R' = C_6H_5$

49 b, $R = H$; $R' = \underline{p}\text{-}C_6H_4{\cdot}CH_3$

49 c, $R = CH_3$; $R' = C_6H_5$

49 d, $RR'NH$ = 

49 e, $R = H$; $R' = \underline{o}\text{-}C_6H_4{\cdot}CO{\cdot}OCH_3$

49 f, $R = H$; $R' = \underline{m}\text{-}C_6H_4{\cdot}CO{\cdot}OCH_3$

49 g, $R = H$ $R' = \underline{p}\text{-}C_6H_4{\cdot}CO{\cdot}OCH_3$

49 h, $R = H$ $R' = \underline{p}\text{-}C_6H_4{\cdot}N(CH_2CH_2Cl)_2$

Table I

Characteristic infrared absorptions, in chloroform solution, of compounds (49a to 49h).

COMPOUND	C-O $cm.^{-1}$ (m)-(s)	C=C * $cm.^{-1}$ (w)	C=O $cm.^{-1}$ (s)	NH $cm.^{-1}$ (m)
49a	1180	1635	1745	3420
49b	1195	1620	1755	3410
49c	1140	1635	1720	-
49d	1175	1630	1720	-
49e	1180	1650(shoulder)	1693,1760	3300
49f	1190	1640	1718,1750	3420
49g	1190	1615	1720,1765	3450
49h	1175	1615-1640	1745	3400

* Spectra in this region were run on a Grub Parsons double beam grating instrument.

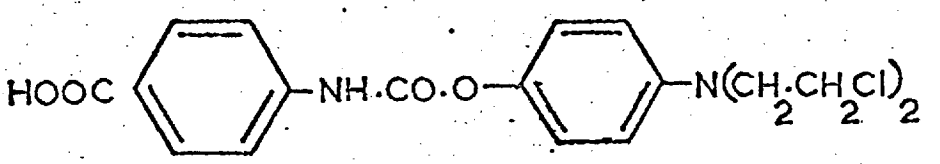
Table II

Characteristic ultraviolet absorptions, in ethanol solutions of compounds (49a to 49h).

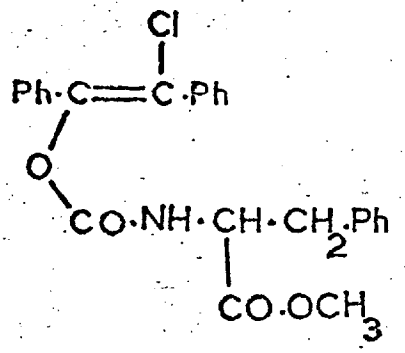
COMPOUND	λ_1 mμ (ε)	λ_2 mμ (ε)	λ_3 mμ (ε)
49a	210 (8,620)	233 (15,145)	273 (6,060)
49b	210(13,880)	235 (21,810)	276 (9,250)
49c	-	227 (16,960)	275 (9,690)
49d	210(13,670)	223 (14,210)	278 (7,650)
49e	225(48,210)	245 (34,630)	292(18,330)
49f	-	230 (21,220)	280(12,510)
49g	210 (3,570)	-	270 (5,100)
49h	208(16,690)	223 (10,311)	270(17,185)

Enol Carbamates of the types (49g) and (49h) should prove very interesting from the point of biological activity. The compound (49h) can give rise to two cytotoxic molecules, viz., desyl chloride and (N,N-di-2-chloroethyl)-p-phenylenediamine (25), upon enzymic cleavage of the carbamate linkage, though a somewhat more promising compound would be one in which the enol of an α-halo-ketone more reactive than desyl chloride is involved. Interest in the enol carbamate (49g) which was obtained by the action of the chloroformate (47) on methyl p-aminobenzoate, stems from the biological importance of the p-aminobenzoyl moiety present in the molecule. This will be evident from later discussion (page 71), about the effective anti-tumour agent (50) which contains the p-aminobenzoic acid unit.

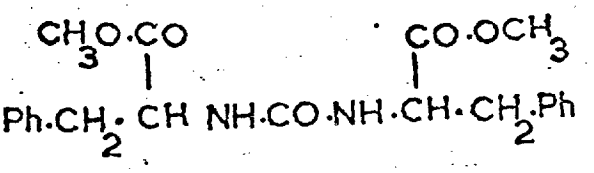
The reaction of the chloroformate (47) with methyl phenylalanate (generated from the hydrochloride by the action of triethylamine) in the presence of triethylamine as condensing agent, did not give the expected carbamate (51), but yielded the urea (52) which was identical with an authentic sample prepared by the action of water on methyl 2-isocyanato-3-phenylpropionate. The reaction of methyl glycinate on the chloroformate (47) likewise did not yield the expected carbamate (53) but in this instance gave the carbonate (54). Considering the



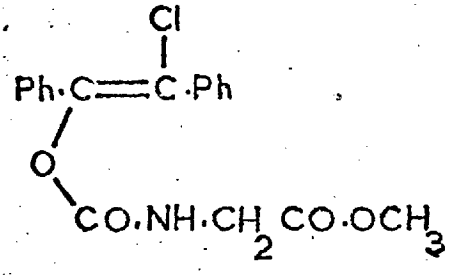
(50)



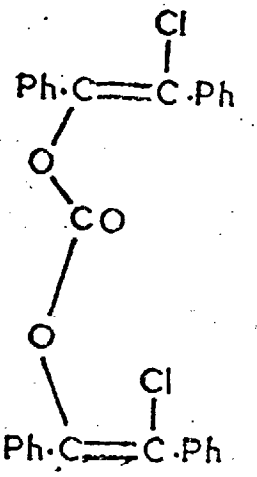
(51)



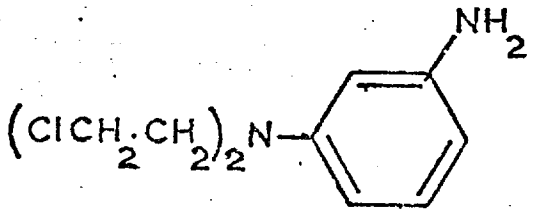
(52)



(53)



(54)



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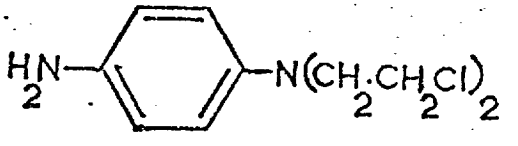
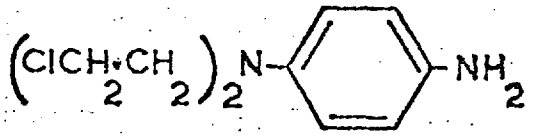


25

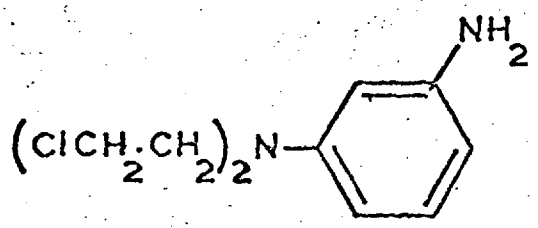
results obtained from the last two anomalous reactions, it seems likely that the chloroformate (47) undergoes disproportionation into the carbonate (54) and phosgene. This would explain the formation of the urea (52) and the isolation of the carbonate (54) from the reaction of methyl glycinate. Such disproportionation is known to occur on heating chloroformates.[105]

SYNTHESES OF SOME CARBAMATES DERIVED FROM (N,N-DI-2-CHLOROETHYL)-p-PHENYLENEDIAMINE AND ITS m-ANALOGUE

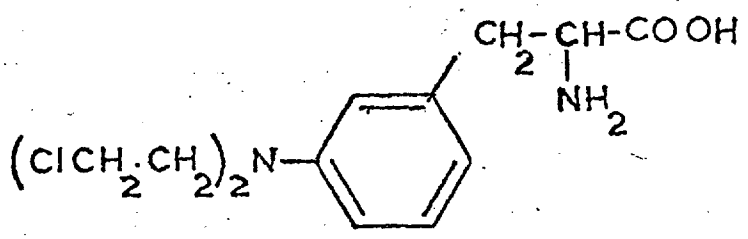
The major part of the present research involved the syntheses of carbamates derived from the cytotoxic mustard amines (25) and (55), which for convenience will be referred to as the p-amino mustard and the m-amino mustard respectively. It has been pointed out in the introduction (see page 31) that the selectivity of a nitrogen mustard such as (25) towards a tumour cell can be increased by acylation of the primary amino group to give a compound susceptible to enzymic activation. Of special interest were carbamate derivatives, because their action would be potentiated by prior administration of a non-mustard urethane (see page 38).[78] The toxicity of the p-amino mustard (25) is greatly reduced by conversion into a carbamate, because the electron attracting nature of the carbonyl group considerably reduces the mesomeric effect of the amino group and lowers the basicity of the nitrogen atom in the mustard moiety. Such a reduction in toxicity is not so likely in the case of the m-amino mustard (55) because the mesomeric effect of the substituent group would be ineffective at the m-position.



(25)



(55)



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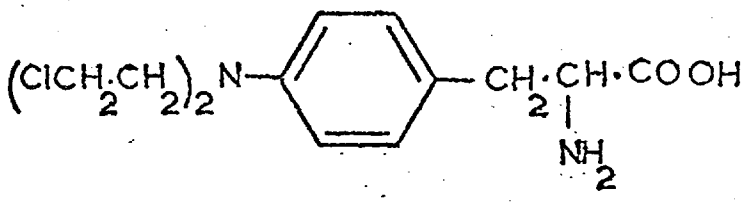


(31)

Although derivatives of the m-amino mustard would therefore seem to be much less likely to show favourable chemotherapeutic indices, it was considered worthwhile to synthesise a few derivatives of the m-compound for the following reasons:-

(i) The m-analogue (56), of sarcolysine (31) (see page 36) is ten times as effective as sarcolysine while it is only three times as toxic.[106]

(ii) The derivatives of the m-amino mustard (55) can be used in tissue culture studies where damage to host cells is not a serious problem. Knowledge gained from tissue culture studies must be confirmed by tests on animals because the blood supply to a diseased site cannot be simulated in tissue cultures. However, such tests on animals could be restricted to the more effective of the m-amino mustard derivatives.

A few analogous model compounds containing the phenyl group instead of the aromatic nitrogen mustard were prepared, for use as potentiators in enzymic activation and sometimes for the characterisation of certain intermediate chloroformates (which were seldom isolated in a pure state).

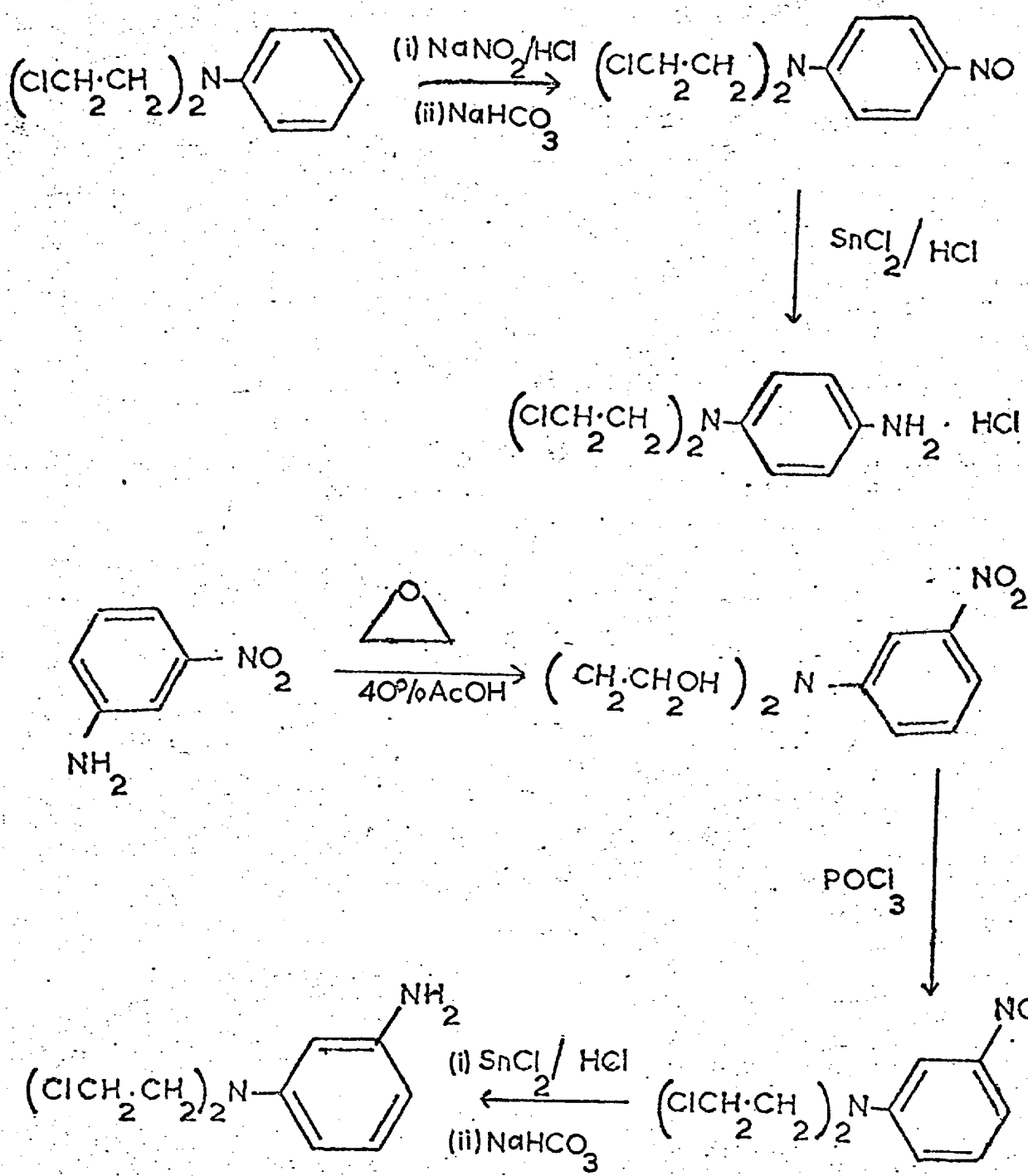
The hydrochloride of the p-amino mustard (25) was synthesised from (N,N-di-2-hydroxyethyl)aniline

according to the scheme due to Everett and Ross,[107] shown on page 70.

(N,N-Di-2-hydroxyethyl)-m-phenylenediamine was prepared from m-nitroaniline by the scheme given on page 70 which is essentially the same as that adopted by Bieksa and Degutis[108] except for the modification that phosphorus oxychloride was used instead of thionyl chloride in the chlorination step. The reason for this change in procedure was that the use of thionyl chloride is apt to give a cyclic sulphite as a by-product when the reaction is conducted on a diol.[108,109]

The mustard amines (25) and (55) reacted with phenyl chloroformate in the presence of anhydrous pyridine to form the carbamates (57) and (58) respectively, of which the former had been previously synthesised by a different route by Benn.[59]

p-Methoxycarbonylphenyl chloroformate (59) was prepared by the action of phosgene on methyl p-hydroxybenzoate in the presence of quinoline as described by Einhorn et al.[110] Quinoline, in addition to removing hydrogen chloride formed in the reaction, presumably forms a complex with phosgene (which is reported to be a good

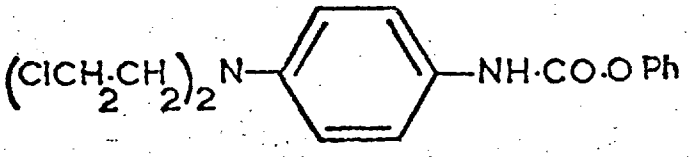

(ClCH·CH2)2N
(i) NaNO2/HCl
(ii) NaHCO3
NO
SnCl2/HCl
NH2 · HCl
NO2
40% AcOH
(CH2·CHOH)2N
POCl3
NH2
(i) SnCl2/HCl
(ii) NaHCO3

(55)

chloroformylating agent for phenols.).[111]

p-Methoxycarbonylphenyl chloroformate was condensed with the p-amino mustard (25) to obtain the expected carbamate (60). The ester group in (60) was selectively hydrolysed to the acid (61) by refluxing with a (1:1) mixture of glacial acetic acid and concentrated hydrochloric acid for 35 minutes. This procedure had been used with considerable success by Owen et al.[112, 113] for the selective hydrolysis of methyl esters in compounds containing thiolcarbamate or carbamate group. The synthesis of (61) was motivated by the promising biological results which emerged from tests on (50) containing the p-aminobenzoic acid moiety, synthesised by Owen et al.[112] It is important to note that the positions of the nitrogen and oxygen atom of the carbamate link are reversed in (61) and (50).

It may be reasonable to assume that the activities of (60) and (61) should not be much different from one another, because the former would be converted into the latter by esterase activity in vivo. A possible advantage of (61) over (60) would be the solubilising effect of the carboxyl group, thus allowing the drug to be administered in buffered solution rather than as an insoluble suspension.



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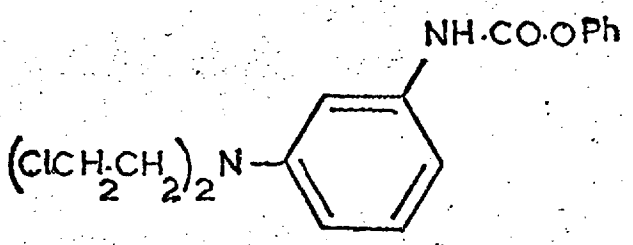

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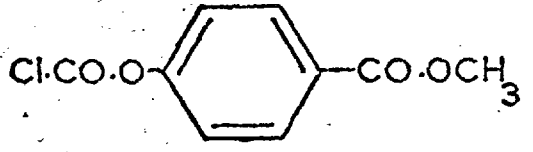

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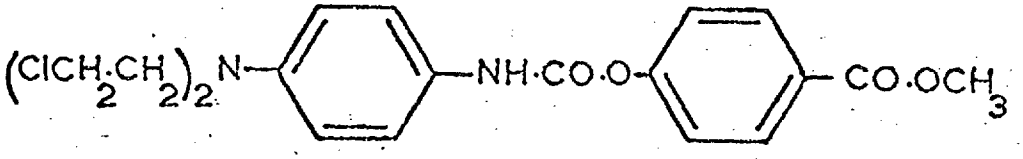
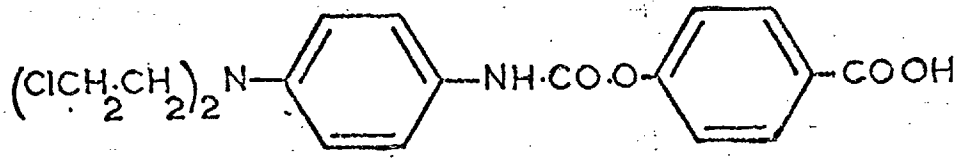
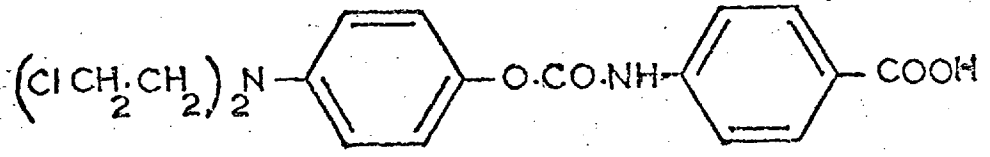


(60)



(61)

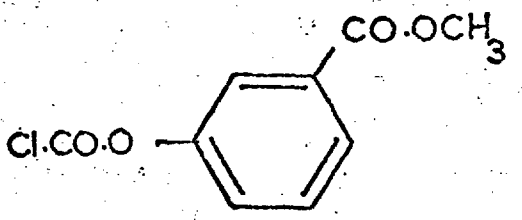


(50)

Since there is no reason to suppose that the p-position is necessarily the best for the carboxyl group to be in, syntheses of other structural isomers were investigated.

m-Methoxycarbonylphenyl chloroformate (62) was prepared by the action of phosgene on methyl m-hydroxy-benzoate in the presence of quinoline. The chloroformate, which was not isolated pure, was converted into m-methoxycarbonylphenyl N-phenylcarbamate (63), both for characterisation of the chloroformate and for possible use as an enzymic potentiator. Reaction of the crude chloroformate with the p-amino mustard and with the m-amino mustard gave the carbamates (64) and (65) respectively. Both of these compounds were obtained as crystalline solids, and they were individually selectively hydrolysed by refluxing with a (1 : 1) mixture of glacial acetic acid and concentrated hydrochloric acid for 45 minutes to obtain the corresponding free acids (66) and (67) respectively.

o-Methoxycarbonylphenyl chloroformate was prepared from methyl salicylate by the action of phosgene, using quinoline as catalyst, according to the method of Einhorn et al.[114] The carbamate (68) was obtained in very good yield by the action of this chloroformate on



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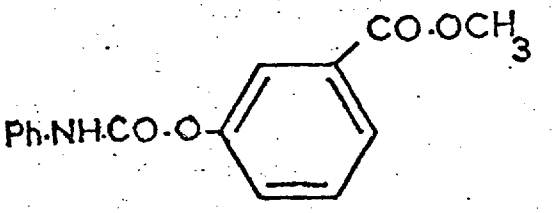

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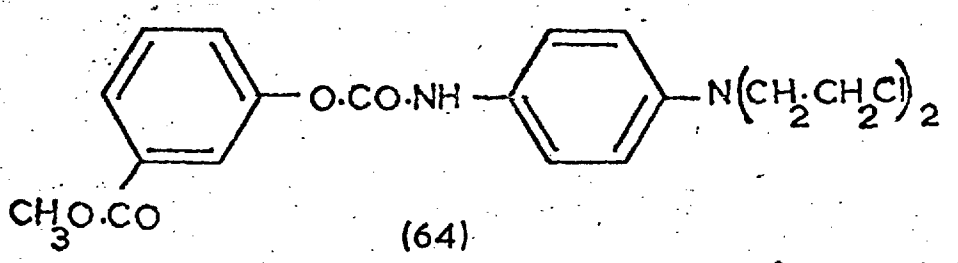

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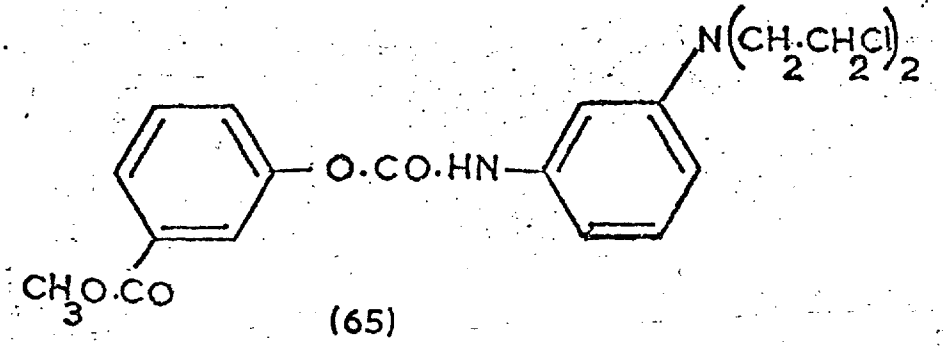
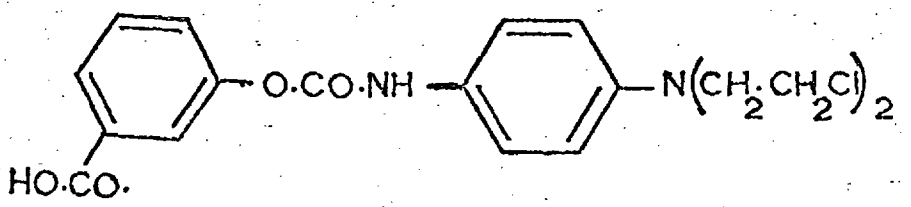


(65)



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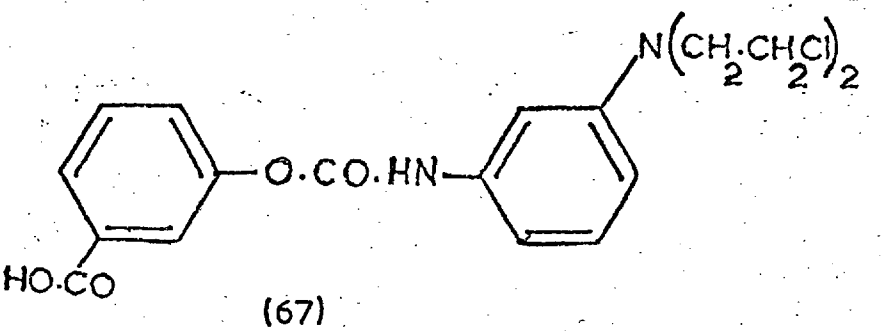
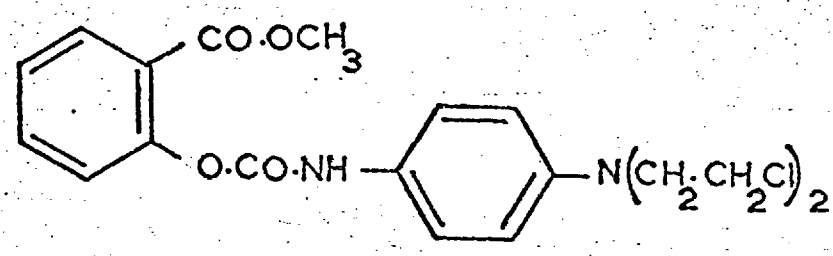


(67)

the p-amino mustard (25). Attempts to obtain the free acid (69) from the methyl ester (68) were unsuccessful. This difficulty in the hydrolytic step can be attributed to the steric hindrance due to the o-substituent . Because of the difficulty experienced in this hydrolysis, it was decided to prepare the benzyl ester (70) which should yield the desired acid (69) on hydrogenolysis. For this purpose benzyl salicylate was converted into the chloroformate (71) which was not isolated but characterised as the N-phenylcarbamate (72). The crude chloroformate (71) was then condensed with the p-amino mustard (25) and the m-amino mustard (55) to obtain the corresponding carbamates (70) and (73) respectively. The hydrogenolysis of (70) using 5% palladium on charcoal gave a poor yield of the acid (69), which crystallised with half a mole of benzene of crystallisation. The hydrogenolysis of the isomeric benzyl ester (73) was not attempted.

Ethyl and isopropyl chloroformate were condensed with the m-amino mustard (55) to obtain the carbamates (74) and (75) respectively. These are the m-analogues of the p-compounds originally prepared by Benn[59] and it will be of much interest to discover whether they likewise undergo activation at the tumour site by the



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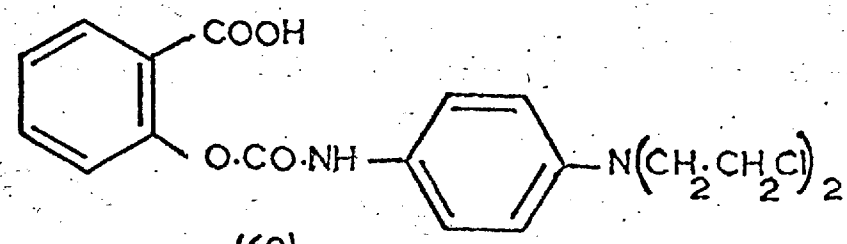

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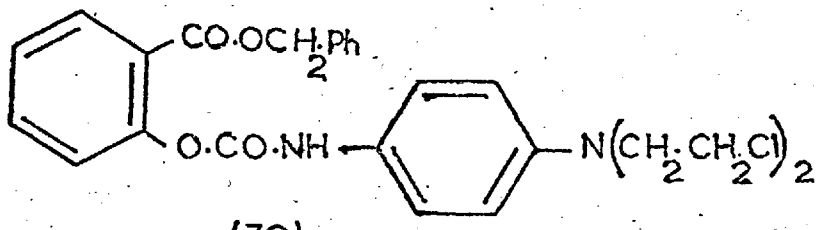

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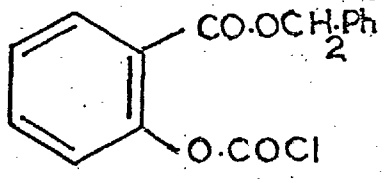

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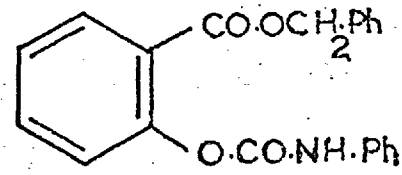

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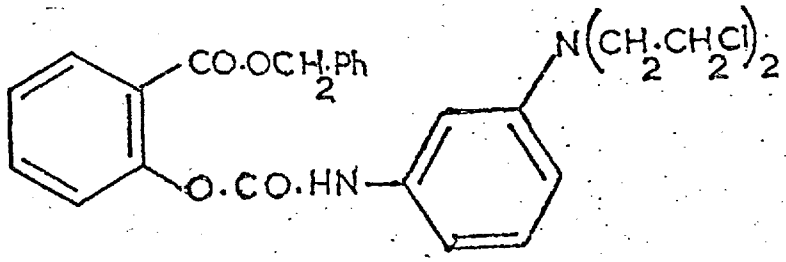


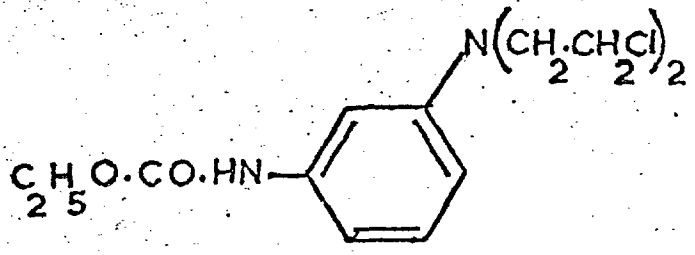
(73)

adaptive enzyme induced by prior administration of a potentiator such as (36).

In order to examine the effect of a non-carboxylic substituent in place of the carboxyl group present in the carbamate (61) (page 71), 4-cyclohexylphenyl chloroformate (76) was prepared by the reaction of a solution of 4-cyclohexylphenol in 2N sodium hydroxide with phosgene. The chloroformate was not obtained pure but was characterised by converting it into the N-phenylcarbamate (77) by the action of aniline. In the chloroformate preparation a small amount of di-4-cyclohexylphenyl carbonate was obtained as a pure crystalline by-product. The crude chloroformate (76) was converted into the carbamates (78) and (79) by reaction with the p-amino mustard (25) and the m-amino mustard (55), respectively.

Derivatives of some amino-acids

The liver, lungs and kidneys of animals are rich in glycine and serine; and these amino-acids are known to be interconvertible under physiological conditions.[115] Thus it may be expected that nitrogen mustards containing glycine and serine moieties will be actively transported to the cells in the liver, lungs and kidneys of animals. Further, it is known that the following substances can act as precursors of t



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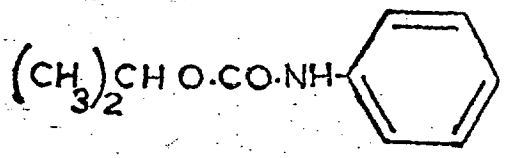

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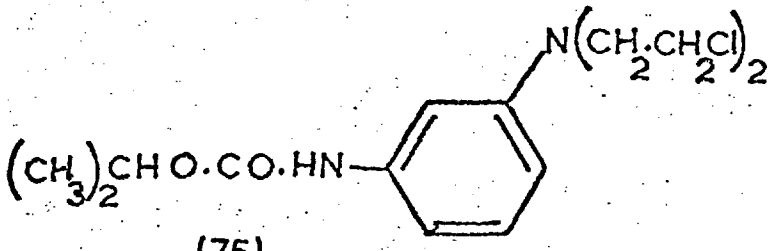

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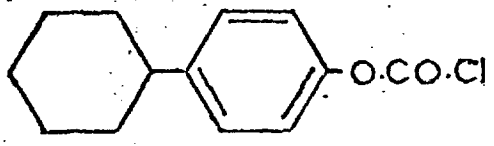

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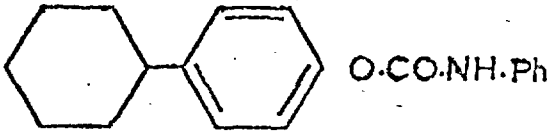

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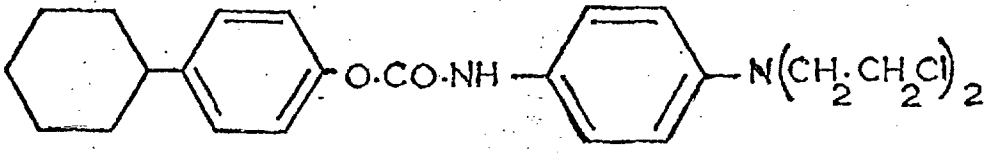

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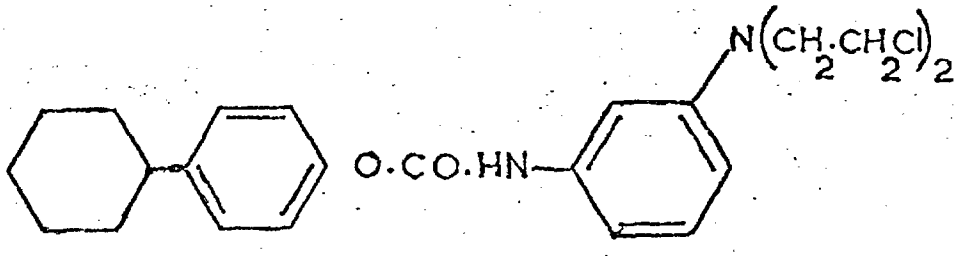


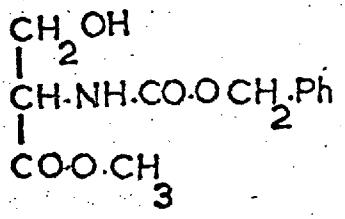
(79)

β-carbon atom of serine, viz., the α-carbon atom of glycine, formate, formaldehyde and the methyl groups of methionine, choline, acetone and sarcosine.[115]

It is, therefore, reasonable to assume that by careful dietary control, it should be possible to induce an artificial deficiency of glycine and serine in an animal. Such an animal which has been rendered deficient in glycine and serine may assimilate a nitrogen mustard derived from glycine or serine by a modified active transport mechanism. These facts together with the knowledge that glycine and serine can serve as precursors in the biogenesis of many biologically important molecules such as glutathione, cysteine, proteins, porphyrins, purines and nucleic acids have led to the synthesis, by various workers, of effective nitrogen mustards containing serine[70,71] or glycine[64,65,66,67] as carrier. Further, Skinner and co-workers[116,117] found that O-carbamoyl serine and O-hydrazinocarbamoylserine acted as antagonists of glutamine in various bacterial systems. Recently, Bergel and Wade[71] have shown that derivatives of aliphatic nitrogen mustards attached to serine through a carbamate linkage are effective anti-tumour agents. In this context it was considered worthwhile to synthesise derivatives of the aromatic mustard amines (25) and (55)

attached to the hydroxyl group of serine through a carbamate linkage.

DL-Serine was converted into the methyl ester hydrochloride by Fischer and Suzuki's[118] method using methanol saturated with hydrogen chloride. Treatment of DL-serine methyl ester hydrochloride with benzyl chloroformate in the presence of sodium hydroxide as described by Bergel et al.[71], gave N-benzyloxycarbonyl-DL-serine methyl ester (80), which was converted into O-chloroformyl-N-benzyloxycarbonyl-DL-serine methyl ester (81), using phosgene as described by Bergel and Wade.[71] The chloroformate (81) was not obtained pure but was characterised by converting it into a crystalline derivative (82) by the action of aniline. The condensation of (81) with the p-amino mustard (25) in the presence of anhydrous pyridine led to the formation of the carbamate (83). Hydrogenolysis of (83) using 5% palladium on charcoal as catalyst gave the α-amino ester (84). The infrared spectrum of (84) in nujol showed characteristic absorption at 1680 (shoulder), 1735, 2400 to 3600 (very broad peak) $cm.^{-1}$. The selective hydrolysis of the methyl ester group in (84) to obtain the free acid (85) could not be achieved, and an alternative scheme, using a benzyl ester,



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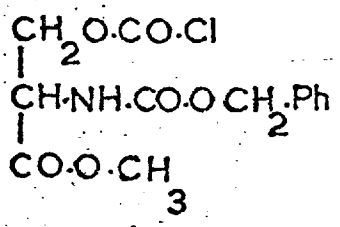

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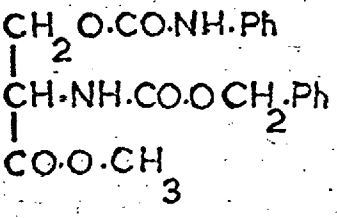

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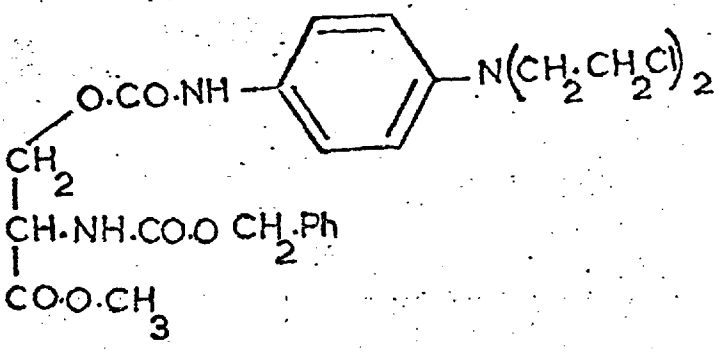

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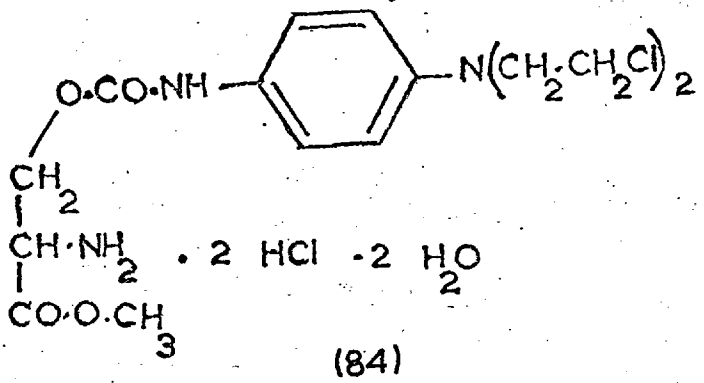

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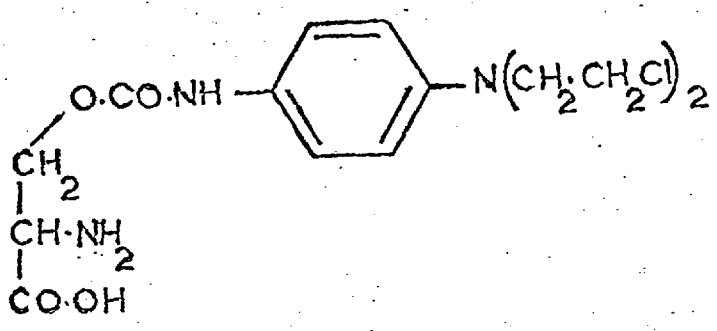
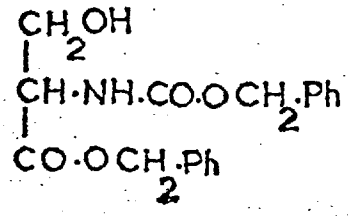


(85)

was therefore investigated.

DL-Serine was converted into N-benzyloxycarbonyl--DL-serine by the action of benzyl chloroformate on a solution of DL-serine in 4 N sodium hydroxide according to the method of Bergmann et al.[119] This product was converted into the benzyl ester (86) by the method of Ben-Ishai and Berger[120] using toluene-p-sulphonic acid as catalyst and benzyl alcohol in benzene; the water being removed azeotropically using a Dean and Stark water separator. This ester was converted by the action of phosgene in benzene into the chloroformate (87) from which the N-phenylcarbamate (88) was made, by the action of aniline, for the purpose of characterisation of the chloroformate. The chloroformate was then made to react with the p- and m- mustard amines (25) and (55), under the influence of anhydrous pyridine, to obtain the crystalline carbamates (89) and (90) respectively. The catalytic hydrogenation of (89) in the presence of 5% palladium on charcoal using methanol-ethyl acetate as solvent gave (91) in which only one of the protecting groups had been removed. It had characteristic infrared absorption at 1715, 3400, 3670 cm.$^{-1}$. However, catalytic hydrogenation using 10% palladium on charcoal using methanol containing concentrated hydrochloric acid gave the product (92) in



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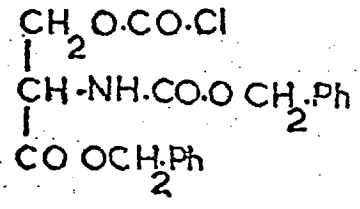

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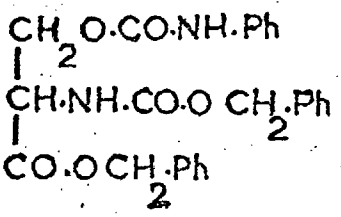

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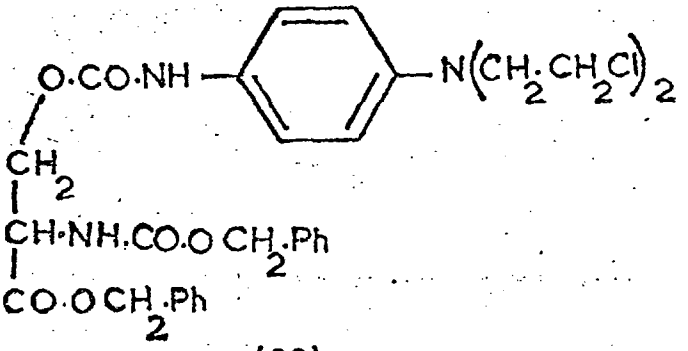

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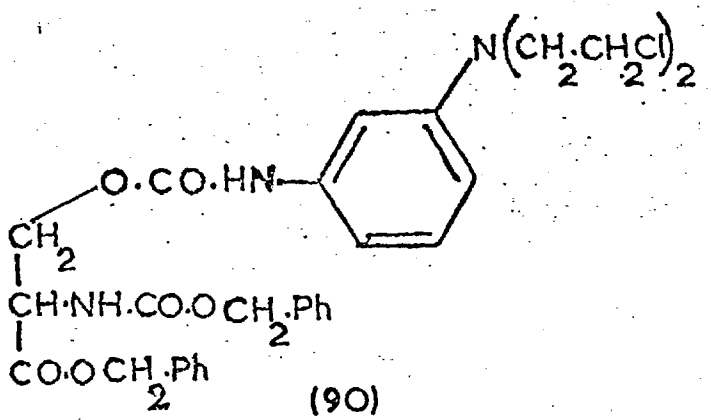

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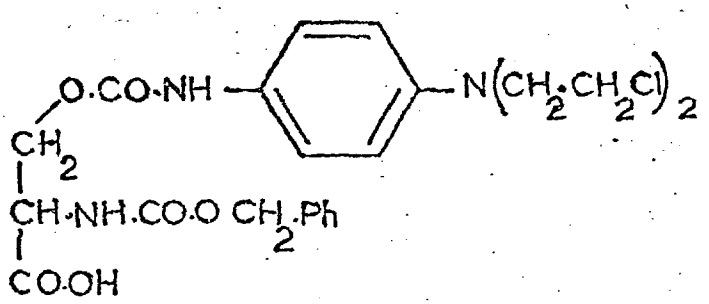
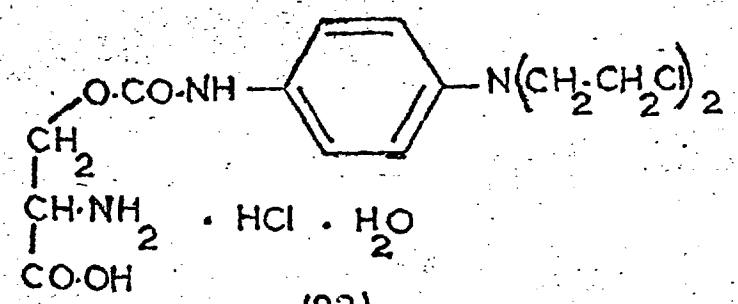


(91)

which both the protecting groups had been removed. The monohydrochloride monohydrate (92) showed characteristic infrared absorption (nujol mull) at 1680-1735 (broad peak), 2400-3600 (broad peak) cm.$^{-1}$.

Bergel et al.[71] have found that whereas serine derivatives of aliphatic nitrogen mustards attached through a carbamate linkage possessed anti-tumour activity, the corresponding threonine derivatives were inactive. It was decided to synthesise a threonine derivative of the aromatic p-amino mustard (25) to compare its anti-tumour activity with that of the corresponding serine derivative.

The nitrogen atom in DL-threonine was protected by conversion into N-benzyloxycarbonyl-DL-threonine according to the method of Riley et al.[121] / then esterified using benzyl alcohol and the ester was treated with phosgene in the presence of N,N-di-methylaniline to obtain the chloroformate (93). This was characterised by converting it into the solid derivative (94) by the action of aniline. The chloroformate reacted with the p-amino mustard (25) to give the carbamate (95) in the usual manner. Catalytic hydrogenation of (95) using 10% palladium on charcoal in methanol containing concentrated hydrochloric acid removed both protecting groups and gave the required compound as the dihydrochloride dihydrate (96). It showed



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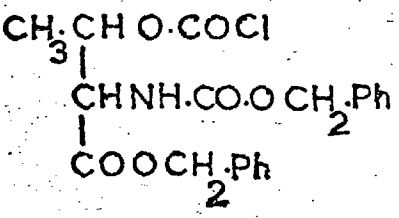

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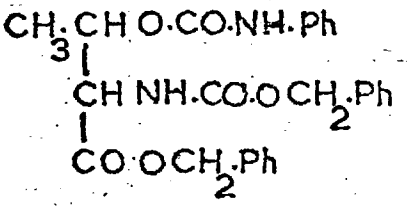

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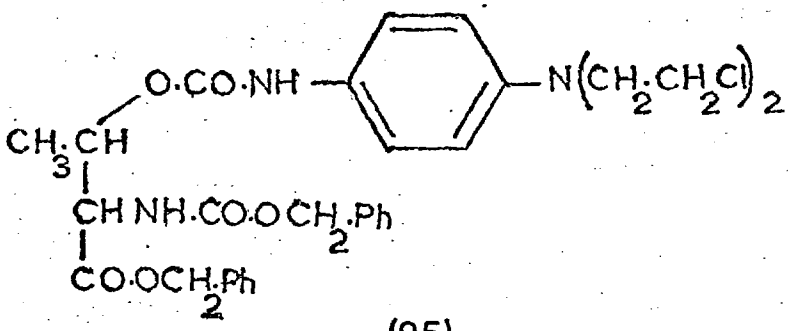

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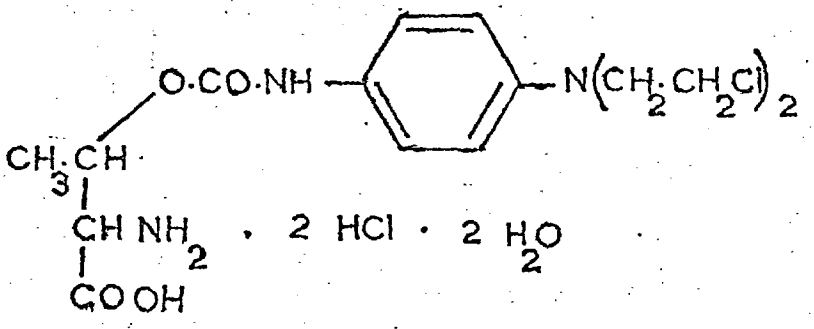


(96)

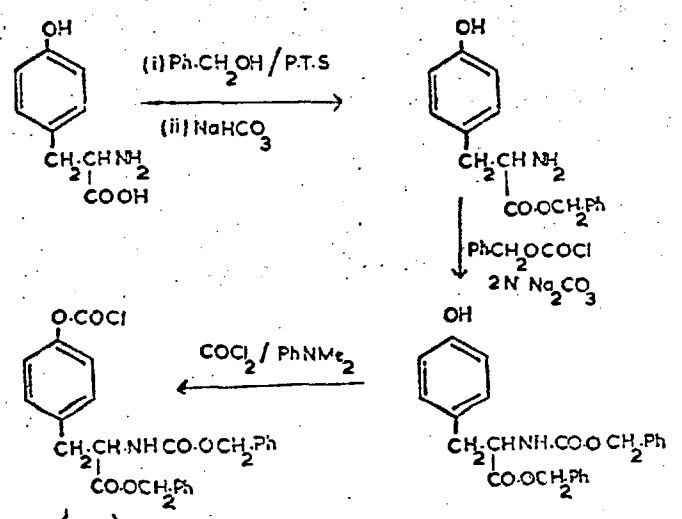
characteristic infrared absorption (nujol mull) at 1680-1740 (broad peak), 2400-3600 (broad peak) cm.$^{-1}$.

Tyrosine can serve as a precursor of several biologically important substances such as adrenaline, 3,4-dihydroxyphenylalanine (DOPA) and thyroxine.[115,122] It is also known that in animals phenylalanine is oxidised to tyrosine even when they are fed an excess of tyrosine. The reverse, i.e., the conversion of tyrosine to phenylalanine does not occur even in phenylalanine deficient animals. Thus it is clear that tyrosine is not essential if an adequate supply of phenylalanine is available in the diet.[115]

Thus, tyrosine deficiency can be induced in a tumour bearing animal by feeding it on a diet low in phenylalanine-content. Now if a nitrogen mustard derivative attached to a tyrosine moiety is administered to the animal, greater specificity through a modified active transport mechanism may result. Bearing this in mind several workers have synthesised derivatives of nitrogen mustards attached to tyrosine.[65,72] Continuing on a similar line of thought, an attempt was made to synthesise a derivative of the p-amino mustard (25) linked to L-tyrosine through a carbamate bridge.

L-Tyrosine was converted into O-chloroformyl-N-benzyloxycarbonyl-L-tyrosine benzyl ester (97) according to the method of Bergel and Wade [72] which is shown on page 88. The chloroformate (97) was not purified and was characterised as the solid N-phenylcarbamate (98) obtained by the action of aniline. Condensation of (97) with the p-amino mustard gave a mixture of products. Chromatography of the mixture yielded two pure substances, viz., the mustard urea (99) and the expected carbamate (100) in a very poor yield, insufficient for catalytic hydrogenation. The formation of the urea (99) might have resulted from nucleophilic attack by excess of the mustard amine (25) on the carbamate (100) as depicted.

Alternatively, the chloroformate (97) might have undergone a disproportionation reaction (as previously discussed, page 65) to yield phosgene and a carbonate. The urea (99) could then have resulted from the action of phosgene on the amine (25).



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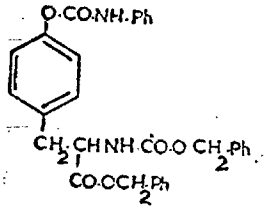

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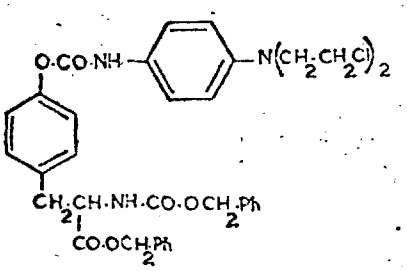

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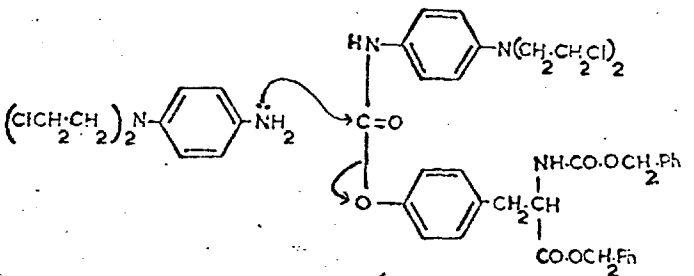
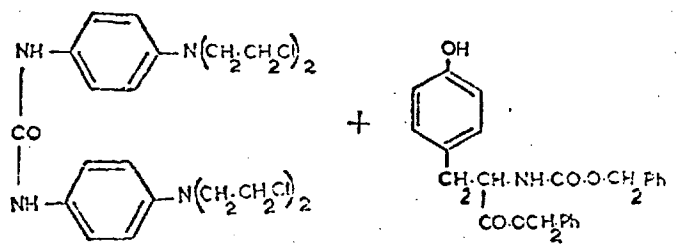


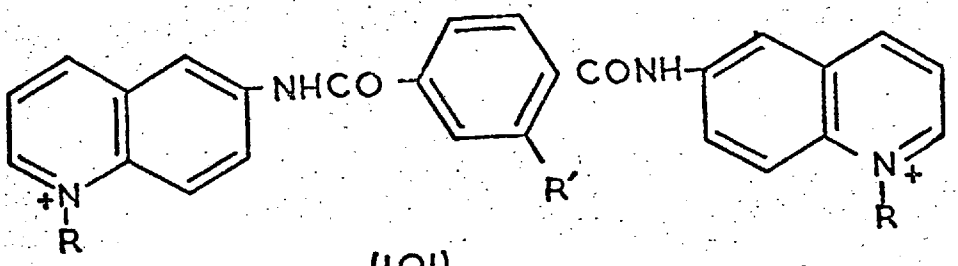


(99)

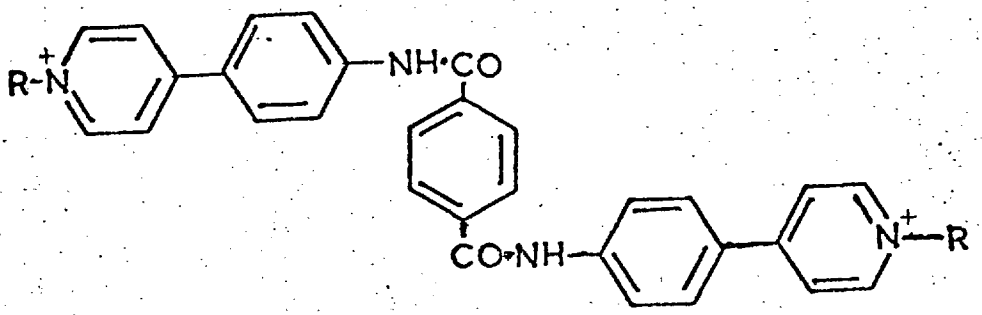
SYNTHESES OF SOME AMIDES DERIVED FROM (N,N-DI-2-CHLOROETHYL)-p-PHENYLENEDIAMINE

Amides prepared by acylation of the primary amino group in the highly cytotoxic p-amino mustard (25) are considerably deactivated because of the electron-attracting effect of the acyl group, as previously explained. A certain degree of amidase activity is often found in biological systems and there is good evidence that certain amides derived from the mustard (25) undergo enzymic activation at the tumour site.[78,123] Owen et al.[112] have synthesised several amides by the action of the p-amino mustard (25) on various aliphatic acid chlorides. It was decided to extend this work to some aromatic amides, because certain derivatives of terephthalic amide such as (101) and (102) have been synthesised by Atwell et al.[124,125] and shown to be potential anti-tumour agents. This motivated the synthesis of the mustard derivatives (103) and (104) of terephthalic amides.

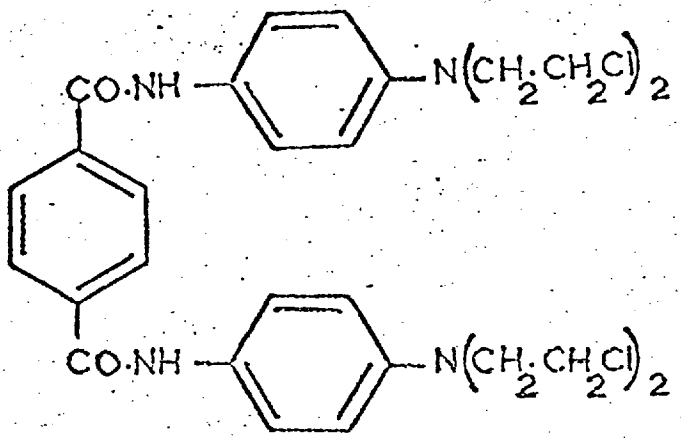
Terephthaloyl chloride was condensed with the p-amino mustard (25) in the presence of pyridine to obtain the diamide (103) in good yield.



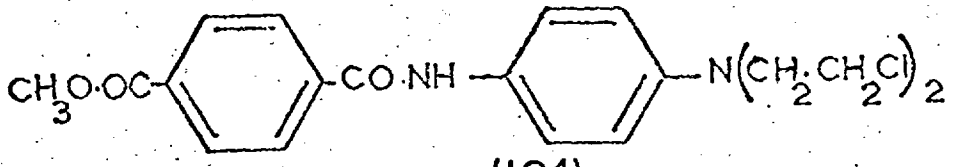
(101)



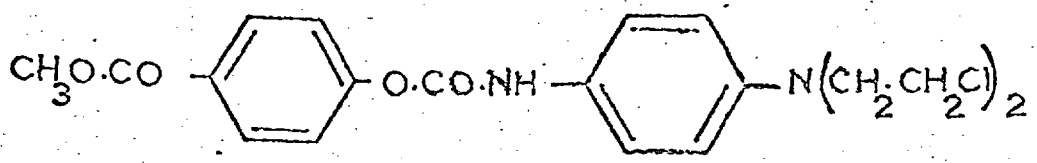
(102)



(103)



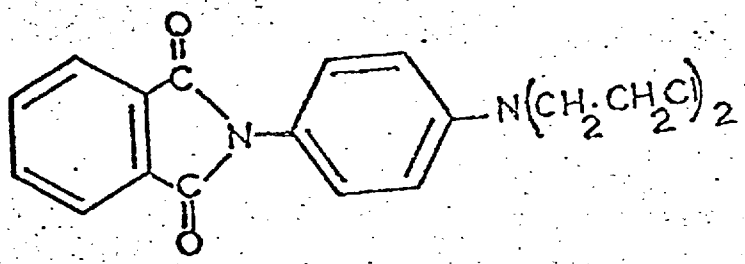
(104)



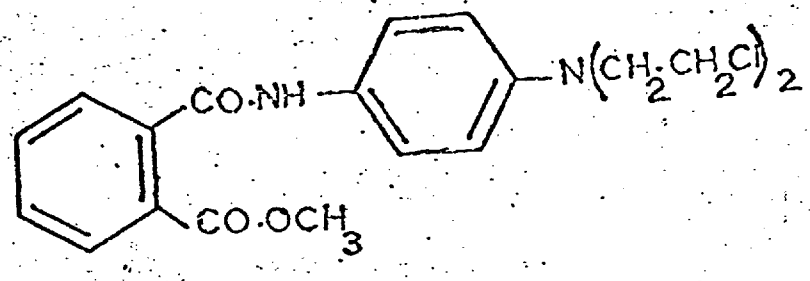
(60)

The preparation of monomethyl terephthalate, required for the synthesis of the monoamide (104) proved rather difficult and none of the methods described in the literature could be repeated. These involved partial hydrolysis of dimethyl terephthalate using various solvents and alkali. However, the acid chloride derived from monomethyl terephthalate was obtained in a poor yield by distillation of a mixture obtained by treating terephthaloyl chloride (2 moles) in benzene with methanol (slightly less than 1 mole). The p-methoxycarbonylbenzoyl chloride obtained in this way was contaminated with terephthaloyl chloride. It was used immediately for condensing with the p-amino mustard (25) and the resultant product was purified by repeated chromatography (silica gel, chloroform) to obtain crystalline p-methoxycarbonylbenzoic N-(p-N,N-di-(2-chloroethylamino)phenyl)amide (104).
A noteworthy feature about the amide (104) is that it differs from the carbamate (60) by only one oxygen atom and comparison of the activities of (104) and (60) may yield some interesting results.

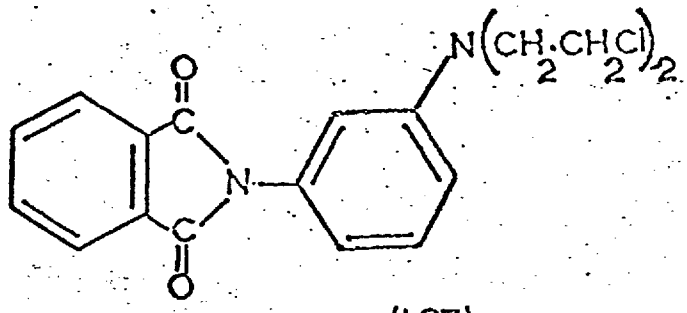
o-Methoxycarbonylbenzoyl chloride was obtained by the action of thionyl chloride on monomethyl phthalate according to the method of Eliel et al.[126] The reaction



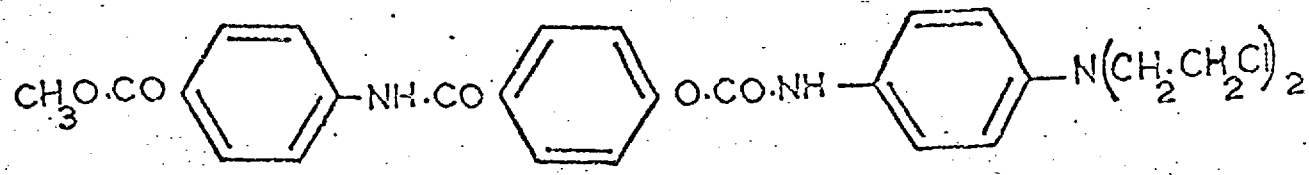
(105)



(106)



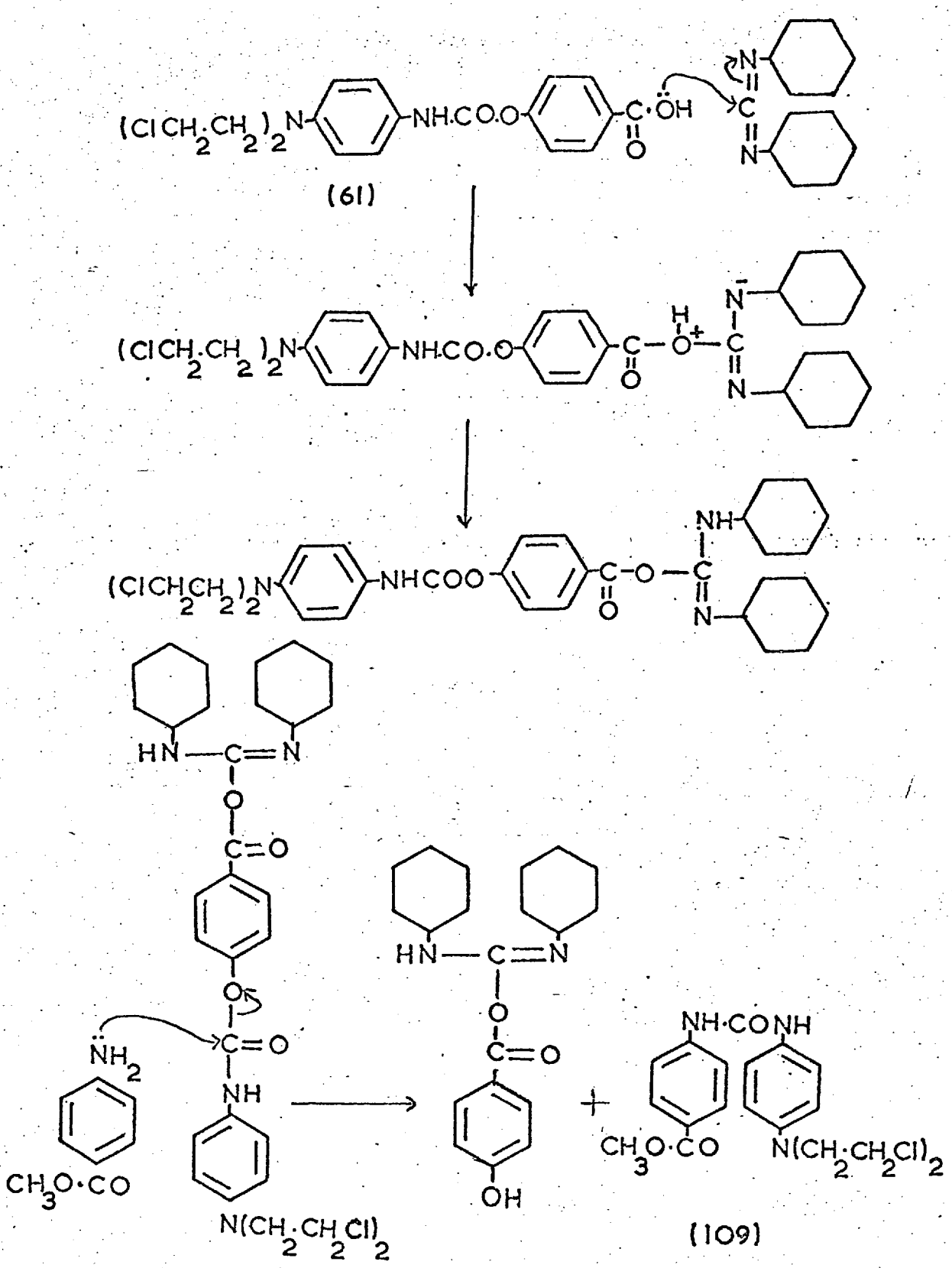
(107)



(108)

between o-methoxycarbonylbenzoyl chloride (1 mole) and the p-amino mustard (25) (1 mole) yielded the cyclic imide (105) instead of the amide (106) which was intended for comparison with the p-isomer (104). The cyclic imide (107) resulted likewise from the reaction of the m-amino mustard (55) with o-methoxycarbonylbenzoyl chloride.

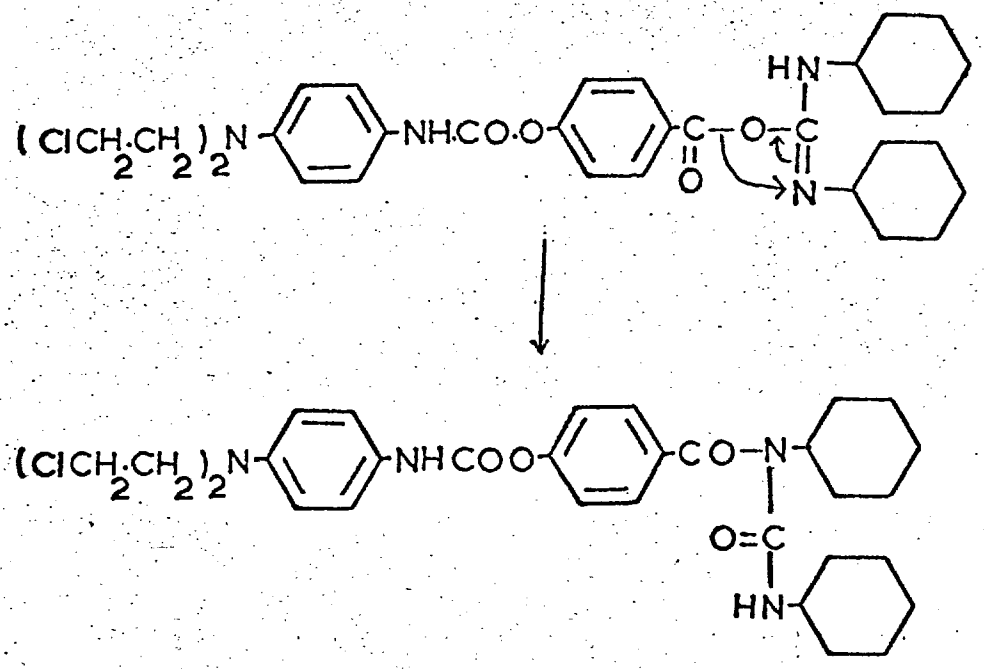
The biological tests on the mustard carbamate (61) and its methyl ester (60) (the syntheses of which were described on page 71) were very encouraging, and attempts were made to modify the carboxyl group and so obtain another mustard derivative (108), which contains an amido link in addition to the carbamate bridge already present in (61). Condensation of methyl p-aminobenzoate and the mustard (61) in the presence of dicyclohexylcarbodiimide gave a mixture of products, whose separation was rather difficult, and on chromatography (alumina, chloroform), only one substance could be isolated pure. This substance which showed a single spot on t.l.c. was identified as the urea (109) on the basis of microanalytical data. The formation of (109) could have taken place by either of the two schemes, (i) and (ii), given on pages 94 and 95 respectively.

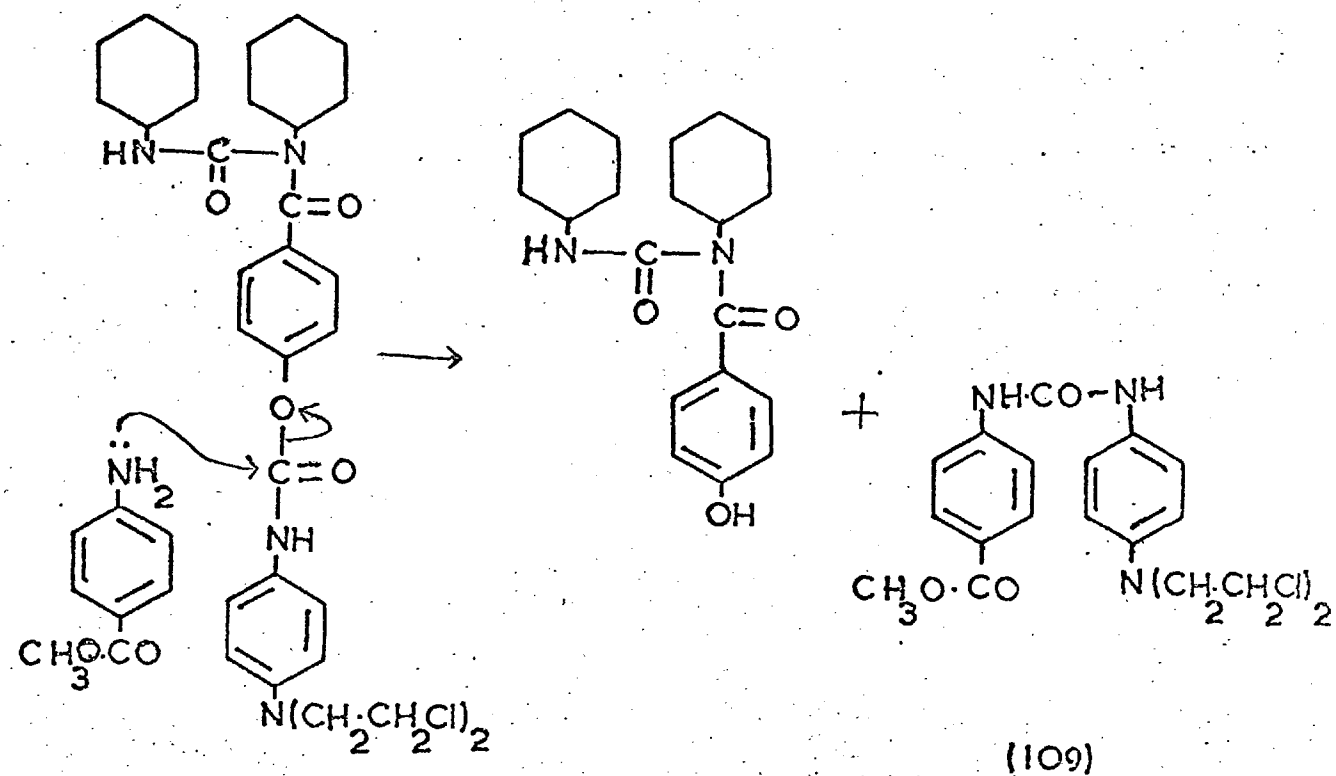


(61)

(109)

SCHEME (i)





(109)

SCHEME (ii)

An attempt to effect the condensation of the mustard (61) with methyl p-aminobenzoate by the mixed anhydride method, using ethyl chloroformate, also resulted in a mixture of products, the purification of which gave only one pure substance, viz., the mustard urea (99) which was found to be identical with an authentic sample in all respects.

BIOLOGICAL RESULTS

Certain of the compounds were sent to the Department of Biochemical Pharmacology of the State University of New York at Buffalo, for biological tests. The results of these tests, which are summarised in the tables A and B on the following page, were made available through the kindness of Professor Hebborn.

The results have been very encouraging and four of the compounds tested had promisingly high chemotherapeutic indices. It is interesting to note that the methyl esters, I.C. 220, has a higher chemotherapeutic index than the corresponding free acid, I.C. 213 which would be considerably easier to administer in aqueous solution. More accurate figures on I.C. 214 are still awaited; this compound is of some interest because it can give rise to the p-amino mustard (25) and desyl chloride as a result of enzymic cleavage of the carbamate link.

No figures are yet available for the derivatives of the m-amino mustard (55).

Table A.

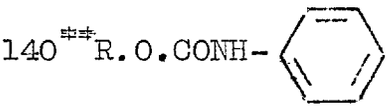
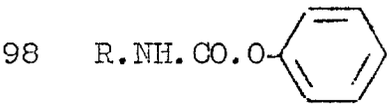
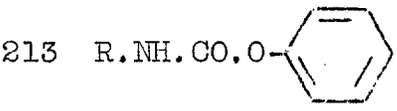
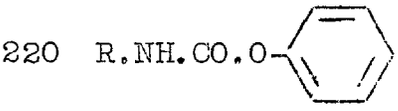
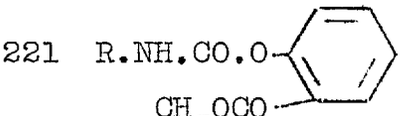
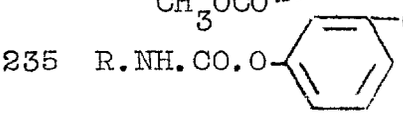
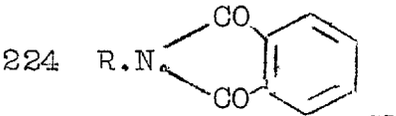
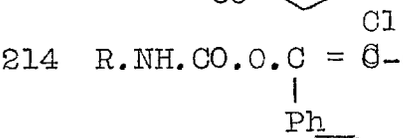
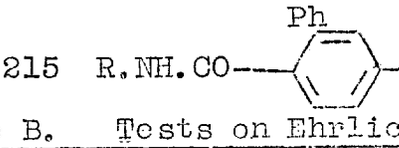
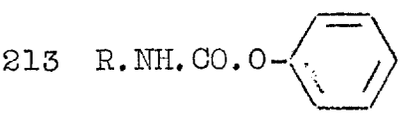
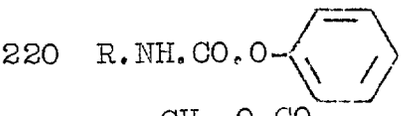
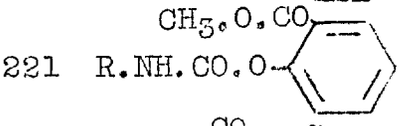
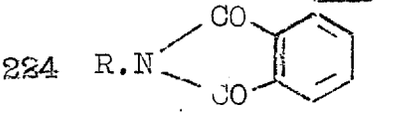
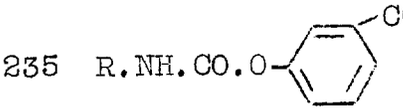
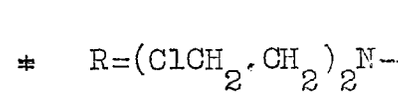
Code No.	Formula of the Drug*	Acute LD_{50} mg./kg. Mouse	Acute LD_{50} mg./kg. Rat	Walker ED_{90}	$\frac{LD_{50}}{ED_{90}}$ = C.I
I.C. 140**		187	56	3.3	17.0
I.C. 98		58	200	30.0	6.6
I.C. 213		43	58	1.2	46.0
I.C. 220		135	110	0.84	131.0
I.C. 221		230	115	0.5 to 1.0	about 100.0
I.C. 235		115	68	0.84	81.0
I.C. 224		480	500	500	?
I.C. 214		1000	500	250	2.0
I.C. 215		1000	500	500	?

Table B. Tests on Ehrlich Ascites Carcinoma

Code No.	Formula of the Drug*	Single Dose, 1 Day LD_{50} mg/kg.	Single Dose, 1 Day ED_{90} mg/kg.	Single Dose, 1 Day $\frac{LD_{50}}{ED_{90}}$	5 Dosed Days 1-5 LD_{50} mg/kg.	5 Dosed Days 1-5 ED_{90} mg/kg.	5 Dosed Days 1-5 $\frac{LD_{50}}{ED_{90}}$
I.C. 213		50	16	3.1	2.3	4.2	5.5
I.C. 220		150	46	3.3	?	9.5	?
I.C. 221		145	12.5	?	60	5.0	?
I.C. 224		310	225				
I.C. 235		88	21.5	4.1	44	5.4	8.1

* 

** This compound was made by Owen *et al.*[112]

EXPERIMENTAL

Numbers, in parentheses, following the headings throughout this chapter refer to the page numbers of the original laboratory record note books.

Infrared spectra were run on a Unicam S.P.200 spectrometer and ultraviolet spectra on a Unicam S.P.800 instrument. In certain cases, the infrared spectra in the 1600-1650 $cm.^{-1}$ region were run by Mr. Rashid Iqbal on a Grub Parsons double beam grating instrument. Values thus obtained are indicated by an asterisk. Proton magnetic resonance spectra were measured by Mrs. A.I. Boston using a Varian A-60 spectrometer.

Melting points were determined on a Kofler block and are uncorrected. In this chapter (N,N-di-2-chloroethyl)-p-phenylenediamine and (N,N-di-2-chloroethyl)-m-phenylenediamine are referred to as mustard amines A and B respectively.

SOME ENOL CARBAMATES DERIVED FROM 2-CHLORO-2-PHENYLACETOPHENONE

2-Chloro-2-phenylacetophenone (29)

This compound was prepared from benzoin, by the action of thionyl chloride in pyridine, using the method of Ward.[127] It had m.p. 66-68°; Ward[127] gives m.p. 66-67°.

N,N-Diethylcarbamoyl chloride (1)

This compound was prepared by the action of diethylamine on an excess of phosgene. It had b.p. 186-190°. (Yield 64%). Lit.,[128] b.p. 186°.

Attempted preparation of (2-chloro-1,2-diphenyl)vinyl N,N-diethylcarbamate (3)

A stirred suspension of sodium hydride (0.48 g.) in dry ether (20 ml.) was treated with anhydrous methanol (0.32 g.). The well-stirred suspension was cooled to -20° and treated with a solution of 2-chloro-2-phenylacetophenone (2.3 g.) in anhydrous ether (10 ml.); hydrogen was evolved and the solution turned deep yellow. When the evolution of hydrogen had ceased (after about 30 minutes), N,N-diethylcarbamoyl chloride (5.0 g.) was added in three lots (4.0, 0.5, and 0.5 g.). The deep yellow colour was not discharged even after three hours at room temperature. The reaction mixture

was washed with (i) sodium bicarbonate solution and (ii) water; and dried (sodium sulphate). Evaporation of the solvent under reduced pressure yielded a yellow oil (1.3 g.). Purification by chromatography (silica gel column; chloroform-petroleum ether (b.p. 40-60°) (90 : 10) yielded 2-chloro-2-phenylacetophenone (0.9 g.), m.p. 68°, identical with an authentic sample in all respects. Further, the infrared spectrum of the crude yellow oil obtained above indicated the absence of enol carbamate (no peak in the 1700-1770 $cm.^{-1}$ region).

Attempted preparation of (2-chloro-1,2-diphenyl)vinyl N-phenylcarbamate (7, 9)

Anhydrous methanol (0.08 g.) was added to a well-stirred suspension of sodium hydride (0.12 g.) in dry benzene (15 ml.). A solution of 2-chloro-2-phenylacetophenone (0.58 g.) in anhydrous benzene (10 ml.) was added to the above suspension. After 30 minutes, the resultant deep yellow solution was treated with phenyl isocyanate (0.8 g.). The mixture was protected from moisture by a calcium chloride guard tube, and refluxed for three hours, then washed with water and dried (sodium sulphate). Evaporation of the solvent yielded a solid. Extraction of the solid with hot

petroleum ether (b.p. 60-80°) left behind an insoluble solid. This was recrystallised from ethanol to obtain needles of sym-diphenylurea, m.p. 244°, identical with an authentic sample in all respects. Cooling of the petroleum ether extract yielded shiny plates (0.46 g.) of 2-chloro-2-phenylacetophenone, m.p. 66-68°, identical in all respects to an authentic sample.

(2-Chloro-1,2-diphenyl)vinyl chloroformate (31,19)

A suspension of sodium methoxide (prepared from sodium hydride (1.416 g.) and anhydrous methanol (0.944 g.) in anhydrous ether was treated with a solution of 2-chloro-2-phenylacetophenone (6.8 g.) in anhydrous ether to obtain the deep yellow enolate. The resultant slurry containing the enolate was added dropwise to liquid phosgene (4 ml.) accompanied by stirring and cooling. The colour of the enolate was discharged immediately. The stirring was continued overnight. The inorganic material was removed by filtration and the excess of phosgene and solvent were removed under reduced pressure to obtain an oil. Distillation under reduced pressure then gave a pale yellow oil (5.0 g.), b.p. 140-160°/1 mm.; which was a mixture (2 spots on t.l.c.), but contained the required chloroformate. $\nu_{max.}$ in chloroform 1110, 1625* (w), 1765 cm.$^{-1}$.

(2-Chloro-1,2-diphenyl)vinyl N-phenylcarbamate (21)

A solution of aniline (0.55 g.) in anhydrous ether (5.0 ml.) was added dropwise with stirring to a solution of crude (2-chloro-1,2-diphenyl)vinyl chloroformate (0.87 g.) in anhydrous ether (5.0 ml.). Immediately white crystals of aniline hydrochloride began to separate out. These were filtered off after allowing the reaction mixture to stand for one hour at room temperature; and the filtrate was washed successively with (i) 2 N hydrochloric acid, (ii) sodium bicarbonate solution and (iii) water. The ethereal solution was dried (sodium sulphate) and concentrated under reduced pressure to obtain a viscous oil which solidified on trituration with petroleum ether (b.p. 40-60°). The solid was recrystallised four times from petroleum ether (b.p. 100-120°) to obtain the pure carbamate (0.3 g.), m.p. 154-158° (Found: C, 71.68; H, 4.46; N, 4.05. $C_{21}H_{16}ClNO_2$ requires C, 71.83, H, 4.58; N, 4.00%), ν max. in chloroform 1095, 1180, 1635* (w), 1745, 3420 cm.$^{-1}$; λ max. in ethanol 210 (ε = 8,620), 233 (ε = 15,145), 273 mμ (ε = 6,060).

(2-Chloro-1,2-diphenyl)vinyl N-p-tolylcarbamate (23)

Crude (2-chloro-1,2-diphenyl)vinyl chloroformate (0.72 g.) and p-toluidine (0.53 g.) were made to react under the same conditions as described for

(2-chloro-1,2-diphenyl)vinyl N-phenylcarbamate. Recrystallisation from petroleum ether (b.p. 100-120°) gave colourless needles (0.35 g.) of the carbamate, m.p. 151°. (Found: C, 72.90; H, 5.08; Cl, 9.54. $C_{22}H_{18}ClNO_2$ requires C, 72.63; H, 4.95; Cl, 9.77%), ν max. in carbon tetrachloride 1095, 1195, 1620* (w), 1755, 3410 cm.$^{-1}$; λ max. in ethanol 210 (ε = 13,880), 235 (ε = 21,810), 276 mμ (ε = 9,250).

(2-Chloro-1,2-diphenyl)vinyl N-methyl-N-phenylcarbamate (27)

Crude (2-chloro-1,2-diphenyl)vinyl chloroformate (0.72 g.) was made to react with N-methylaniline (0.53 g.) by the method described above. Recrystallisation from petroleum ether (b.p. 80-100°) yielded colourless chunky crystals (0.195 g.) of the carbamate, m.p. 113-115°. (Found: C, 72.47; H, 4.80; Cl, 10.00. $C_{22}H_{18}ClNO_2$ requires C, 72.63; H, 4.95; Cl, 9.77%), ν max. in chloroform 1140, 1635* (w), 1720 cm.$^{-1}$; λ max. in ethanol 227 (ε = 16,960), 275 mμ (ε = 9,690).

N-(2-Chloro-1,2-diphenyl)vinyloxycarbonylpyrrolidine (137,138)

Crude (2-chloro-1,2-diphenyl)vinyl chloroformate (2.0 g.) was condensed with pyrrolidine (1.0 g.) by the general method described above. Recrystallisation from

petroleum ether (b.p. 100-120°) gave fine needles (0.95 g.) of N-(2-chloro-1,2-diphenyl)vinyloxycarbonylpyrrolidine, m.p. 143-145°. (Found: C, 69.91, H, 5.53; N, 4.00; Cl, 10.82. $C_{19}H_{18}ClNO_2$ requires C, 69.91; H, 5.53; N, 4.27; Cl, 10.81%), ν max. in chloroform 1100, 1175, 1630* (w), 1720 cm.$^{-1}$; λ max. in ethanol 210 (ε = 13,670), 223 (ε = 14,210), 278 mμ (ε = 7,650).

(2-Chloro-1,2-diphenyl)vinyl N-o-methoxycarbonylphenylcarbamate (205)

Crude (2-chloro-1,2-diphenyl)vinyl chloroformate (1.0 g.) in anhydrous benzene (10 ml.) was condensed with methyl o-aminobenzoate (0.5 g.) in the presence of pyridine (0.6 g.). On working up in the usual way an oil was obtained. Purification of this by chromatography (silica gel column; chloroform-petroleum ether (b.p. 60-80°) (70:30)) yielded a solid (0.5 g.). Recrystallisation from petroleum ether (b.p. 100-120°) gave fine needles of the required product, m.p. 127-129° (softens at 119-120°). (Found: C, 67.67; H, 4.54; N, 3.37; Cl, 8.73. $C_{23}H_{18}ClNO_4$ requires C, 67.72; H, 4.45; N, 3.43; Cl, 8.69%), ν max. in chloroform 1100, 1180, 1635* (shoulder), 1693, 1760, 3300 cm.$^{-1}$; λ max. in ethanol 225 (ε = 48,210), 245 (ε = 34,630), 292 mμ (ε = 18,330).

(2-Chloro-1,2-diphenyl)vinyl N-p-methoxycarbonylphenylcarbamate (203)

Crude (2-chloro-1,2-diphenyl)vinyl chloroformate (1.0 g.) in anhydrous benzene (10 ml.) was condensed with methyl p-aminobenzoate (0.5 g.) in the presence of anhydrous pyridine (0.6 g.) as described for the o-isomer. On working up, a solid was obtained. Recrystallisation from petroleum ether (b.p. 100-120°) gave colourless needles (0.6 g.) of the carbamate, m.p. 198-200°. (Found: C, 67.87; H, 4.37; N, 3.26; Cl, 8.84. $C_{23}H_{18}ClNO_4$ requires C, 67.72; H, 4.45; N, 3.43; Cl, 8.69%), ν max in carbon tetrachloride 1080, 1190, 1615* (w), 1720, 1765, 3450 cm.$^{-1}$; λ max. in ethanol 210 (ε = 13,570), 270 mμ (ε =5,100).

(2-Chloro-1,2-diphenyl)vinyl N-m-methoxycarbonylphenylcarbamate (227)

Crude (2-chloro-1,2-diphenyl)vinyl chloroformate (1.0 g.) was condensed with methyl m-aminobenzoate (from the hydrochloride (0.7 g.)) by the general method described above, and gave an oil which crystallised on trituration with petroleum ether (b.p. 40-60°). Recrystallisation from petroleum ether (b.p. 100-120°) yielded needles (0.7 g.) of the required product, m.p. 186-188°. (Found: C, 67.51; H, 4.54; N, 3.17; Cl, 8.55. $C_{23}H_{18}ClNO_4$ requires

C, 67.72; H. 4.45; N, 3.43; Cl, 8.69%), ν max. in chloroform 1093, 1190, 1640* (w), 1718, 1750, 3420 cm.$^{-1}$; λ max. in ethanol 230 (ε = 21,220), 280 mμ (ε = 12,510).

(2-Chloro-1,2-diphenyl)vinyl N-p-(N,N-di-2-chloroethylamino) phenylcarbamate (113, 125, 126)

Crude (2-chloro-1,2-diphenyl)vinyl chloroformate (3.0 g) was treated with the hydrochloride of mustard amine A (2.7 g.) in the presence of pyridine (1.6 g.) in anhydrous ether (30 ml.). On working up in the usual way an off-white solid was obtained. This was decolourised with activated charcoal and recrystallised from petroleum ether (b.p. 100-120°) to obtain needles (1.1 g.), m.p. 133-139°. This was further purified by column chromatography (alumina, Spence grade H; chloroform) followed by recrystallisation from petroleum ether (b.p. 100-120°) to obtain needles of the carbamate, m.p. 158-160°. (Found: C, 60.60, 61.28; H, 4.61, 4.59; N, 5.45; 5.69; Cl, 23.08, 23.48. $C_{25}H_{23}Cl_3N_2O_2$ requires C, 60.73; H, 4.69; N, 5.67; Cl, 21.66%), ν max. in carbon tetrachloride 1090, 1175, 1615 to 1640* (w), 1745, 3400 cm.$^{-1}$; λ max. in ethanol 208 ((ε = 16,690), 223 (ε = 10,311), 270 mμ (ε = 17,185).

Attempted condensation of (2-chloro-1,2-diphenyl)vinyl chloroformate and DL-phenylalanine methyl ester (127)

The crude chloroformate (1.5 g.) in anhydrous ether (20 ml.) was added dropwise with stirring to a solution of DL-phenylalanine methyl ester (from the hydrochloride (1.2 g.)) in ether containing triethylamine (1.4 g.). On working up in the usual way, a solid (0.7 g.) was obtained. Recrystallisation from benzene-petroleum ether (b.p. 100-120°) yielded chunky crystals of N,N -di-(1-methoxycarbonyl-2-phenyl)ethylurea, m.p. 131°, identical with an authentic sample. (The authentic sample was prepared by the action of water on methyl 2-isocyanato-3-phenylpropionate*). (Found: C, 65.47; H. 6.24; N, 7.20; Cl. 0.00. Calculated for $C_{21}H_{24}N_2O_5$: C, 65.60; H, 6.29; N, 7.29; Cl, 0.00%), ν max. in chloroform 1110, 1190, 1678, 1738, 3420 cm.$^{-1}$.

(* This compound was kindly supplied by Mr. M.J. Pringle)

Attempted condensation of (2-chloro-1,2-diphenyl)vinyl chloroformate and DL-glycine methyl ester (45)

The crude chloroformate (0.9 g.) in anhydrous ether was added dropwise with stirring to a solution of DL-glycine methyl ester (generated from the hydrochloride

0.38 g.)) in ether containing pyridine (0.2 g.).
On working up in the usual way an oil was obtained.
This oil (0.1 g.) crystallised on standing for a long time to yield colourless prisms of <u>di-(2-chloro-1,2-diphenyl) vinyl carbonate</u>, m.p. 154°. (Found C, 71.09; H, 3.98; N, trace. $C_{29}H_{20}Cl_2O_3$ requires C, 71.46; H, 4.6%).

CARBAMATES DERIVED FROM (N,N-DI-2-CHLOROETHYL)-p-PHENYLENEDIAMINE AND ITS m-ANALOGUE

N,N-Di-(2-chloroethyl)aniline (225)

This compound was prepared from N,N-di-(2-hydroxyethyl)aniline by the action of phosphorous oxychloride according to the method of Robinson and Watt.[129] The reaction product was purified by fractional distillation, the fraction b.p. 115-118°/0.1 mm., solidified into a white crystalline mass (yield 80%), m.p. 46°. Robinson and Watt[129] quote b.p. 164°/14 mm., m.p. 45° (yield 75%).

N,N-Di-(2-chloroethyl)p-nitrosoaniline (229)

This compound was prepared by the action of an ice-cold solution of sodium nitrite in water, added dropwise with stirring to an ice-cold solution of N,N-di-2-(chloroethyl) aniline as described by Everett and Ross.[107] The pure product was obtained by recrystallisation from benzene as lustrous blue-green prisms, (yield 80%), m.p. 80-81°. Everett and Ross[107] quote m.p. 79-80°.

N,N-Di-(2-chloroethyl)-p-phenylenediamine hydrochloride (231)

This was prepared from N,N-di-(2-chloroethyl)-p-nitrosoaniline according to the method of Everett and Ross[107]

by the action of stannous chloride in concentrated hydrochloric acid, in a yield of 60%, m.p. 245-250°(d.). Everett and Ross[107] quote m.p. 250-260°(d.).

N,N-Di(2-hydroxyethyl)-m-nitroaniline (241, 243)

m-Nitroaniline (5.0 g.), acetic acid (40%, 40 ml.) and ethylene oxide (25 ml.) were mixed together at 0° and kept stirred overnight to obtain a clear solution. The reaction mixture was neutralised with aqueous sodium carbonate solution when a lemon yellow solid began to separate. This solid was filtered off and recrystallisation from ethanol gave yellow plates of N,N-di-(2-hydroxyethyl)-m-nitroaniline (4.3 g.), m.p. 98°. Lit.,[130] m.p. 98°

N,N-Di-(2-chloroethyl)-m-nitroaniline (243, 245, 287)

Phosphorus oxychloride (5.0 g.) was added dropwise with stirring to a suspension of N,N-di-(2-hydroxyethyl)-m-nitroaniline (4.0 g.). After the addition was complete, the reaction mixture was refluxed over a water bath for 6 hours. The excess of phosphorus oxychloride and the solven were distilled off under reduced pressure to obtain a yellow gum. Extraction of this gum with hot ethylene dichloride, followed by cooling of the extract, gave deep yellow needles (3.2 g.), of N,N-di-(2-chloroethyl)-m-nitroaniline, m.p. 116°.

Bieksa and Degutis[108] who used thionyl chloride in ethylene dichloride for chlorination, quote m.p. 112-113°, for this compound.

N,N-DI-(2-chloroethyl)-m-phenylenediamine (247, 287)

This compound was prepared from N,N-di-(2-chloro ethyl)-m-nitroaniline according to the method of Bieksa and Degutis[108] using stannous chloride in concentrated hydrochloric acid as the reducing agent and liberating the free amine from the resultant hydrochloride by the action of sodium bicarbonate solution. The amine was obtained as a dark grey solid, m.p. 60°. Bieksa and Degutis[108] give m.p. 59-60°.

Phenyl chloroformate (70, 249)

This compound was prepared according to the method of Crosby and Niemann[131] by the action of a solution of phenol (6.0 g.) in 2N sodium hydroxide solution (40 ml.) added dropwise with stirring to a cooled solution of phosgene (8.0 ml.) in toluene (50 ml.). The toluene layer after separation, drying (sodium sulphate) and evaporation under reduced pressure gave, on distillation, phenyl chloroformate (6.1 g.) b.p. 59°/4 mm., Crosby and Niemann[131] quote 63°/7 mm.

This compound was also prepared by the action of phosgene on phenol in the presence of quinoline. The product had b.p. 80°/~ 22 mm. (Yield 70%).

Phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate (71,72)

Phenyl chloroformate (1.57 g.) was added dropwise with stirring to a cooled mixture of the hydrochloride of mustard amine A (2.69 g.), pyridine (1.6 g.) and benzene (50 ml.). The stirring was continued for one hour after the addition was complete. Then the reaction mixture was washed with (i) 2N hydrochloric acid, (ii) sodium bicarbonate solution and (iii) water. The organic layer on drying (sodium sulphate) and evaporation, gave an oil which solidified on trituration with petroleum ether (b.p. 40-60°). Recrystallisation from benzene yielded needles (2.4 g.) of phenyl N-p-(N,N-di-2-chloroethylamino) phenylcarbamate, m.p. 124-125°. Benn,[59] who prepared the same compound by a different route, quotes m.p. 124-125°.

Phenyl N-m-(N,N-di-2-chloroethylamino)phenylcarbamate (249)

Phenyl chloroformate (0.8 g.) was condensed with mustard amine B (1.15 g.) in the presence of pyridine (1.0 g.) as described for the p-isomer. Recrystallisation from benzene yielded needles (1.1 g.) of phenyl N-m-(N,N-di-2-chloroethylamino)phenylcarbamate, m.p. 107-108°.

(Found: C, 58.07; H, 5.16; N, 7.83; Cl, 19.90. $C_{17}H_{18}Cl_2N_2O_2$ requires C, 57.79; H, 5.13; N, 7.93; Cl, 20.06%), ν max. in chloroform 1745, 3450 cm.$^{-1}$.

p-Methoxycarbonylphenyl chloroformate (39)

This compound was prepared by the action of a saturated solution of phosgene in benzene on methyl m-hydroxybenzoate in the presence of quinoline as described by Einhorn et al.[110]. Recrystallisation from petroleum ether (b.p. 60-80°) gave off-white crystals, m.p. 56°. Einhorn et al.[110] quote m.p. 58°. ν max. 1720, 1765 cm.$^{-1}$.

p-Methoxycarbonylphenyl N-p-(N.N-di-2-chloroethylamino) phenylcarbamate (41)

p-Methoxycarbonylphenyl chloroformate (0.8 g.) was added to a stirred mixture of the hydrochloride of the mustard amine A (1.0 g.) and pyridine (0.58 g.) in benzene. On working up as described for phenyl N-p-(N,N-di-2-chloro ethylamino)phenylcarbamate a solid was obtained. Recrystallisation from benzene gave needles (1.5 g.) of the required product, m.p. 143-145°. (Found: C, 55.30; H, 4.71; N, 6.80. $C_{19}H_{20}Cl_2N_2O_4$ requires C, 55.47; H, 4.87; N, 6.81%), ν max. in chloroform 1170, 1705, 1740, 3410 cm.$^{-1}$.

p-Carboxyphenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate (43)

The above carbamate (1.2 g.) was refluxed with a mixture of glacial acetic acid - concentrated hydrochloric acid (1 : 1 mixture; 15 ml.) for 35 minutes. The cooled reaction mixture was poured into ice-cold water (100 ml.) and the precipitated solid was filtered off and air-dried. Recrystallisation from ethyl acetate-petroleum ether (b.p. 40-60°) gave needles (1.0 g.) of the acid, m.p. 178-180.5°. (Found: C, 54.63; H, 4.43; Cl, 17.75. $C_{18}H_{18}Cl_2N_2O_4$ requires C, 54.30; H, 4.56; Cl, 17.80%), ν max. in nujol 1680, 1720, 3340 cm.$^{-1}$.

m-Methoxycarbonylphenyl chloroformate (211,213,257)

A mixture of methyl m-hydroxybenzoate (3.8 g.) and quinoline (3.3 g.) was added dropwise with stirring to an ice cold solution of phosgene in benzene (30 ml.). The stirring was continued overnight and then the excess of phosgene was removed by bubbling nitrogen through the reaction mixture. The phosgene-free reaction mixture was washed with 2N hydrochloric acid. Drying (sodium sulphate) followed by evaporation of the solvent under reduced pressure at $< 30°$ yielded the crude chloroformate as a pungent smelling oil (3.9 g.) ν max. (liquid film) 1712, 1770 cm.$^{-1}$.

The chloroformate was used without further purification for subsequent reactions, and it was characterised by conversion into a solid N-phenylcarbamate, described below.

m-Methoxycarbonylphenyl N-phenylcarbamate (213)

The above chloroformate (0.2 g.) in benzene (5 ml.) was added to aniline (0.2 g.) in benzene (5 ml.). On working up the reaction mixture in the usual way, a crystalline solid (0.09 g.) was obtained. Recrystallisation from benzene-petroleum ether (b.p. 40-60° gave needles of the carbamate, m.p. 113-115°. (Found: C, 66.59; H. 4.81; N, 5.18. $C_{15}H_{13}NO_4$ requires C, 66.40; H, 4.83; N, 5.16%), ν max. in chloroform 1720, 1750, 3450 cm.$^{-1}$.

m-Methoxycarbonylphenyl N-p-(N,N-di-2-chloroethylamino) phenylcarbamate (215)

m-Methoxycarbonylphenyl chloroformate (2.14 g.) was added to a mixture of the hydrochloride of mustard amine A(2.7 g.), pyridine (1.6 g.) and benzene (30 ml.) as described in the case of phenyl N-p-(N,N-di-2-chloroethylamino)phenyl carbamate. On working up in the usual way a crystalline solid (2.9 g.) was obtained. Recrystallisation from benzene gave needles of the product,

m.p. 137-138°. (Found: C, 55.45; H, 4.82; N, 6.68; Cl, 16.96. $C_{19}H_{20}Cl_2N_2O_4$ requires C, 55.47; H, 4.87; N, 6.81; Cl, 17.24%), ν max. in chloroform 1105, 1190, 1720, 1740, 3420 cm.$^{-1}$.

m-Carboxyphenyl (N-p-(N,N-di-2-chloroethylamino)phenylcarbamate (217)

The above carbamate (0.9 g.) was hydrolysed as described for p-carboxyphenyl N-p-(N,N-di-2-chloroethylamino) phenylcarbamate, using glacial acetic acid-concentrated hydrochloric acid (1 : 1) mixture. Recrystallisation from benzene yielded colourless needles, (0.7 g.), of the acid, m.p. 160-162°. (Found: C, 54.43; H, 4.54; N, 7.12; Cl, 17.78. $C_{18}H_{18}Cl_2N_2O_4$ requires C, 54.30; H, 4.56; N, 7.05; Cl, 17.80%), ν max. in chloroform 1105, 1190, 1700 to 1740 (broad peak), 3430 cm.$^{-1}$.

m-Methoxycarbonyl N-m-(N,N-di-2-chloroethylamino)phenyl carbamate (291)

m-Methoxycarbonylphenyl chloroformate (2.1 g.) was condensed with the mustard amine B (2.3 g.) in the presence of pyridine (1.6 g.) as described for phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate. Recrystallisation from benzene yielded needles (2.2 g.) of the product containing half a mole of benzene of crystallisation, m.p. 118°. (Found: C, 58.18; H, 4.93; N, 5.99; Cl, 15.84. $C_{19}H_{20}Cl_2N_2O_4 + \frac{1}{2} C_6H_6$ i.e., $C_{22}H_{23}Cl_2N_2O_4$ requires C, 58.68; H, 5.15; N, 6.22; Cl, 15.75%).

Recrystallisation of the above solid from petroleum ether (b.p. 100-120°) gave the solvent-free compound, m.p. 114°. (Found: C, 55.78; H, 4.80; N, 6.72; Cl, 16.97; $C_{19}H_{20}Cl_2N_2O_4$ requires C, 55.49; H, 4.90; N, 6.81; Cl, 17.24%), ν max. 1110, 1195, 1720, 1745, 3450 $cm.^{-1}$.

m-Carboxyphenyl N-m-(N,N-di-2-chloroethylamino)phenylcarbamate (295)

m-Methoxycarbonylphenyl (N-m-(N,N-di-2-chloroethylamino)phenylcarbamate was hydrolysed by refluxing with a 1:1 mixture of glacial acetic acid-concentrated hydrochloric acid as described for p-carboxyphenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate. On working up in the usual way a crystalline off-white solid was obtained. Recrystallisation from ethyl acetate-petroleum ether (b.p. 40-60°) gave needles of the acid, m.p. 128-131°. (Found: Cl, 17.96; $C_{18}H_{18}Cl_2N_2O_4$ requires Cl, 17.80%), ν max. in nujol 1680, 1720, 3400 $cm.^{-1}$.

o-Methoxycarbonylphenyl chloroformate (157, 297)

This compound was prepared by the action of methyl salicylate (5.1 g.) on phosgene in the presence of quinoline according to the directions of Einhorn et al.[114] On working up an oil was obtained. This gave, on distillation, a colourless liquid (6.8 g.), b.p. 140°, which solidified on storage in the refrigerator to a mass of colourless crystals, m.p. 24°. Einhorn et al.[114] quote b.p. 142° ν max. (liquid film) 1720, 1780 $cm.^{-1}$.

o-Methoxycarbonylphenyl N-p-(N,N-di-2-chloroethylamino) phenylcarbamate (157)

o-Methoxycarbonylphenyl chloroformate (1.3 g.) was added to a mixture of the hydrochloride of mustard amine A (1.35 g.), triethylamine (1.0 g) and benzene (25 ml.); and the reaction mixture was worked up as described for the preparation of phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate to obtain a crystalline solid (1.8 g.). Recrystallisation from benzene gave fine needles of the carbamate, m.p. 107-108°. (Found: C, 55.54; H, 4.60; N, 7.04; Cl, 17.10. $C_{19}H_{20}Cl_2N_2O_4$ requires C, 55.49; H, 4.90; N, 6.81; Cl, 17.24%) ν max. 1095, 1190, 1720 to 1740 (broad peak), 3450 cm.$^{-1}$.

Attempted hydrolysis of o-methoxycarbonylphenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate (159)

The above carbamate (1.0 g.) was refluxed with a mixture of glacial acetic acid and concentrated hydrochloric acid (1:1;25 ml.) for 2 hours. On working up the reaction mixture in the usual way, the starting material was obtained as fine needles (0.4 g.), m.p. 107-108°, identical with the sample prepared above.

o-Benzyloxycarbonylphenyl chloroformate (207, 209)

Benzyl salicylate (5.7 g.) and quinoline (3.3 g.) were added dropwise to a stirred solution of phosgene in benzene while cooling was applied externally by an ice-bath. On working up the reaction mixture after three days as described for m-methoxycarbonylphenyl chloroformate, a pungent smelling oil (6.0 g.) was obtained. ν max. (liquid film) 1705, 1775 cm.$^{-1}$. The above oil was used without further purification in subsequent reactions. The crude o-benzyloxycarbonylphenyl chloroformate was characterised as its solid N-phenylcarbamate derivative described below.

o-Benzyloxycarbonylphenyl N-phenylcarbamate (209)

o-Benzyloxycarbonylphenyl chloroformate (0.3 g.) was condensed with aniline (0.3 g.) in the usual way. On working up, a colourless solid (0.25 g.) was obtained. Recrystallisation from petroleum ether (b.p. 100-120°) gave chunky crystals of the derivative, m.p. 88-91° (softens at 86°). (Found: C, 72.56; H, 5.00; N, 3.91. $C_{21}H_{17}NO_4$ requires C, 72.59; H, 4.94; N, 4.03%), ν max. in chloroform 1095, 1180, 1700 to 1750 (broad peak), 3430 cm.$^{-1}$.

o-Benzyloxycarbonylphenyl N-p-(N,N-di-2-chloroethylamino) phenylcarbamate (179)

o-Benzyloxycarbonylphenyl chloroformate (2.1 g.) was added to a mixture of the hydrochloride of mustard amine A (2.0 g.) and pyridine (1.2 g.) in benzene (30 ml.) according to the general method described for phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate. On working up, an oil was obtained. Purification of this by chromatography (silica gel column; chloroform-petroleum ether (b.p. 60-80°) (7:3)) yielded a crystalline solid (2.0 g.). Recrystallisation from benzene-petroleum ether (b.p. 40-60°) gave needles of the required product, m.p. 78-80°. (Found: C, 61.68; H, 4.91; N, 5.68; Cl, 14.76. $C_{25}H_{24}Cl_2N_2O_4$ requires C, 61.60; H, 4.96; N, 5.75; Cl, 14.54%). ν max. 1095, 1180, 1720 to 1750 (broad peak), 3450 cm.$^{-1}$.

o-Benzyloxycarbonylphenyl N-m-(N,N-di-2-chloroethylamino) phenylcarbamate (289)

o- Benzyloxycarbonylphenyl chloroformate (2.0 g.) was treated with mustard amine B (1.8 g.) in the presence of pyridine (1.2 g.). On working up in the usual way, an oil (2.1 g.) was obtained. This oil crystallised on trituration with petroleum ether (b.p. 40-60°). Recrystallisation from benzene yielded fine needles of the required product, m.p. 99°. (Found: C, 61.93; H, 4.93; N, 5.94; Cl, 14.37. $C_{25}H_{24}Cl_2N_2O_4$ requires C, 61.60; H, 4.96; N, 5.75; Cl, 14.54%), ν max. in chloroform 1090, 1185, 1720, 1750, 3440 cm.$^{-1}$.

o-Carboxyphenyl N-p-(N,N-di-2-chloroethylamino) phenylcarbamate (219)

o-Benzyloxycarbonylphenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate (1.0 g.) was dissolved in methanol (40 ml.) containing a drop of concentrated hydrochloric acid and subjected to hydrogenation at atmospheric pressure in the presence of 10% palladium on charcoal as catalyst. The hydrogen uptake was complete in 6 hours. Removal of the catalyst, followed by evaporation of the filtrate yielded a recalcitrant gum. This was taken up in benzene and washed with (i) sodium bicarbonate solution, and (ii) water. Drying (sodium sulphate) followed by evaporation of benzene gave a solid (0.3 g.). Recrystallisation from benzene gave colourless needles of the acid, containing 1 mole of benzene of crystallisation, m.p. 73-74°. (Found: C, 59.74; H, 5.07; N, 5.70; Cl, 15.61. $C_{24}H_{24}Cl_2N_2O_4$ i.e., $C_{18}H_{18}Cl_2N_2O_4 + C_6H_6$, requires C, 60.63; H, 5.09; N, 5.90; Cl, 14.91%), ν max. in chloroform 1095, 1185, 1680 to 1740 (broad peak sharpening at 1740) 3450 $cm.^{-1}$.

Di-4-Cyclohexylphenyl carbonate and 4-cyclohexylphenyl chloroformate (277, 279)

A solution of 4-cyclohexylphenol (5.8 g.) in 2N sodium hydroxide solution (20 ml.) was added dropwise with stirring to an ice-cold saturated solution of phosgene in toluene (40 ml.). The toluene layer was separated and dried (sodium sulphate). Evaporation of the solvent under reduced pressure gave a pungent smelling oil, part of which solidified on storing overnight in a refrigerator. The solid was isolated by filtration and when recrystallised from petroleum ether (b.p. 60-80°), yielded needles (1.2 g.) of

di-4-cyclohexylphenyl carbonate, m.p. 80-81°. (Found: C, 79.68; H, 8.30; $C_{25}H_{30}O_3$ requires C, 79.98; H, 7.99%), ν max. in chloroform 1110, 1180, 1775, 1895 cm.$^{-1}$.

The unsolidified portion of the above mixture (2.5 g.) was identified as 4-cyclohexylphenyl chloroformate on the basis of the infrared spectrum, ν max. in chloroform 1690, 1770, 1895 (very weak; due to the carbonate impurity), cm.$^{-1}$; and by its conversion into the N-phenylcarbamate derivative described below.

4-Cyclohexylphenyl N-phenylcarbamate (281)

The above chloroformate (0.2 g.) in anhydrous ether (10 ml.) was condensed with aniline (0.2 g.) as described in the case of m-methoxycarbonylphenyl N-phenylcarbamate. Recrystallisation from petroleum ether (b.p. 100-120°) gave fine needles (0.15 g.) of the carbamate, m.p. 142-143°. (Found: C, 77.44; H, 7.28; N, 4.62. $C_{19}H_{21}NO_2$ requires C, 77.25; H, 7.16; N, 4.74%), ν max. in chloroform 1010, 1185, 1745, 3460 cm.$^{-1}$.

4-Cyclohexylphenyl N-p-(N,N-di-2-chloroethylamino) phenyl carbamate (283)

4-Cyclohexylphenyl chloroformate (0.6 g.) was added to a mixture of the hydrochloride of mustard amine A (0.7 g.), pyridine (0.4 g.) and benzene (25 ml.) in the manner described for phenyl (N-p-(N,N-di-2-chloroethylamino) phenylcarbamate. Recrystallisation from petroleum ether (b.p. 100-120°) gave needles (0.8 g.) of the product, m.p. 124-125°. (Found: C, 64.14; H, 6.82; N, 6.12; Cl, 16.14. $C_{23}H_{28}Cl_2N_2O_2$ requires C, 63.45; H, 6.48; N, 6.43; Cl, 16.29%), ν max. in chloroform 1180, 1740, 3450 cm.$^{-1}$.

4-Cyclohexylphenyl N-m-(N,N-di-2-chloroethylamino) phenylcarbamate (285)

4-Cyclohexylphenyl chloroformate (0.6 g.) was made to react with mustard amine B (0.6 g.) in the presence of pyridine (0.4 g.) as described for mustard amine A. Recrystallisation of the product from benzene-petroleum ether (b.p. 60-80°) gave needles (0.6 g.) of the carbamate, m.p. 138-139°. (Found: C, 63.02; H, 6.42; N, 6.54; Cl, 16.29. $C_{23}H_{28}Cl_2N_2O_2$ requires C, 63.45; H, 6.48; N, 6.43; Cl, 16.29%), ν max. in chloroform 1195, 1740, 3420 cm.$^{-1}$.

Ethyl N-m-(N,N-di-2-chloroethylamino)phenylcarbamate (251)

Ethyl chloroformate (0.27 g.) and mustard amine B (0.5 g.) were condensed in the presence of pyridine (0.6 g.) as described under phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate. Recrystallisation from benzene-petroleum ether b.p. 40-60°) gave needles (0.2 g.) of the carbamate, m.p. 86-87°. (Found: C, 51.37; H, 5.74; N, 9.17; Cl, 23.25. $C_{13}H_{18}Cl_2N_2O_2$ requires C, 51.14; H, 5.94; N, 9.18; Cl, 23.25%), ν max. in chloroform 1190, 1720, 3450 cm.$^{-1}$.

Isopropyl N-m-(N,N-di-2-chloroethylamino)phenylcarbamate (255)

Isopropyl chloroformate (0.12 g.) was condensed with mustard amine B (0.25 g.) by the general method described above. Recrystallisation from petroleum ether (b.p. 100-120°) gave fluffy crystals (0.22 g.) of the isopropyl compound, m.p. 104-105°. (Found: C, 52.46; H, 6.39; N, 8.72; Cl, 22.22. $C_{14}H_{20}Cl_2N_2O_2$ requires C, 52.67, H, 6.32; N, 8.77; Cl, 22.21%), ν max. in chloroform 1110, 1180, 1720, 3440 cm.$^{-1}$.

Some amino-acid derivatives

DL-Serine methyl ester hydrochloride (59)

This compound was prepared from DL-serine by the action of methanol saturated with hydrogen chloride according to the method of Fischer and Suzuki.[118] It had m.p. 132° (decomp.). Fischer and Suzuki quote m.p. 114° (decomp.). King,[132] and Bergel and Wade[71] quote m.p. 134° (decomp.).

N-Benzyloxycarbonyl-DL-serine methyl ester (61)

This compound was prepared by the action of benzyl chloroformate on DL-serine methyl ester (from the hydrochloride) in the presence of sodium bicarbonate according to the directions of Bergel et al.[71]

It had b.p. 178-185°/1 mm., Bergel et al.[71] quote 178-183°/1 mm.

O-Chloroformyl-N-benzyloxycarbonyl-DL-serine methyl ester (63)

This compound was prepared according to the method of Bergel and Wade[71] by the action of phosgene on N-benzyloxycarbonyl-DL-serine methyl ester. The product was obtained as a pungent smelling oil and was used without further purification for subsequent reactions. ν max. (liquid film) 1735, 1765, 3350 cm.$^{-1}$.

O-(N-Phenylcarbamoyl)-N-benzyloxycarbonyl-DL-serine methyl ester (87)

The above chloroformate (1.0 g.) was made to react with aniline as described under m-methoxycarbonylphenyl N-phenylcarbamate. On working up, an oil (0.5 g.) was obtained. This was triturated with petroleum ether (b.p. 40-60°) to induce crystallisation. Recrystallisation from benzene-petroleum ether (b.p. 40-60°) gave colourless crystals of the required compound, m.p. 74-75°. (Found: C, 60.91, H, 5.36; N, 7.33; $C_{19}H_{20}N_2O_6$ requires C, 61.28; H. 5.41; N, 7.52%), ν max. in chloroform 1715 (shoulder) 1735, 3420 cm.$^{-1}$.

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-N-benzyloxycarbonyl-DL-serine methyl ester (67, 89)

The preceding chloroformate (3.0 g.) was added to a mixture of the hydrochloride of mustard amine A (2.6 g.), pyridine (1.6 g.) and benzene (40 ml.) according to the general method described under phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate. The oily product was purified by column chromatography (alumina, Spence grade H; chloroform) to obtain a colourless solid. Recrystallisation from benzene-petroleum ether (b.p. 40-60°) gave fine needles (2.3 g.) of the required compound, m.p. 100-102°. (Found: C, 54.09; H, 5.20; N, 7.79; Cl, 13.92. $C_{23}H_{27}Cl_2N_3O_6$ requires C, 53.91; H, 5.31; N, 8.23; Cl, 13.84%), ν max. in chloroform 1718 (shoulder) 1735, 3410 cm.$^{-1}$.

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-DL-serine methyl ester (107)

The above carbamate (2.5 g.) was dissolved in methanol (75 ml.) containing concentrated hydrochloric acid (0.75 g.) and subjected to hydrogenation in the presence of 5% palladium on charcoal catalyst. The hydrogen uptake was complete in 12 hours. Removal of the catalyst followed by concentration of the filtrate under

vacuum yielded a gummy mass. This recalcitrant gum was dissolved in a small amount of methanol and precipitated out by the addition of anhydrous ether. The precipitate was collected by filtration and vacuum dried to obtain pink crystals (1.6 g.) of the hydrogenolysis product, as a dihydrochloride dihydrate. It had no sharp melting point. (Found: C, 37.36; H, 5.72; N, 8.60; Cl, 29.27. $C_{15}H_{27}Cl_4N_3O_6$, i.e., $C_{15}H_{21}N_3O_4Cl_2 \cdot 2HCl.2H_2O$, requires C, 37.0; H, 5.59; N, 8.63; Cl, 29.13%), ν max. in nujol 1680 (shoulder), 1735, 2400 to 3600 (very broad peak) cm.$^{-1}$.

Attempted preparation of O-(N-p-(N,N-di-2-chloroethylamino) phenylcarbamoyl)-DL-serine (108)

The above compound was refluxed for 35 minutes with a (1 : 1) mixture of glacial acetic acid and concentrated hydrochloric acid. The reaction product was worked up in the usual way, to obtain an intractable mixture which could not be purified.

N-Benzyloxycarbonyl-DL-serine (109)

This compound was prepared by the action of benzyl chloroformate on DL-serine in the presence of sodium hydroxide according to the method of Bergmann and Zervas.[119] It had m.p. 125-126°. Lit.,[119] m.p. 125°.

N-Benzyloxycarbonyl-DL-serine benzyl ester (111)

This compound was obtained by the esterification of N-benzyloxycarbonyl-DL-serine with benzyl alcohol in the presence of toluene-p-sulphonic acid as catalyst; the water formed in the reaction being removed with the help of benzene, using a Dean and Stark water separator. This method is due to Ben Ishai and Berger.[120] The compound had m.p. 72°. Lit.,[71] m.p. 73-74°.

O-Chloroformyl-N-benzoyloxycarbonyl-DL-serine benzyl ester (117, 263)

This compound was prepared according to the method of Bergel and Wade[71] by the action of phosgene on N-benzyloxycarbonyl-DL-serine benzyl ester. The chloroformate was obtained as a pungent smelling oil which was used without further purification in subsequent reactions. $\nu_{max.}$ (liquid film) 1718, 1765, 3400 cm.$^{-1}$.

O-(N-m-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-N-benzyl oxycarbonyl-DL-serine benzyl ester (265)

O-Chloroformyl-N-benzyloxycarbonyl-DL-serine benzyl ester (0.6 g.) was condensed with mustard amine B (0.5 g.) in the presence of pyridine (0.5 g.) according to the general procedure described for the preparation of phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate. Purification of the resulting oil (alumina, Spence grade H, column; chloroform-petroleum ether (b.p. 60-80°) (7:3)) gave a crystalline solid (0.4 g.). Recrystallisation from benzene-petroleum ether (b.p. 40-60°) gave fine needles of the carbamate, m.p. 95-96°. (Found: C, 59.19; H, 5.28; N, 7.05; Cl, 11.97. $C_{29}H_{31}Cl_2N_3O_6$ requires C, 59.19; H, 5.31; N, 7.14; Cl, 12.05%), ν max. in chloroform 1180, 1705 to 1745 (broad peak), 3430 cm.$^{-1}$.

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-N-benzyloxycarbonyl-DL-serine benzyl ester (117)

The same chloroformate (prepared from N-benzyloxycarbonyl-DL-serine benzyl ester (4.8 g.)) was dissolved in benzene and added dropwise with stirring to a mixture of the hydrochloride of mustard amine A (3.9 g.), pyridine (2.4 g.) and benzene (40 ml.). On working up the reaction mixture in the usual way,

an oil was obtained. Trituration of this oil with petroleum ether (b.p. 40-60°) gave a crystalline solid (5.2 g.). Recrystallisation from benzene, followed by recrystallisation from carbon tetrachloride, gave needles of the carbamate, m.p. 129°. (Found: C, 59.05; H, 5.27; N, 6.73; Cl, 12.51. $C_{29}H_{31}Cl_2N_3O_6$ requires C, 59.19; H, 5.31; N, 7.14; Cl, 12.05%), ν max. in chloroform 1720 (broad peak), 3410 cm.$^{-1}$.

O-(N-p(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-N-benzyloxycarbonyl-DL-serine (131, 132)

The above carbamate (1.5 g.) was dissolved in a mixture of methanol (25 ml.) and ethyl acetate (25 ml.); and subjected to hydrogenation at atmospheric pressure in the presence of 5% palladium on charcoal catalyst (0.03 g.). On working up in the usual way, a crystalline solid (0.82 g.) was obtained. Recrystallisation from ethyl acetate gave the acid, m.p. 133-135°. (Found: C, 53.13; H, 4.98; N, 7.86; Cl, 15.10. $C_{22}H_{25}Cl_2N_3O_6$ requires C, 53.01; H, 5.01; N, 8.43; Cl, 14.22%), ν max. in chloroform 1715, 3400, 3670 cm.$^{-1}$.

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-DL-serine (133)

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-DL-serine benzyl ester (1.0 g.) was dissolved in methanol (50 ml.) containing concentrated hydrochloric acid (0.17 ml.) and subjected to hydrogenation in the presence of 10% palladium on charcoal (0.02 g.). The catalyst was filtered off and the filtrate concentrated under vacuum to obtain a gum. Recrystallisation of this gum from methanol-anhydrous ether gave pink needles (0.45 g.) of the hydrogenolysis product, as a monohydrochloride monohydrate, m.p. 123-126°. (Found: C, 40.63; H, 5.16; N, 9.98; Cl, 25.19. $C_{14}H_{19}Cl_2N_3O_4$. $HCl.H_2O$ i.e., $C_{14}H_{22}Cl_3N_3O_5$ requires C, 40.16; H, 5.30; N, 10.03; Cl, 25.41%), ν max. in nujol 1680 to 1735 (broad peak) 2400 to 3600 (broad peak) cm.$^{-1}$.

N-Benzyloxycarbonyl-DL-threonine (121)

This compound was prepared from DL-threonine by the action of benzyl chloroformate according to the method of Riley et al.[121] It had m.p. 73°. Riley et al.,[121] quote m.p. 74-75°.

N-Benzyloxycarbonyl-DL-threonine benzyl ester

N-Benzyloxycarbonyl-DL-threonine was esterified with benzyl alcohol in the presence of toluene-p-sulphonic acid as catalyst; the water formed in the reaction being removed azeotropically with benzene, using a Dean and Stark water separator, as described by Bergel and Wade.[71] The compound had m.p. 63°. Lit.,[71] m.p. 63-64°.

O-Chloroformyl-N-benzyloxycarbonyl-DL-threonine benzyl ester (129, 267)

This compound was obtained from N-benzyloxycarbonyl-DL-threonine benzyl ester by the action of phosgene in the presence of N,N-dimethylaniline according to the method of Bergel and Wade.[71]. The chloroformate, which was obtained as a pungent smelling oil, was used without further purification in subsequent reactions. ν max. (liquid film) 1720, 1765 cm.$^{-1}$.

O-(N-Phenylcarbamoyl)-N-benzyloxycarbonyl-DL-threonine benzyl ester (130)

The above chloroformate (0.2 g.) was condensed with aniline (0.2 g.) as described for the preparation of m- methoxycarbonylphenyl N-phenylcarbamate.

Recrystallisation from benzene-petroleum ether (b.p. 40-60°) gave needles (0.05 g.) of the carbamate, m.p. 107-108°. (Found: C, 67.75; H, 5.55; N, 5.91. $C_{26}H_{26}N_2O_6$ requires C, 67.53; H, 5.67; N, 6.06%), ν max. in chloroform 1715 to 1735 (broad peak) 3410 cm.$^{-1}$.

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-N-benzyloxycarbonyl-DL-threonine benzyl ester (129)

O-Chloroformyl-N-benzyloxycarbonyl-DL-threonine benzyl ester (prepared from N-benzyloxycarbonyl-DL-threonine benzyl ester (4.65 g.)) was added to a mixture of the hydrochloride of mustard amine A (4.15 g.), pyridine (2.8 g.) and benzene (50 ml.). On working up the reaction mixture in the usual way, an oil was obtained. This yielded a crystalline solid on trituration with petroleum ether (b.p. 40-60°). Recrystallisation of this solid from benzene gave needles (5.9 g.) of the mustard carbamate, m.p. 91°. (Found: C, 59.71; H, 5.48; N, 6.65; Cl, 12.02. $C_{30}H_{33}Cl_2N_3O_6$ requires C, 59.79; H, 5.52; N, 6.97; Cl, 11.76%), ν max. in chloroform 1700 to 1735 (broad peak) 3410 cm.$^{-1}$.

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-DL-threonine (166)

The above carbamate (3.0 g.) was dissolved in methanol (150 ml.) containing concentrated hydrochloric acid (0.54 ml, and subjected to hydrogenation in the presence of 10% palladium on charcoal. On working up as described for the corresponding serine compound, an off-white solid (1.2 g.) was obtained. Recrystallisation from methanol-anhydrous ether gave pink crystals of the hydrogenolysis product, as a dihydrochloride dihydrate which had no sharp melting point. (Found: C, 37.79; H, 5.49; N, 8.56; Cl, 28.05. $C_{15}H_{27}Cl_4N_3O_6$ i.e., $C_{15}H_{21}Cl_2N_3O_4.2HCl.2H_2O$ requires C, 37.0; H, 5.59; N, 8.63; Cl, 29.13%), ν max. in nujol 1680 to 1740 (broad peak), 2400 to 3600 (broad peak) $cm.^{-1}$.

L-Tyrosine benzyl ester (141, 235)

This compound was prepared according to the method of Zervas et al.[133] The compound had m.p. 120°. Lit.,[72] m.p. 121-122°.

N-Benzyloxycarbonyl-L-tyrosine benzyl ester (143, 237)

This compound was prepared from L-tyrosine benzyl ester by the action of benzyl chloroformate according to the method of Bergel and Wade.[72] The compound had m.p. 118-119°. Bergel and Wade[72] quote m.p. 118-119°.

O-Chloroformyl-N-benzyloxycarbonyl-L-tyrosine benzyl ester (151,237)

This compound was prepared by the action of phosgene on N-benzyloxycarbonyl-L-tyrosine benzyl ester in the presence of N,N-dimethylaniline according to the method of Bergel and Wade.[72] The chloroformate was obtained as a pungent smelling oil which was used without further purification in the subsequent reactions.

O-(N-Phenylcarbamoyl)-N-benzyloxycarbonyl-L-tyrosine benzyl ester (151)

The above chloroformate (0.5 g.) was condensed with aniline (0.5 g.) as described for phenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate. On working up, an oil was obtained. Chromatography

(alumina, Spence grade H, column; chloroform) of this oil gave a crystalline solid (0.1 g.). Recrystallisation from benzene-petroleum ether (b.p. 40-60°) gave fine needles of the carbamate, m.p. 135-136°. (Found: C, C, 70.97; H, 5.29; N, 5.29. $C_{31}H_{28}N_2O_6$ requires C, 70.96; H, 5.38; N, 5.34%), ν max. in chloroform 1700 to 1745 (broad peak), 3410 cm.$^{-1}$.

O-(N-p-(N,N-Di-2-chloroethylamino)phenylcarbamoyl)-N-benzyloxycarbonyl-L-tyrosine benzyl ester (151, 161)

A solution of O-chloroformyl-N-benzyloxycarbonyl-L-tyrosine benzyl ester (from N-benzyloxycarbonyl-L-tyrosine benzyl ester (3.0 g.)) was added to a mixture of the hydrochloride of mustard amine A (2.1 g.), pyridine (1.2 g.) and benzene (25 ml.) by the general method previously described. On working up an oil (2.2 g.) was obtained. Chromatography (silica gel column; chloroform-petroleum ether (b.p. 60-80°) (7:3)) gave fine needles (0.8 g.) of N,N-di-p-(N,N-di-2-chloroethylamino)phenylurea, m.p. 207-208°, identical with an authentic sample in all respects; and a granular solid (0.75 g.)

O-(N-p-(N,N-di-2-chloroethylamino)phenylcarbamoyl)-N-benzyloxycarbonyl-L-tyrosine benzyl ester, m.p. 38-42° (softens at 35°). (Found: C, 63.34; H, 5.34; N, 6.17; Cl, 10.56. $C_{35}H_{35}N_3Cl_2O_6$ requires C, 63.24; H, 5.31; N, 6.32; Cl, 10.67%), ν max. in chloroform 1720, 1740, 3450 cm.$^{-1}$.

SOME AMIDES DERIVED FROM (N,N-DI-2-CHLOROETHYL-p-PHENYLENE DIAMINE

Terephthalic N,N -di-(p-N,N-di-(2-chloroethylamino phenyl)diamide (145)

A solution of terephthaloyl chloride (1.0 g.) in benzene was added dropwise with stirring to a mixture of the hydrochloride of mustard amine A (2.7 g.), pyridine (1.6 g.) and benzene (30 ml.) according to the general method previously described. On working up, a dull yellow solid was obtained. This was purified by chromatography (alumina, Spence grade H, column; chloroform) to obtain a bright yellow solid (2.4 g.) which was recrystallised from acetone to give shiny platelets of the diamide, m.p. 187-190° (softens at 176°). (Found: C, 56.32; H, 5.03; N, 9.29; Cl, 23.05. $C_{28}H_{30}Cl_4N_4O_2$ requires C, 56.39; H, 5.07; N, 9.40; Cl 23.78%), ν max. in nujol 1640, 3300 cm.$^{-1}$.

p-Methoxycarbonylbenzoyl chloride (167)

Anhydrous methanol (0.8 ml.) was added dropwise to an ice-cold solution of terephthaloyl chloride (4.06 g.) in anhydrous benzene (50 ml.). The reaction mixture was

stored overnight in a refrigerator. Then benzene and hydrogen chloride formed in the reaction, were removed under reduced pressure in a rotary evaporator. The residual oil was distilled, 130° (bath)/0.8 mm., to give a colourless liquid (2.7 g.) which solidified on standing to yield a crystalline mass, m.p. 38°. Lit.,[134] m.p. 38-40°, lit.,[135] m.p. 130°; and lit.,[136] m.p. 54-55°.

p-Methoxycarbonylbenzoic N-(p-N,N-di-2-chloroethylamino phenyl)amide (168)

A solution of the above acid chloride (2.0 g.) in benzene (25 ml.) was added to a mixture of the hydrochloride of mustard amine A (2.7 g.), pyridine (1.6 g.) and benzene (25 ml.) according to the general method. On working up, a yellow solid (2.8 g.) was obtained. This solid, on chromatography (alumina, Spence grade H, column; chloroform), gave crystals of terephthalic N,N -di-(p-N,N-di-(2-chloroethylamino)phenyl)diamide (2.1 g.), m.p. 187-190°, identical with an authentic sample obtained previously and pale yellow prisms (0.5 g.) of p-methoxycarbonylbenzoic N-p-(N,N-di-2-chloroethylamino phenyl)amide, m.p. 158-159°, after recrystallisation from chloroform. (Found: C, 57.25; H, 5.08; N, 6.90; Cl, 18.56. $C_{19}H_{20}Cl_2N_2O_3$ requires C, 57.73; H, 5.10; N, 7.09;

Cl, 17.94%), ν max. in chloroform 1670, 1715, 3410 cm.$^{-1}$.

o-Methoxycarbonylbenzoic acid (149)

This compound was prepared according to the method of Eliel et al.[126] from phthalic anhydride and methanol. It had m.p. 82°, Eliel et al., quote m.p. 82-82.5°.

o-Methoxycarbonylbenzoyl chloride (149)

The above compound was refluxed with thionyl chloride for 1 hour as described by Eliel et al.[126] Removal of the excess of thionyl chloride, and the hydrogen chloride formed in the reaction, gave the acid chloride which was used without further purification in the following reactions.

N-p-(N,N-Di-2-chloroethylamino)phenylphthalimide (149)

The above acid chloride (from o-methoxycarbonyl benzoic acid (1.8 g.) was added to a mixture of the hydrochloride of mustard amine A (2.7 g.), pyridine (1.6 g.) and benzene (30 ml.) according to the general method described previously. The bright yellow solid (2.95 g.) so obtained was recrystallised from chloroform to obtain bright yellow needles of the imide, m.p. 169-170°. (Found: C, 59.26; H, 4.34; N, 7.94; Cl, 19.29.

$C_{18}H_{16}Cl_2N_2O_2$ requires C, 59.53; H, 4.44; N, 7.71; Cl, 19.52%), ν max. in chloroform 1090, 1110, 1190 1360, 1385, 1700, 1730 (shoulder), 1750 (shoulder), 1775 cm.$^{-1}$. The nuclear magnetic resonance spectrum in deuteriochloroform showed τ 2.0 (4H, multiplet, aromatic protons in phthaloyl ring); τ 2.73 and 3.27 (4H, pair of doublets, aromatic protons in p-phenylenediamine ring); and τ 6.31 (8H, multiplet, protons of 2-chloroethylamino groups).

N-m-(N,N-Di-2-chloroethylamino)phenylphthalimide

o-Methoxycarbonylbenzoyl chloride (from o-methoxycarbonylbenzoic acid (0.9 g.) was condensed with mustard amine B (1.1 g.) by the general method. On working up, an oil was obtained. This was chromatographed (alumina, Spence grade H, column; chloroform) to give almost colourless plates of the imide, m.p. 138-139°. (Found: C, 59.54; H, 4.43; N, 7.32; Cl, 19.75. $C_{18}H_{16}Cl_2N_2O_2$ requires C, 59.53; H, 4.44; N, 7.71, Cl, 19.52%), ν max. in chloroform 1085, 1110, 1180, 1360, 1385, 1705 (shoulder), 1710, 1760, 1780 cm.$^{-1}$. The nuclear magnetic spectrum in deuteriochloroform showed τ 2.39 (4H, multiplet,

aromatic protons in phthaloyl ring); τ 2.92 and 3.43 (4H, two multiplets, aromatic protons in m- phenylenediamine ring); and τ 6.53 (8H, multiplet, protons in 2-chloroethylamino groups).

Attempted condensation of p-carboxyphenyl N-p-(N,N-di-2-chloroethylamino)phenylcarbamate and methyl p-amino benzoate (78, 79, 101, 102)

(a) using dicyclohexylcarbo-di-imide

The carbamate (0.21 g.), dicyclohexylcarbo-di-imide (0.11 g.) and methyl p-aminobenzoate (0.15 g.) were shaken with a mixture of dry N,N-dimethyl formamide (0.5 ml.) and anhydrous dichloromethane (0.5 ml.) at 5°C. After standing overnight at room temperature, the solvent was removed under reduced pressure at <40°. The solid residue was taken up in chloroform and the clear solution obtained after filtration was washed with (i) dilute hydrochloric acid and (ii) water. Drying (sodium sulphate), followed by evaporation of the solvent gave a white solid (0.076 g.), m.p. 165-170°. This solid was purified by chromatography (alumina column; chloroform) to obtain a solid which gave needles (0.035 g.) on recrystallisation from ethyl acetate. This solid was rechromatographed (alumina; chloroform)

using a fraction cutter for collecting the eluate, to obtain needles (0.008 g.) of pure N-p-methoxycarbonyl phenyl-N -p-(N,N-di-2-chloroethylamino)phenylurea, m.p. 174-180°. (single spot on t.l.c.). (Found: C, 55.17; H, 5.15; N, 10.04. $C_{19}H_{21}Cl_2N_3O_3$ requires C, 55.61; H, 5.16; N, 10.24%).

(b) using ethyl chloroformate

The carbamate (0.1 g.) was dissolved in dry dichloromethane (0.5 ml.) and treated with ethyl chloroformate (0.027 g.) at 0°C. Triethylamine (0.025 g.) was added to the cooled mixture. After 20 minutes when a few crystals (presumably triethylammonium chloride) appeared; a solution of methyl p-aminobenzoate (0.04 g.) in dichloromethane (0.5 ml.) was added. After standing overnight, the reaction mixture was diluted with ether (5 ml.) and filtered. The filtrate was washed successively with (i) 2N hydrochloric acid, (ii) sodium bicarbonate solution and (iii) water. The washed filtrate was dried (sodium sulphate) and concentrated under reduced pressure to obtain a mixture of solids (0.08 g.), m.p. 184-198°. Chromatography (alumina, chloroform)

of this solid gave a crystalline solid (0.015 g.), m.p. 200-203°. Recrystallised from chloroform to obtain pure N,N -di-p-(N,N-di-2-chloroethylamino) phenylurea, m.p. 207°, identical with an authentic sample prepared previously, in all respects. (Found: C, 51.31; H, 5.46; N, 10.86. Calculated for $C_{21}H_{26}Cl_4N_4O$. C, 51.22; H, 5.32; N, 11.37%).

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