

A Thesis entitled

"SOME SYNTHESSES OF COMPOUNDS OF THE NITROGEN MUSTARD
TYPE"

submitted by

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This thesis is dedicated to my mother

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I also wish to thank the staff of the Microanalytical Laboratories for analyses, and Mr. G. Foster for help in the interpretation of the N.M.R. spectra.

Finally, I thank my mother for her continual help and encouragement which has enabled me to reach this stage in my education.

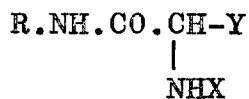
Armstrong Laboratory,
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B. J. Johnson.

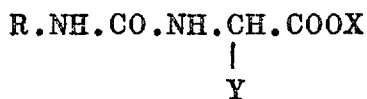
June, 1963.

ABSTRACT

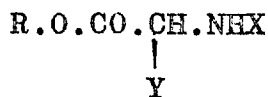
Some new aromatic nitrogen mustards have been synthesized for possible chemotherapeutic use as anti-tumour agents. The work has been mainly concerned with the modification of known nitrogen mustards in order to make them more biologically specific in their action. With this object in view, some amino-acids which take part in metabolic pathways (glycine, glutamic acid, methionine and tyrosine) have been attached to an aromatic nitrogen mustard, NN-di-2-chloroethyl-p-phenylenediamine (RNH_2) or p-(NN-di-2-chloroethylamino)phenol (ROH) via a peptide, urea, ester or carbamate linkage to give compounds of the types (A), (B), (C) or (D). In order to produce extra



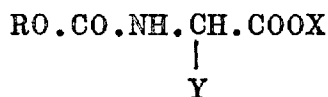
(A) where X = H or
protecting group.



(B) where X = alkyl.



(C) where X =
protecting group.



(D) where X = alkyl.

selectivity, the amino group of the amino-acid moiety in some of the compounds of types (A) and (C) was protected with enzymically fissionable groups such as formyl, carbamato, dichloroacetyl or acetyl. Some analogous derivatives were also synthesized from the antimetabolite, ethionine.

Compounds containing other known or potential anti-metabolites attached to nitrogen mustard moieties have also been prepared, including a number of sulphonamides. Some Schiff base derivatives of NN-di-2-chloroethyl-p-phenylenediamine, and the bifunctional nitrogen mustard, methyl p-hydroxy-m-(NN-di-2-chloroethylamino)benzoate, were also synthesized.

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PREFACE

'And their teaching will eat its way like cancer
or spread like gangrene'

2 Timothy 2:17.

St. Paul was of course referring to the heresy being preached by Phygelus and Hermogenes but the fact is that cancer had been known and recognised for a long time. Hippocrates (460?-377 B.C.) likened the visible form of the tumour to that of a crab; hence the name cancer. By 300 B.C. it was differentiated from carbuncles etc., and treated by surgical means wherever possible. Ointments such as of the arsenical family, and of strong corrosive action, have been used. With the advent of X rays, which were found to have anti-tumoural effects, has come a very powerful ally in the therapy of cancer. Chemotherapy has only become a force, and that a junior one, in the last two or three decades. The observation by Ward¹ and Prelog et al.² that tri-2-chloroethyl amine (HN3) and methyl di-2-chloroethylamine (HN2) respectively, were vesicant and analogous to mustard gas (of World War I fame) led to a large amount of research into nitrogen mustards being conducted during the Second World War. It was found that besides their vesicant

action the mustards also attacked the haemopoietic organs and produced a drop in the white blood cell level (leucopenic effect). This effect led to clinical trials of various nitrogen mustards for the treatment of leukaemia. From these results, and the fact that nitrogen mustards attack rapidly dividing cells, a surge of interest in them was produced for the treatment of cancer. Today a large number of them are being prepared and tested in the hope of reducing their toxicity and increasing their specificity, and this thesis will record some attempts by the author to attain these ends.

CHAPTER 1

CHEMOTHERAPY OF CANCER

Chemotherapy is simply the use of chemicals to cure or prevent disease. In cancer, chemotherapy involves the administration of chemicals to a host bearing a tumour, such that the tumour is affected (i.e. inhibited or regressed) while the host is not.

The superiority of one drug over another is frequently measured by its Chemotherapeutic Index (C.I.) which is the ratio of the Lethal Dose (LD_{50}) to the Median Effective Dose (MED). Thus the larger the C.I. of a drug when compared to another the better it is, other things being equal.

That there are very few differences between normal and cancerous cells has been shown by Warburg³ who in a biochemical investigation concluded that all the known chemical constituents of normal tissues were also present in cancerous tissues. No single morphological feature specific to the cancer cell has been demonstrated by optical and electron microscopy, although one must immediately say that certain features when taken together are characteristic of tumour cells. However, it has been found that there are alterations⁴, always compared with the corresponding normal cell, in the levels of enzymic activities or in the

functionally available amounts of coenzymes and co-factors in tumour cells.

Because these differences are small selective cytotoxicity is very hard to achieve. Thus if the anti-tumoural drug depends on only one difference the agent will not show a very great specificity. But should a drug be designed in such a way that its action depends on two or more differences, it will show a greater specificity.

DRUG DESIGN.

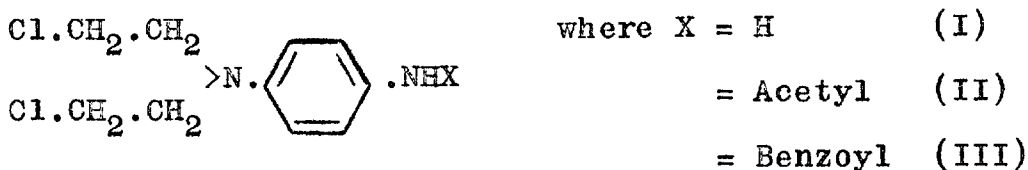
There are many biological variables concerned in determining the selectivity of a drug, and these must be fully exploited to obtain the highest degree of therapeutic efficiency⁵.

Among such variables are: enzymic constitution, permeability properties, facilitated diffusion, active transfer, chromosome *fusion*, protein synthesis, nucleic acid synthesis, gene reproduction, adaptation to drugs, resistance to drugs, etc. However, due to a lack of knowledge of many of these variables the only fields which can be exploited are limited to enzymic constitution, permeability properties and certain small areas of knowledge in connection with other variables.

Enzyme Activation of Drugs.

The principle is to use an active nitrogen mustard in an inert form, such as a derivative, from which, by enzymic action, the free active nitrogen mustard will be liberated.

If we consider the nitrogen mustard (I)

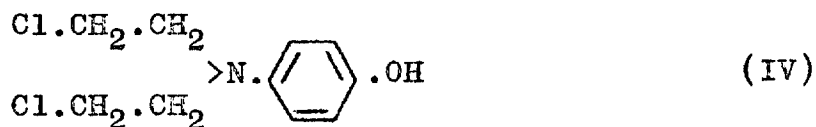


the N-acetyl compound, due to the electron withdrawing effect, as predicted, lowered the toxicity and produced more damage to the tumour, for a given toxicity, than the parent compound (I); the N-benzoyl compound was even less toxic but had little action on the weight of the animal or size of the tumour, even when the dose was sufficient to kill the animal. This was explained when the enzymic content of the tumour was investigated, for it was found to contain an enzyme which would liberate the nitrogen mustard (I) from (II) but not from (III). Thus it can be seen that should a tumour possess enzymes at levels higher than in normal tissue then they can be used to liberate an active nitrogen mustard from its inactive derivative. However in a large number of cases these higher levels of enzymes have not been found and so only partial selectivity has been obtained, but should a non-toxic substance when used in pre-treatment

of a tumour cause formation of an adaptive enzyme, then it could be used to potentiate the action of the drug designed to be activated by the adaptive enzyme^{6,7} (see p.16).

If in the general design R.  .N(CH₂.CH₂.X)₂, group R is chosen to incorporate a substance which when released will be toxic, then two toxic substances instead of one will be released when the compound is activated in vivo.

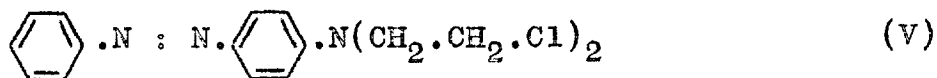
A large number of compounds have been designed to be activated by enzymic hydrolysis, reduction and oxidation, and also by the formation of more effective partially alkylated products, some^{of} which may function as irreversible antimetabolites. Hebborn and Danielli⁸ have shown that the Walker tumour contains enzymes capable of hydrolysing the acetate and benzoate ester of the nitrogen mustard (IV).



Aliphatic nitrogen mustard amides are also hydrolysed in vivo⁹, but the analogous sulphonamides were found not to be readily attacked by hydrolytic enzymes and so they were inactive. Two aromatic nitrogen mustard phosphoramides have been described by Ross et al.¹⁰ but only the diethyl ester showed any anti-tumour activity.

Compounds activated by reduction are the N-oxides (Nitromin) and the S-oxides. Azobenzene derivatives (V)

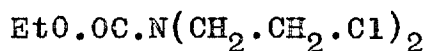
have been used and these are presumably reduced in vivo



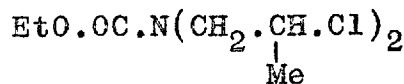
to the nitrogen mustard I. Enzymic oxidation activation was shown with the compound (VI)^{11a}, which is oxidized to the



carboxylic acid derivative, and presumably exists as the anionic form at physiological pH. The chemical reactivity of (VI) measured by its rate of hydrolysis was very low¹¹, but it showed moderate anti-tumour activity which can only be explained by the oxidation of the aldehyde group occurring in vivo. The simple urethane derivatives (VII) and (VIII) inhibit the growth of the Walker tumour. Although of low chemical reactivity, they probably owe their anti-tumoural activity to the formation of nor HN2 in vivo.



(VII)



(VIII)

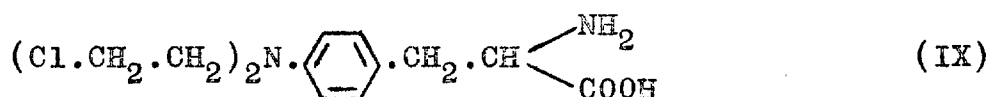
Nor HN2 could be derived by the hydrolysis of the C-N bond or by reduction of the ethoxycarbonyl group.

Active Transport (Facilitated Diffusion).

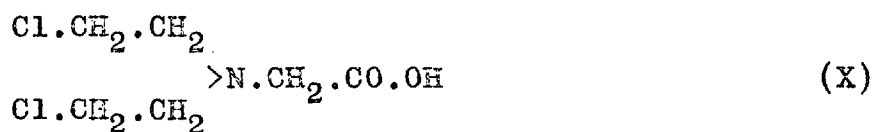
Insufficient knowledge is known about the ability of molecules to diffuse into cells by thermal agitation, to be of use in drug design. However it is known that patches

of cell membranes selectively facilitate the passage of sugars and amino-acids without expenditure of energy.

In this laboratory a number of sugar derivatives have been made^{12,13} in order to obtain the extra selectivity afforded by facilitated diffusion of carbohydrates, but these compounds have shown disappointing results. A large number of amino acids have been attached to nitrogen mustards, one of the first being (IX), the L-form of which is known as Melphalan^{14,15}.



The D-form (Medphalan) of (IX) was much less active than the L-form and the DL-form (Merphalan) or Sarcolysine¹⁶ had an intermediate activity. It would seem reasonable that the carboxyl and α-amino groups of the amino acid portion should be free as in (IX), if only that the metal-pyridoxal complex with the α-amino group, which is the diffusing entity, can be formed. However, some Japanese workers¹⁷ have made derivatives involving the amino group of glycine and alanine, e.g. (X), which have inhibited the growth of several animal tumours.



When Melphalan (IX) is condensed with optically pure amino acids^{18,19} to give simple peptides there are indications that the C.I. of the compounds is increased.

Other amino acid derivatives which have been prepared are those of serine and threonine^{20,21}.

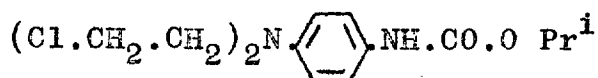
Exploitation of Adaptation and Resistance.

Cells may become adapted to drugs by various mechanisms, namely by developing an enzyme or enzymes to detoxify the drug, develop an active transport system which removes the drug from the site as fast as it gets there, or by eliminating the activating enzyme or enzymes.

If the adaptability or resistance of a tumour cell to a drug is due to changes in the enzymic constitution and, or, active transfer, then these effects can be made use of in designing drugs. Thus Danielli²² suggested that compounds, such as ethyl carbamate, to which the tumour had become resistant due to the formation of an adaptive enzyme, could be replaced by the nitrogen mustard carbamate. Thus the adaptive enzyme would then release the nitrogen mustard in an active form; the enzyme would in fact be acting as an activating enzyme. This principle has been used extensively with the drugs synthesized in this laboratory. An example is afforded by Danielli^{6,7,23}.



(XI)



(XII)

Benn²⁴ made a number of compounds similar to (XII) which gave growth inhibition of the Walker tumour. The simple carbamate (XI) when administered alone gave little decrease in tumour growth. However, if after administration of (XI) for several days, the nitrogen mustard (XII) was given, complete regressions were obtained.

Some other factors to be considered in Drug Design.

We have considered the biological factors which can influence the suitability of a nitrogen mustard for selective anti-tumoural effects. However other factors such as solubility, hydrolysis rate, substituents in the aromatic nucleus, mono or difunctional nitrogen mustards and polarity, etc., might increase the alkylating power of a nitrogen mustard.

Solubility and Polarity.

Obviously a drug which is soluble at physiological pH should be more useful than one which is insoluble. However, there is at least one proviso, namely that if slow release of the drug is required injection in an oil will be preferable and for even slower release implantation of the drug may be necessary.

However, for rapid transfer of the drug to the site of action White²⁵ prepared a series of nitrogen mustard carboxylic acid derivatives which could be easily

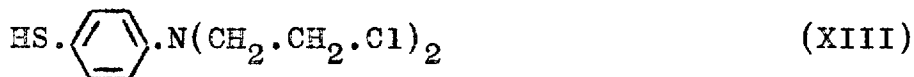
administered intravenously at pH 7.5. It was found that some were better than others and this apparent anomaly can be explained by the degree of ionization. Work and Work²⁶ have stated that the undissociated form of a carboxylic acid would diffuse through a cell membrane more easily than the anionic form. However once inside the cell an equilibrium is set up between the dissociated and undissociated forms, and so the more reactive anionic state will be available.

Although Warburg³ observed no differences in the chemical constituents between normal and cancerous tissues he did find that lactic acid was being produced by cancerous cells even in the presence of oxygen. Thus there could be an accumulation of lactic acid and so the pH of the cell would be lowered. It has been found to be the case, for when glucose is administered to a tumour its pH is reduced from 6.9 to 6.3. Because the rate of diffusion of ions through a cell membrane is far less than that of the non-ionic species, the more basic nitrogen mustards, which would be in the cationic form at this pH, would not be expected to be so effective from this point of view. However, nitrogen mustards with a suitable pK_a value have been prepared, and their anti-tumoural properties enhanced by prior administration of glucose.

Variation of β halogen atoms and change in hydrolysis rate.

Ross²⁷ has correlated the rate of hydrolysis, under standard conditions, of a nitrogen mustard with its effectiveness as an antitumoural drug. It would appear²⁸ that a compound having a "rate of hydrolysis" of 20% or more has the ability to stop the growth of the Walker tumour, but those compounds with a lower rate of hydrolysis show little or no activity.

One of the ways to increase the rate of hydrolysis is to use bromine instead of chlorine as the β halogen atoms of a nitrogen mustard. If iodine is used instead of bromine there is, however, a decrease in the hydrolysis rate²⁷. Benn²⁴ prepared the bromo analogue of (XIII) and found it had a hydrolysis rate of 70% compared with 15% for



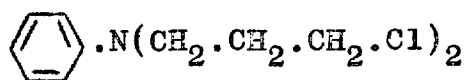
the chloro compound; it was also much more biologically active.

Variation of substituent groups in the aromatic ring and on the nitrogen mustard chain.

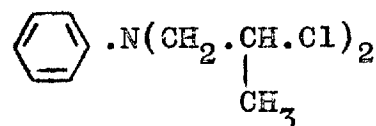
The hydrolysis rate is lowered by the normal electron attracting groups when substituted on a benzene ring. This is in keeping with chemical theory, e.g. nitro and carboxylic acid groups. The electrons are withdrawn from

the tertiary nitrogen atom of the nitrogen mustard. The hydrolysis rate is raised by the substitution of electron donating groups such as methoxy or methyl groups.

The reactive side chain of the nitrogen mustard can be varied, and this can have very large effects on the rate of hydrolysis. An increase in the alkyl chain length of the nitrogen mustard, i.e. from 2 to 3 carbons as in (XIV), reduces the rate of hydrolysis. However, an introduction of a methyl group as in (XV) increases its hydrolysis rate



(XIV)



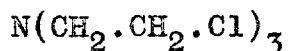
(XV)

from 20%, for the unsubstituted analogue, to 90% for (XV). However, the derived carbonium ion from such a compound has reduced alkylating properties to nucleophiles other than water²⁹.

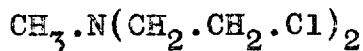
Mono or Difunctional Nitrogen Mustards.

Haddow, Kon and Ross³⁰ have shown that monofunctional aromatic nitrogen mustards, i.e. those carrying one 2-haloethylamino group, had no effect on the Walker tumour, whereas the difunctional (di-2-haloethylamino) nitrogen mustard had. However, increasing the number of alkylating groups beyond two does not seem to cause any corresponding increase in effectiveness. Thus HN3 was no more effective

than HN2.



HN3



HN2

It was the requirement of difunctionality which prompted Goldacre, Loveless and Ross³¹ to put forward their cross linking hypothesis. It is found that administration of a nitrogen mustard results in characteristic anti-mitotic effects³², chromosome alteration, such as fragmentation and bridging at anaphase occurring as well as mitotic arrest. Thus it seemed possible that the nitrogen mustards were cross-linking the chromosomes. However, the exceptions found to this theory rendered it insufficient as a total explanation. Ross³³ had in mind the possibility that one arm of the agent could first react with an exposed receptor and then the second arm could seek out and react with a more screened group.

The nitrogen mustards, like radiation, appear to act during the resting stage between cell divisions, when D.N.A. (deoxyribonucleic acid) is being synthesized. It would be tempting to think of the nitrogen mustards interfering with the synthesis of D.N.A. or with the D.N.A.-protein association which comprises the chromosome.

The reader is directed to the American Association for the Advancement of Science's publications "Approaches to Tumour Therapy"^{33a} and "Antimetabolites and Cancer"^{34a} for further references.

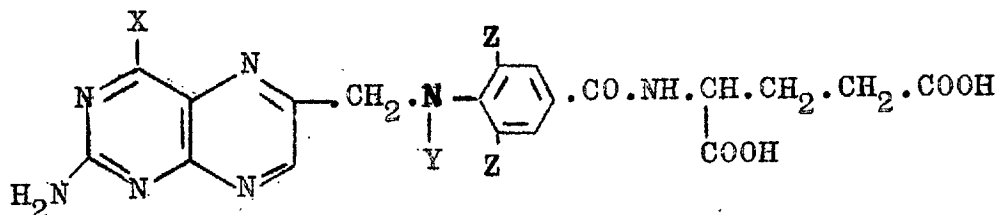
CHAPTER II

CHEMOTHERAPEUTIC AGENTS

The following is a very brief review of the two main classes of drugs used in the chemotherapy of cancer; these are the antimetabolites and the biological alkylating agents. Consideration of the hormones is excluded.

The Antimetabolites.

Folic acid plays an essential part in the production and maturation of red blood cells, and as a consequence has been used in the treatment of some forms of anaemia. The folic acid antagonists, which have been used to produce temporary remission of acute leukaemia, are drugs which resemble the true vitamin chemically but are sufficiently different to be able to interfere with essential metabolic processes. Four of the antagonists to folic acid (XVI) are Aminopterin (XVII), A-methopterin (XVIII), Aminofol and Dichloromethotrexate (XIX).



(XVI) when X = OH; Y = H and Z = H

(XVII) " X = NH₂; Y = H and Z = H

(XVIII) " X = NH₂; Y = CH₃ and Z = H

(XIX) " X = NH₂; Y = CH₃ and Z = Cl

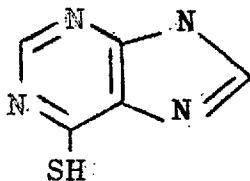
Reports on the first two of these antagonists show that they prevent conversion of folic to folinic acid in various organisms. They have also caused remissions in children with acute leukaemia³⁴. A review from the chemical point of view is given by Timmis³⁵.

Purine Antagonists and antipyrimidines.

Cell divisions depends upon the synthesis of D.N.A., which is required for the duplication of the chromosomes during interphase. Thus purines or pyrimidines are required as precursors, and so if the cell is fed with modified precursors mitosis of the cell may be hindered or stopped.

Anti-tumour activity has been found to be most prominent in compounds containing a 3,4-dihalophenyl group attached to the 5-position of the pyrimidine ring³⁶. This and other similar compounds have been studied extensively³⁷.

6-Mercaptopurine (XX) has been used extensively



(XX)

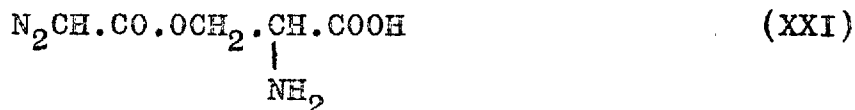
^{37a}
Heidelberg^{37a} has prepared a series of antagonists of pyrimidines, e.g. 5-fluoro-uracil, and its compounds with ribose and deoxyribose. In animal tumours 5-fluoro-uracil has been shown to inhibit the synthesis of D.N.A. For

further information on these types of antagonists Delmonte and Jukes³⁴ have just recently produced an excellent review.

Amino Acid Antagonists.

Because protein synthesis must be an essential factor in the continual growth of tumours, Connors et al.³⁹ have recently prepared α -methylated methionine, ethionine and other amino acids in an attempt to produce antagonists for the naturally occurring amino acids. The α -methyl compounds were chosen because it is believed that these derivatives are strongly concentrated intracellularly. However, no useful tumour inhibiting drugs were found. As a logical extension of the work some cyclic compounds, which could be considered as having been derived from α -alkylated amino acids were prepared.⁴⁰ It was found that 1-amino-cyclopentanecarboxylic acid, although toxic, produced tumour inhibition. In order to reduce the toxicity of the compound derivatives have been prepared, but the biological results are not available. It is interesting to note that 1-aminocyclohexanecarboxylic acid has no anti-tumoural effects.⁴¹

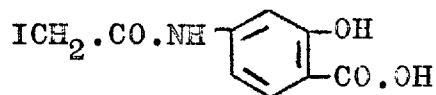
Azaserine (XXI), an antibiotic, was found to act as a glutamine antagonist.



The structure of Azaserine has been modified by replacing the serine moiety with various hydroxyamino acids⁴², and also by variation of the diazoacetic acid portion with various acids.⁴³ However, none of these modifications have produced compounds which have appreciable activity in the regression of growth of tumours.

Other Antimetabolites.

Baker⁴⁴ has produced a class of non classical anti-metabolites of irreversible inhibitors that operate by exo-alkylation. Thus 4-iodoacetamidosalicylic acid



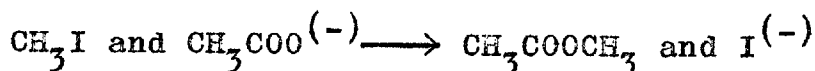
can complex reversibly with an enzyme and then become irreversibly bound within the complex by formation of a covalent bond adjacent to the active site. They inhibit lactic acid dehydrogenase and various other enzymic systems.

Biological Alkylating Agents.

These are simply chemical compounds which act as alkylating agents under physiological conditions.

Alkyl halides.

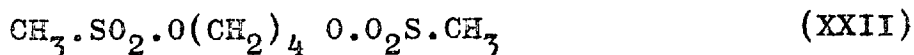
Alkyl halides react essentially by the SN_2 mechanism except for the tertiary alkyl halides which react by the SN_1 mechanism. Thus



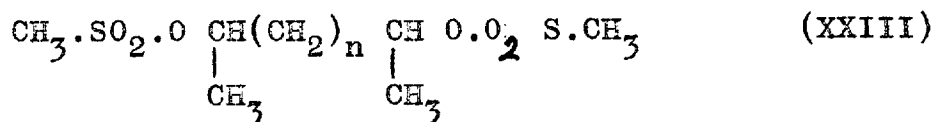
The tertiary alkyl halide is of little interest as a biological alkylating agent, because the carbonium ion is more likely to react with the water of the cell rather than with other nucleophiles. Benzyl halides, which in general are more reactive than simple alkyl halides, tend to react by a mixed mechanism but for details see Grice⁴⁵, Grice and Owen⁴⁶.

Sulphonic Acid Esters and Sulphuric Acid Esters.

The alkylating action of these esters is due to the alkyl-oxygen bond fission. A spectrum of reaction mechanisms has been observed for these compounds, namely SN_2 for the methyl sulphonic esters to SN_1 for the isopropyl esters. Myleran (XXII) has been found to be useful for



the treatment of chronic myeloid leukaemia.⁴⁷ In order to increase the ability to alkylate, the dissecondary compound (XXIII) was prepared, but although its hydrolysis rate was



increased it was not so reactive towards other nucleophiles. It is still active, when $n = 4$, against myeloid leukaemia. Two other sulphonic acid esters, namely ethyl methane-sulphonate^{47a}, and 1,6-dimethanesulphonylmannitol are

active towards the Walker tumour.

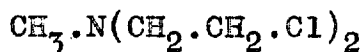
2-Chloroethyl sulphides.

These compounds, of which the original "mustard gas" is a typical example, are highly reactive in water, the mechanism of reaction being wholly SN_1 , and they are capable of alkylating a large variety of groups. Sulphur mustard gas, like X rays, was found to inhibit mitotic activity in the vaginal epithelium in mice.⁴⁸ However, it is highly reactive, toxic, and vesicant, and because the sulphur mustard gas molecule is less suited to modifications than the nitrogen mustard structure, few attempts have been made to do this.

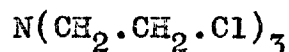
Nitrogen Mustards.

Reference has already been made in earlier sections to a few examples of these compounds, many hundreds of which have been synthesized by various groups of workers. All that can be done here is to mention some which bear reference to the work of the present writer, and to some of those which have had successful clinical trials.

The two prototypes HN2 and HN3 were found to have powerful anti-tumoural effects although they were highly toxic.

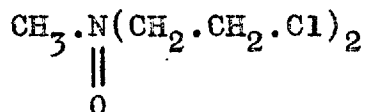


HN2



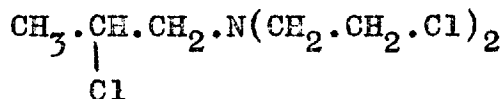
HN3

Nitromin¹⁷, an oxidative product of HN2 has been used by the Japanese. It has a lower toxicity than HN2, and no

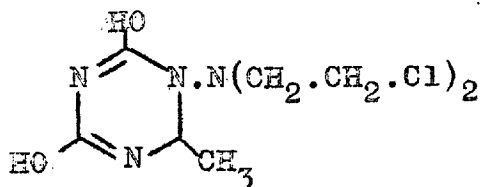


vesicant action, but it has been reported to cause complete regressions of the Yoshida sarcoma. It would seem probable that the N-oxide has to be reduced in vivo¹⁷ and release the free HN2 before any anti-tumoural effect is shown.

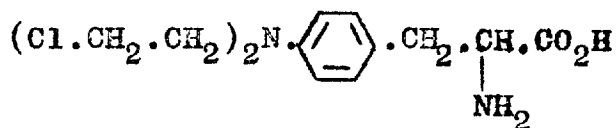
The Russians have used Novoembichin, Dopan, and Sarcolysine (which is similar to Melphalan but with the phenylalanine residue in the DL-form).



Novoembichin



Dopan

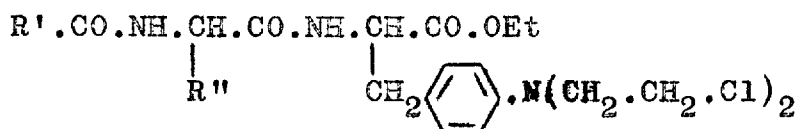


Melphalan

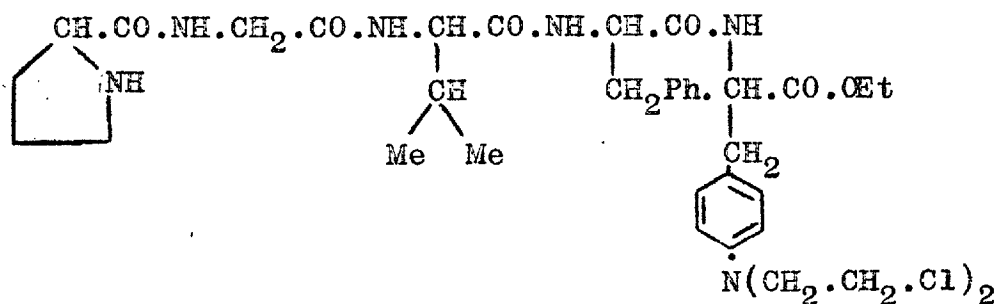
Sarcolysine

Melphalan^{14,15} caused complete inhibition of the Walker tumour. The D-form, known as Medphalan, was far less active (see p.15). It has been shown recently that the m-isomer and the p-isomer of Sarcolysine were even more effective than the p-isomer. m-Sarcolysine is four times more active than Melphalan against adenocarcinoma. The o-Sarcolysine will completely inhibit the Walker tumour and cause 80% inhibition at a dose of 0.1 mg. per kg.⁴⁹

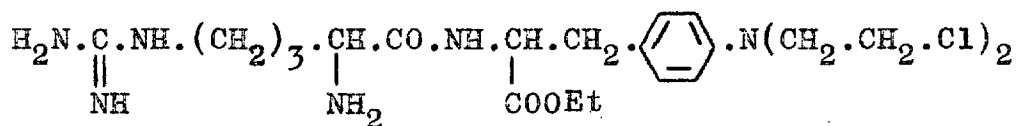
Although Melphalan has this very pronounced anti-tumoural activity, it also affects the blood forming organs, circulating blood elements, and has a generally high toxicity as well. In order to increase the C.I. of this compound Bergel and Stock^{19,50} have recently produced derivatives of melphalan, e.g. N-acyl, amide, ester, di, tri peptides and one tetra peptide. It was found that the compounds which exhibited the greatest anti-tumoural activity were those having a free amino group, even if the free amino group was two or three amino acid residues from the Melphalan molecule. However, these compounds still had the highest general toxicity and haemotoxicity. Acylation reduced the biological activity although the C.I. was the same as Melphalan. It was found⁵⁰ that for a whole series of optically pure N-acetyl-L and D-aminoacyl derivatives of Melphalan ethyl ester, e.g.



that there were considerable variations in anti-tumoural effects and toxicities, although the chemical reactivity of the nitrogen mustard was virtually constant. Some of the compounds, e.g. acetyl-L-alanyl-L-leucyl, and glycyl and formyl-L-phenylalanyl residues had better C.I. values than the parent Melphalan. One of the results of this work was the large differences in anti-tumoural activity between the L-L and D-L stereoisomeric pairs, the L-L pair being more active than the D-L pair by a factor of up to 100. Melphalan was always in the L-form in these compounds. This work was extended⁵¹, because of the effect of the carrier portion of the dipeptide, to the preparation of a pentapeptide prolylglycylvalylphenylalanyl-melphalan, i.e.

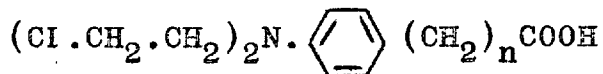


This compound markedly inhibited the growth of the Walker rat carcinoma 256, in a single dose of 5 mg./kg. which is approximately one tenth of the toxic dose. It is active against the mouse sarcoma S.180. Johnson and Stock also prepared a Melphalan derivative containing a basic peptide, i.e. L-arginylmelphalan.



It showed similar activity to that of the pentapeptide, completely inhibiting the Walker tumour at a single dose of 2 mg./kg. which is approximately one twelfth of its toxic dosage.

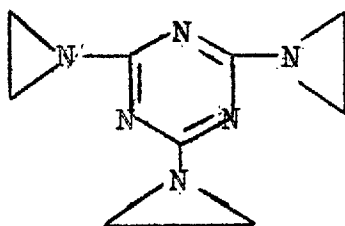
The Chester Beatty Research Institute has synthesized and tested a large number of compounds besides those of Melphalan and its derivatives. Chlorambucil was one of these (when $n = 3$).



A series of compounds⁵² was prepared where $n = 0, 1, 2, 3$ and 4 ; the best was when $n = 3$. Chlorambucil has been found to be a valuable agent in the treatment of chronic lymphocytic leukaemia which was resistant to T.E.M. (see later), and has also given promising results in the treatment of ovarian carcinoma. It has been found that chlorambucil affects predominantly the lymphoid system whereas Myleran (XXII) exerts its depressant action on myeloid elements and has little effect on lymphoid components. For further details of these and other compounds of the nitrogen mustard class see Ross et al.⁵³ and Bergel et al.⁵⁴ and for the biological results see the "Annual Reports of the British Empire Cancer Campaign".

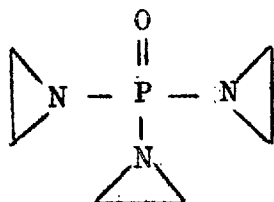
Ethyleneimines.

Because aliphatic nitrogen mustards undergo cyclization on solution to give the ethyleneimines, interest was evoked in drugs with a related chemical structure. Their reactions would be expected to be similar to those of ethylene oxide⁵⁵ except for those reactions in which the greater basicity of the nitrogen atom, as compared to that of the oxygen atom, becomes important. One of the first to be used was T.E.M. triethylenemelamine.⁵⁶

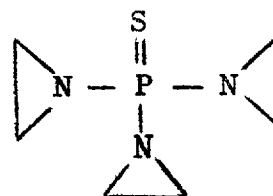


T.E.M.

The results for Hodgkin's disease and chronic myeloid leukaemia were similar to those obtained with HN2 but were somewhat better for lymph⁸sarcoma and chronic lymphatic leukaemia. The related phosphoramides, triethylenethio-phosphoramide⁵⁸ (thio T.E.P.A.) have also proved to be of clinical benefit in Hodgkin's disease, in neuroblastoma and in some cases with disseminated melanoma



T.E.P.A.



Thio T.E.P.A.

Other alkylating agents.

β -lactones, diazoalkanes, activated ethylenic compounds, halogenomethyl ketones and esters, and esters of haloketones, chloro-methyl ethers, ammonium and sulphonium compounds, epoxides, and of course phosphoric esters are all compounds which can cause alkylation, and the majority of them have been used at one time or another. The reader can find more information on these compounds in 'Biological Alkylating Agents' by W. C. J. Ross (1962). A review of the more important chemotherapeutic agents is given by Farber et al. in "Advances in Cancer Research" 1956, 4, 20.

CHAPTER III

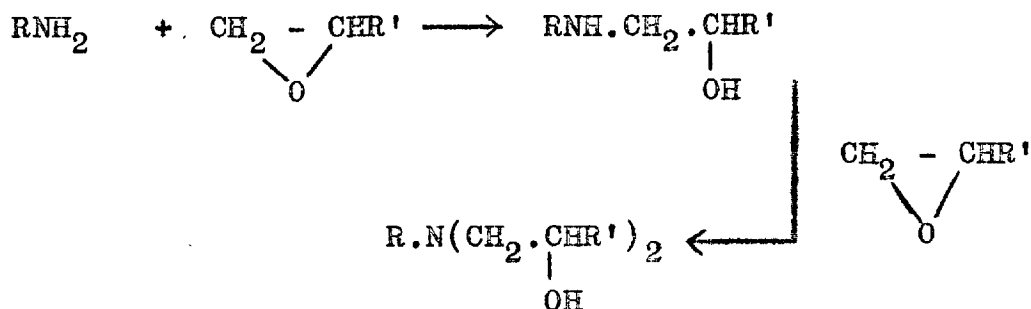
THE CHEMISTRY OF THE NITROGEN MUSTARDS

Methods of preparation.

The nitrogen mustards are usually prepared from the corresponding di-2-hydroxyalkylamine by halogenation of the diol with various halogenating compounds. There are three principal methods of preparing the dialkanolamines, of which the first is the most generally used.

a) The reaction of an amine with an epoxide.

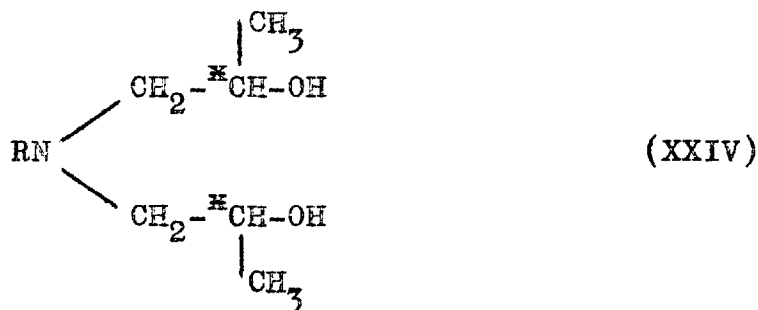
Epoxides react with amines as follows:



Ethylene oxide reacts with alkylamines under anhydrous conditions⁵⁹, and also in aqueous solution⁶⁰. The speed of reaction seems to depend on the basicity of the amine, thus the more basic the amine the more readily hydroxyalkylated it is. Ethylene oxide reacts with hydrazine using acetic acid at room temperatures⁶¹ to give the NN-di-2-hydroxyethylhydrazine compound. Aromatic amines, which

are weaker bases, react with ethylene oxide when heated together in a sealed tube at elevated temperatures⁶². Everett et al. found that below 150⁰, large amounts of mono substituted products were produced²⁹. Benzene can also be added to the reactants for the sealed tube reaction⁵³. Aryl-2-hydroxyalkylamines can also be produced at room temperature from the aromatic amine in aqueous acetic acid and excess ethylene oxide⁶³. It is important to use the theoretical quantities of the reactants when hydroxy-alkylating aliphatic amines by the sealed tube method, otherwise polymers are obtained. The writer always used the aqueous acetic acid method, for the products are clean, more suited to large scale preparation, and excess ethylene oxide can be used.

Everett et al.²⁹ found that propylene oxide in excess could be heated with an aromatic amine without any polymeric material being obtained. The ring opened so as to form (XXIV)



The product which contained two asymmetric centres was a mixture of the various isomers.

Mannich base type reactions are undergone by diethanolamine hydrochloride to give substituted diethanolamines.⁶⁷

Halogenation of the dialkanolamines.

The normal halogenating compounds are used to convert the dialkanolamine into the nitrogen mustard analogue. However, some reagents are better than others in that cleaner and more easily purified products may be obtained. The aliphatic diol hydrochloride is usually used suspended in dry chloroform and halogenated with thionyl chloride.^{1,60} Phosphorus trichloride has been used⁶⁰ but it is definitely inferior to thionyl chloride. Likewise the bromo-mustard is best prepared with thionyl bromide.⁶⁸

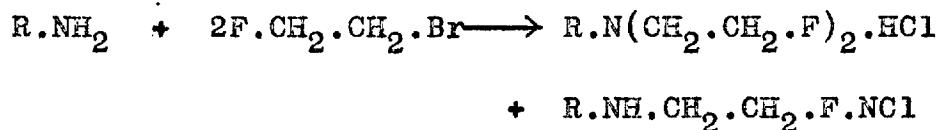
The aromatic nitrogen mustards are better prepared from the free base of the diol, by boiling it under reflux in dry benzene solution with phosphorus oxychloride.⁵³ Phosphorus pentachloride has been used^{53,69} and constitutes a standard method of preparation, but phosphorus trichloride cannot. Thionyl chloride is not such a successful reagent for aromatic nitrogen mustards, although it has been used for carboxylic acid or amino acid derivatives.⁵⁴ Phosphorus tribromide or phosphorus pentabromide reacts with these aryl di-2-hydroxyethylamines in an inert solvent to produce the bromo-nitrogen mustard analogue.^{53,24} The yields in the halogenation step are usually poor and ^{the products} can normally

only be purified by chromatography on alumina. Bromo nitrogen mustards can be obtained from the corresponding chloro-mustards by an exchange reaction with excess lithium bromide in a suitable solvent. Similarly iodo-nitrogen mustards can be obtained from the bromo-compounds by boiling under reflux with sodium iodide⁵³ in acetone.

Other methods which do not employ the intermediate diols but are of limited application only, are the reactions of di-2-chloroethylamine with reactive halogen compounds. A number of NN-di-2-chloroethyl amides have been prepared⁶⁵ by reaction of carboxylic; sulphenic and sulphonic acid chlorides with di-2-chloroethylamine. Trichlorethylamine reacts with some alkanols to give 2-alkoxyethyl di-2'-chloroethylamines⁷⁰



The reaction of 1-bromo-2-fluoro-(or chloro-) ethane with a powerful aliphatic amine gives a mixture of the aliphatic nitrogen mustard⁷¹ and the mono substituted amine e.g.



The two compounds were separated by fractional crystallisation as their hydrochlorides.

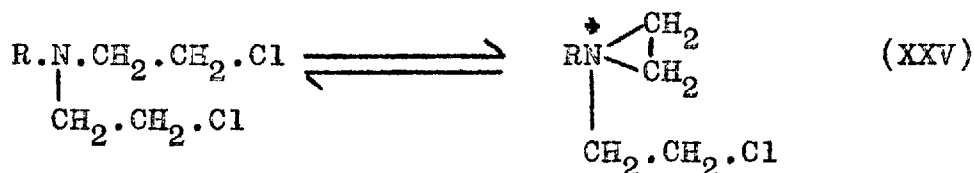
Reactions of the nitrogen mustards.

The reader is referred to an excellent review by Ross²⁷ on this subject.

Aliphatic Nitrogen Mustards

Hydrolysis

Golumbic et al.⁷² found that when methyl di-2-chloroethylamine reacts with water there is a rapid elimination of one equivalent of chloride ion but very little liberation of hydrogen ion. This was interpreted as the formation of an ethylenonium ion (XXV).



which was sufficiently stable such that derivatives of it, e.g. the picrylsulphonate^{72,73} could be isolated.

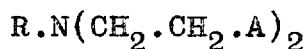
After one equivalent of hydrochloric acid has been produced⁶⁰ hydrolysis ceases due to the formation of the compound (XXVI) which is stabilized as the hydrochloride. However



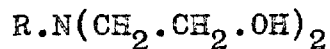
in buffered solutions (XXVI) can be hydrolysed to completion. Golumbic et al. found that there was a rapid reaction of a

Reaction with Anions.

Substituted aminoethyl esters are formed when an aliphatic nitrogen mustard is allowed to react with an aqueous solution containing anions (A^-)(XXVII). However, they are readily hydrolysed to the diol(XXVIII)



(XXVII)



(XXVIII)

Thus Fruton et al.⁷⁶ allowed methyl and ethyl-di-2-chloroethylamine to react with sodium acetate in slightly alkaline solution to produce the diol, which was ascribed to the ester being hydrolysed under these conditions. Tri-2-chloroethylamine reacted in solutions containing sodium acetate, hippurate, acetyldehydrophenyl alanine, and acetyldehydrophenylalanyldehydrophenylalanine under similar conditions giving esters which were isolated and characterised in the case of the last two anions. It has been proved that methyl and ethyl-di-2-chloroethylamine and tri-2-chlorethyl amine react with anions of the following acids: nicotinic, hippuric, acetic, carbobenzoxy-L-glutamic carbobenzoxy-L-aspartic, and DL-methionine. The same technique was used to confirm reaction with phosphate, fructose 1- and 6-phosphate etc. Competition factors for anions in their reactions with aliphatic chloroethylamines

have not been determined by the method which Ogston used for chloroethyl sulphides, whereas they have for the aromatic nitrogen mustards (see later).

Reactions with Amines and Thioethers.

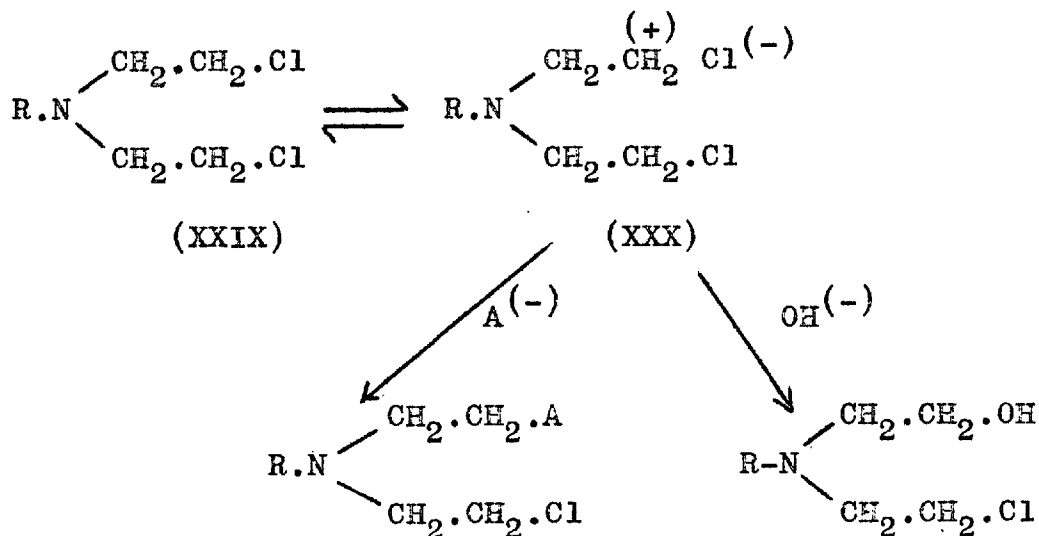
Prelog et al.² found that 4-alkyl-1-phenylpiperazines were formed when alkyl-di-2-chloroethylamines or bromoethylamines were heated with various amino acids by estimation of the decrease in amino nitrogen (Van Slyke method), and found that at pH8 glycine, L-alanine, L-serine, DL-threonine, L-glutamic acid, L-arginine, L-lysine, L-histidine, and β -alanine were alkylated. The pK_a of these amino groups are in the region of 9-10 and so they will be largely unionized at pH8. Gurin et al.⁷⁷ found that tertiary amines were more reactive than secondary amines towards methyl-di-2-chloroethylamine, also compounds containing the nitrogen atom in a cyclic structure were very reactive towards the ethylene-imonium ion. Fruton et al.⁷⁶ has shown that the methylthio ether in methionine was unreactive towards methyl and ethyl-di-2-chloroethylamine but showed signs of sulphonium salt formation with tri-2-chloroethylamine.

Aromatic Nitrogen Mustards.

Hydrolysis.

The nitrogen atom is far less basic in an aromatic nitrogen mustard, when compared to that of an aliphatic

nitrogen mustard; because of this the ethyleneimmonium ion is not formed. The hydrolysis of aromatic nitrogen mustards has been extensively studied by Ross who, because of their low solubility in water, performed their hydrolysis in aqueous acetone.^{27,29,53} During hydrolysis halide and hydrogen ions are liberated at the same rate with no tendency for formation of the half hydrolysis product. The mechanism is judged to be SN_1 based on the following evidence



The kinetics are unimolecular, but the value of the rate coefficient falls as the reaction proceeds due to the increasing importance of the back reaction ($\text{XXX} \rightarrow \text{XXIX}$). Addition of anions other than the halide ion derived from the nitrogen mustard causes an increase in the velocity of reaction, addition of the same halide ion reduces the rate of reaction.

The relative amounts of ester and hydroxy compound formed when NN-di-2'-chloro- and bromo-ethyl-2-naphthylamine are hydrolysed in aqueous solution containing added anions are the same; this would be expected if the carbonium ion (XXX) was the intermediate. The SN_1 rate determining step is the separation of the halide ion and so electron repelling substituents attached to the ring should, and do, accelerate while electron attracting groups slow down this ionization and decrease the reaction rate. This is the reverse of that required if the reaction went by the SN_2 mechanism.

Reaction with Anions.

Ross^{29,78} has made a study of the relative reactivity of aryldi-2-chloroalkylamines and various anions by a method similar to that used by Ogston.⁷⁹ Because anions vary in their nucleophilicity a quantitative measure of their reactivity in ester formation with nitrogen mustards is the 'competition factor' (F_A) of the anion as defined by Ogston

$$F_A = \frac{[\text{Amount of ester formed}]}{[\text{Amount of diol formed}][A^-]}$$

where $[A^-]$ is the concentration of the anion A^- . The competition factors for a number of anions have been found by the reaction of aromatic nitrogen mustards in 1:1 aqueous acetone with a known concentration of anions and then

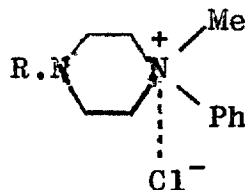
estimating the amounts of hydrogen and chloride ion liberated. It was found that thiosulphate and iodide ions have very high, while fluoride and nitrate ions low competition factors. The formation of esters under the conditions used to determine competition factors has been verified by the isolation and characterization of these derivatives in a number of cases.

Reactions with Amines.

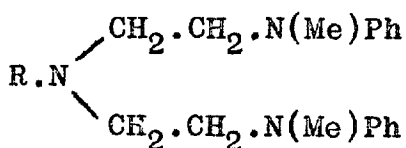
Aryldi-2-halogenoalkylamines react with primary, secondary and tertiary amines (Ross⁷⁸) 2-Naphthyldi-2'-chloroethylamine reacts with aniline to give a good yield of the piperazine; this is a general reaction for the preparation of NN-disubstituted piperazines⁸⁰ (XXXI). Secondary amines, e.g. methyl-aniline, gave both the cyclic quaternary compound (XXXII) and also the di-methyl-anilino compound (XXXIII). The double quaternary salt (XXXIV) is the product obtained in the reaction with dimethylaniline



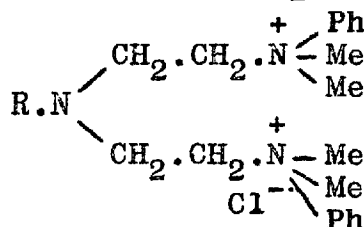
(XXXI)



(XXXII) Cl^-



(XXXIII)



(XXXIV)

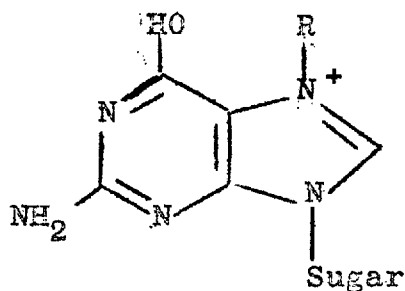
The cationic form of an amine (e.g. lysine) is not nucleophilic and would not be expected to react with a nitrogen mustard. However, if the zwitterion is broken by preparing the ethyl ester then reaction occurs in unbuffered aqueous acetone solution.

Thioethers react with the chloroethylamines giving sulphonium compounds. When NN-di-2-chloroethyl-p-anisidine is reacted with phenyl methyl sulphide and also with dibutyl sulphide, sulphonium salts were obtained.⁸¹ Thiophenol also reacts with aromatic nitrogen mustards, but it is dependent on the pH of the solution, thus as the pH of the solution rises there is an increase in the percentage reacted⁸¹ indicating that the thiol reacts with the aromatic nitrogen mustard when it is ionized.

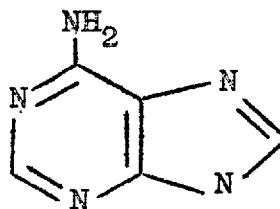
Reactions in Biological Systems in vitro.

The pH of a physiological system is about 7.5, and this puts a severe limitation on the groups which can be alkylated by biological alkylating agents. Thus a carboxylic acid must be in the anionic form to a considerable extent before it could be alkylated. Thus in amino acids, pK_a 9-10 and 2-3, the carboxylic acid portion can be alkylated whereas only a small proportion of the α -amino groups will be in the reactive uncharged form. The pK_a

of the hydroxyl groups of sugars is in the region of 12-13 and so at pH 7.5 the hydroxy group will not be in the reactive form. However, the hexosephosphoric acids can be alkylated to form di and triesters. The phosphoric acid hydroxyl of groups (pK_a 1.5 and 6.2) will be readily alkylated. Substances containing strongly basic groups like adrenaline will not be reactive since they will be protonated at pH 7.5. However, ring nitrogen atoms in heterocyclic bases derived from pyridine, pyrimidine and purine are capable of being alkylated. In nucleic acids the constituents which will be capable of alkylation at pH 7.5 are primary and secondary phosphoryl; ring nitrogen atoms, e.g. guanine, and adenine. The reactive guanine molecule is alkylated at the N_7 position giving a quaternary derivative (XXXV), while the adenine molecule is attacked at the N_1 and N_3 positions (XXXVI). Alkylation of nucleic



(XXXV)



(XXXVI)

acids reduces their stability, such that degradation of the macromolecule to products of lower molecular weight occurs under mild conditions.

^{81a} Timmis has pointed out that difunctional alkylating groups could react with a nucleic acid precursor giving a product which still possessed a reactive group. The precursor would then be able to alkylate irreversibly the enzymes required for nucleic acid synthesis. Craig and Jackson⁸² have shown that the nitrogen mustard (XXXVII) was not retained in tissues but found that there was a relatively high concentration in red blood cells.



It has been reported that labelled HN2 with ¹⁴C in the methyl group was widely distributed throughout the body of mice into which it had been injected, the highest concentration of radioactivity being found in the blood serum. However, the distribution of radioactivity does not necessarily indicate the distribution of the alkylating agent because it has been found that the N-methyl group is demethylated within one hour after an intravenous injection in a rat.

Fruton et al.⁷⁶ have reported that an aqueous solution of HN2 blocked some of the free amino groups of egg albumin and gelatin. Klopp et al.⁸³ have reviewed the effects of HN2 on skin, plasma, hematopoietic system, respiration, gastrointestinal tract, liver, pancreas, kidneys, etc.

CHAPTER IV

SOME METHODS OF FORMING THE PEPTIDE LINKAGE

Part of the work in this thesis is concerned with the preparation of nitrogen mustards containing peptide linkages. A review of the more usual methods of protecting the various functional groups of amino acids, and of forming the peptide bond, follows:-

Protection of functional groups.

Although the synthesis of a peptide only requires the formation of the amide bond by condensation of the respective components, this normally cannot be done with a free amino acid. The proper function of the protecting group is to prevent unwanted condensations occurring, to break the zwitterion leaving a free α -carboxyl or α -amino group, and to be capable of easy removal without rupture of the amide bond after it has been formed.

Protection of α -carboxyl groups.

Protection of α -carboxyl groups as their methyl and ethyl esters is the most widely used method in peptide synthesis. The oldest, and most usual, method of esterification is the use of an alcoholic solution of the amino acid saturated with hydrogen chloride.⁸⁴ The classical method of liberating esterified amino acids from their hydrochlorides is by

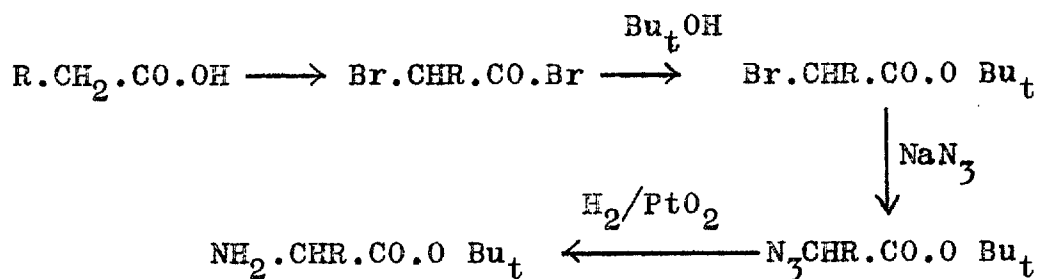
means of ethanolic sodium ethoxide,⁸⁵ another way is the treatment of the salt with a chloroform-ammonia solution, followed by the removal of the ammonium chloride by filtration⁸⁶. Removal of the methyl or ethyl ester may be performed by using alkaline or acid conditions. However, in the case where there is a nitrogen mustard residue White²⁵ has found that the methyl ester is more easily removed than the ethyl ester without hydrolysis of the halogen atoms. Other procedures which have been used for the formation of the ester, ^{are,} from the free amino acid, methanolic thionyl chloride⁸⁷ and from the N-acylamino acids by the use of methanolic sulphuryl chloride,⁸⁸ ester interchange with methyl or ethyl formate or acetate,⁸⁹ and esterification using an ion exchange resin as catalyst.

Tertiary butyl esters have come to the fore in recent years because of their ease of removal. Roeske⁹¹ obtained these esters by the acid (H_2SO_4) catalysed addition of the amino acid to isobutene.



The tertiary butyl esters are preferentially removed by the action of hydrogen chloride in benzene in the presence of N-benzyloxycarbonyl groups. However, both tertiary butyl and N-benzyloxycarbonyl groups are removed by the action of hydrogen bromide in acetic acid. Anderson⁹² has

shown that tertiary butyl esters are removed by *p*-toluene-sulphonic acid, while *N*-benzyloxycarbonyl and trifluoroacetyl groups are stable under these conditions. However, under alkaline conditions the *N*-trifluoroacetyl group can be removed without the tertiary butyl ester being touched. Tertiary butyl esters have been prepared by Vollmar and Dunn⁹³ from the acid bromide e.g.



Tashner et al.⁹⁴ produced the tertiary butyl esters of various amino acids by direct esterification using perchloric acid as catalyst, and also produced these esters, of *N*-protected amino acids, by an ester interchange with tertiary butyl acetate, again using perchloric acid as catalyst.⁹⁵

Benzyl esters are an exceedingly useful tool in this work, for they can be removed by hydrogenolysis over a palladium catalyst at atmospheric pressure.⁹⁶ Their use, however, was limited until satisfactory methods of their preparation were devised by direct esterification using benzene sulphonic acid^{97,99} and *p*-toluene sulphonic acid.⁹⁷ Erlanger and Hall⁹⁸ used polyphosphoric acid as the catalyst and dehydrating agent for the production of benzyl

esters of free amino acids. Usually the water produced by these esterifications is removed by azeotropic distillation. Schwarz^{100,101} has been the advocate for p-nitrobenzyl esters which he has prepared by the action of p-nitrobenzyl chloride or bromide and triethylamine on the N-protected amino acid. These esters are more stable than the unsubstituted benzyl esters towards hydrogen bromide in acetic acid. They are, however, removed by catalytic (10% Pd/C) hydrogenolysis.

Protection of α -amino groups.

There are far more methods of protecting the α -amino group than the α -carboxyl function, although it must be said that the short review on the methods of protecting the α -carboxyl group given above is not exhaustive. In this section only the more common N-protecting groups will be reviewed.

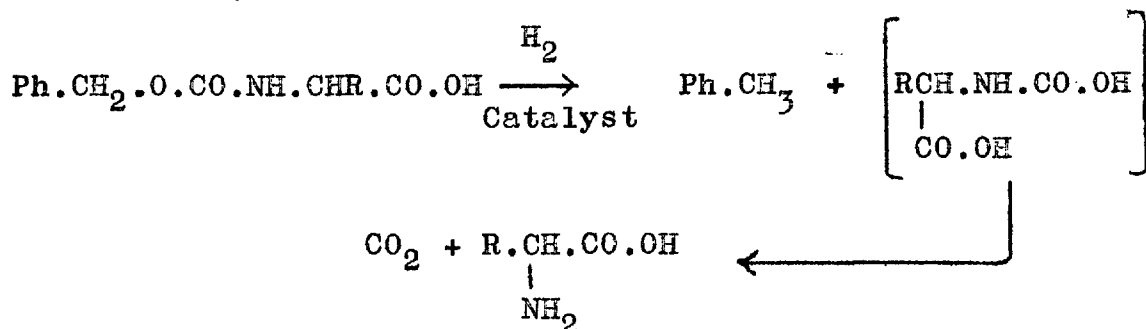
Benzyloxycarbonyl and related derivatives.

This group is the most widely used amino protecting group, and most probably will continue to be so.

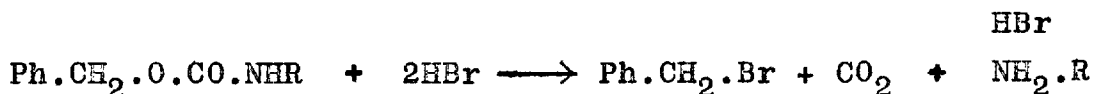
N-Benzyloxycarbonyl derivatives are made by treating the amino acid under Schotten-Baumann conditions¹⁰², with benzyl chloroformate.¹⁰³

Bergmann and Zervas¹⁰²) found that the benzyloxycarbonyl group could be removed by catalytic hydrogenolysis. However, this method of removal is unfortunately not

selective between benzyloxycarbonyl and benzyl ester derivatives.

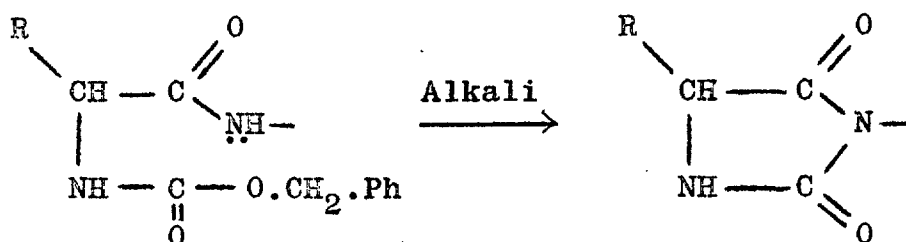


Another method of removing the N-benzyloxycarbonyl group is reduction with sodium in liquid ammonia.¹⁰⁴ It can be removed selectively with N-hydrogen bromide^{105,106} in acetic acid at room temperature, for one hour. Under these mild conditions most other sensitive groups, such as ethyl and benzyl esters, thiol esters and amides are largely unaffected.^{105,106}

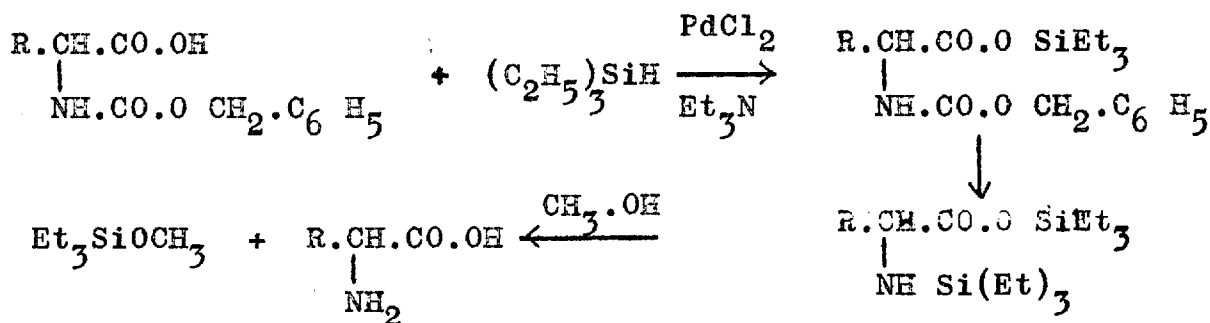


The reaction is usually performed in glacial acetic acid, although nitromethane¹⁰⁷ has been used, and the progress of the reaction may be followed by the evolution of carbon dioxide. If one is lucky the hydrobromide of the free amine may crystallize out of the reaction solution.

One of the disadvantages of this protecting group is that it is not stable to alkali. Under quite strongly alkaline conditions it cyclizes to the substituted imidazolidine derivative.¹⁰⁸

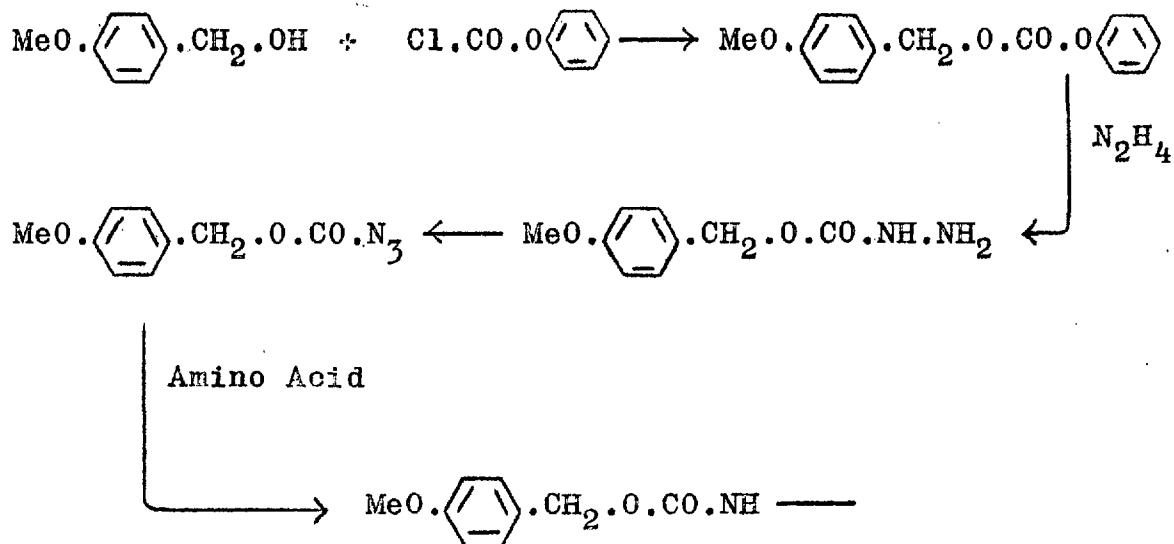


Recently Birkofer et al.¹⁰⁹ have used triethyl silane to remove selectively the N-benzyloxycarbonyl group



Another method of removal is to use anhydrous trifluoroacetic acid.¹¹⁰ Derivatives of N-benzyloxycarbonyl groups have been used, the p-nitro analogue having been advocated by Carpenter¹¹¹ because the chloroformate used in its preparation is a stable crystalline solid, unlike benzyl chloroformate. The p-nitrobenzyloxycarbonyl protecting group is easily removed by hydrogenolysis.

Weygand and Hunger¹¹² have introduced the use of p-methoxybenzyloxycarbonyl azide for the formation of the carbamate by reaction with the amino acid. The azide is prepared as shown.



This protecting group is removed by anhydrous trifluoroacetic acid at 0° , under which conditions the unsubstituted benzyloxycarbonyl derivatives are stable.

Formyl Derivatives.

Although Emil Fischer¹¹³ used the formyl group to protect the α -amino function of an amino acid it was not until Sheehan et al.¹¹⁴ studied it that the formyl group has been used extensively for N-protection. The amino acid is formylated by treatment with formic acid and acetic anhydride to give an optically pure product. The group is readily removed by alcohols containing a slight excess of 0.5N-hydrochloric acid,¹¹⁴ or by oxidation with hydrogen peroxide.¹¹⁵ The formyl group is very resistant to alkaline conditions used for removal of ester groups. It will be remembered that the N-benzyloxycarbonyl group is cyclized under these conditions. The main disadvantage of the

formyl derivative is its tendency to racemize or even decompose under the influence of many coupling procedures, except the dicyclohexylcarbodiimide coupling method.¹¹⁴

Trityl Derivatives.

The group is introduced directly on the amino acid, in the presence of base, by the action of triphenylmethyl chloride. Sometimes the amino acid ester has been used, tritylated, and then the ester removed by alkaline hydrolysis, or by hydrogenolysis in the case of benzyl esters. The trityl group is easily removed by mild acidic hydrolysis e.g. 2 minutes at 100° in 50% aqueous acetic acid. It can also be removed by catalytic hydrogenolysis. Three groups of workers have thoroughly explored this field.^{116,117,118} The only disadvantage of this group is its large size which may cause sufficient steric hindrance to give poor yields in coupling reactions.¹¹⁹

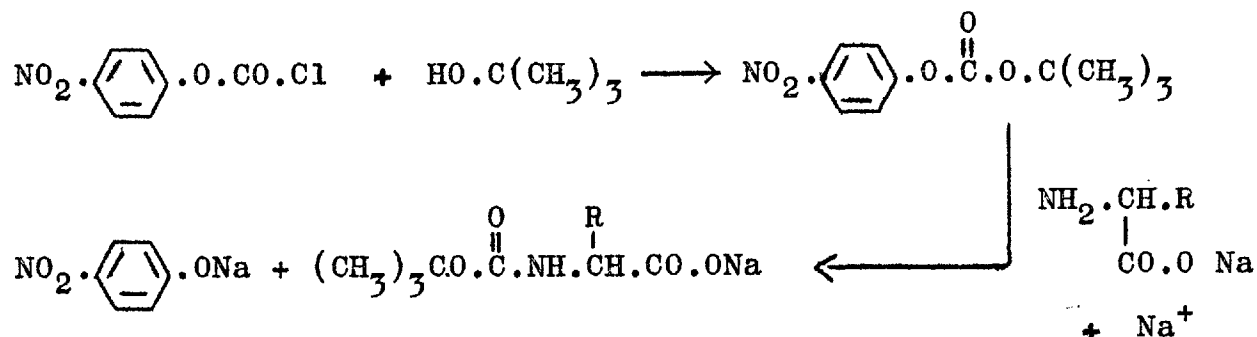
Tosyl Derivatives.

This group only became important when it was found possible to remove it by the reductive action of sodium in liquid ammonia.¹²⁰ The protecting group is introduced by the action of toluene-p-sulphonyl chloride on the amino acid under Schotten-Baumann conditions.

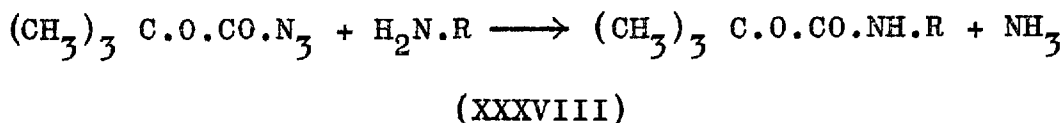
The other way of removing the tosyl group is the action of hydrogen bromide and phenol in acetic acid.¹²¹

Alkoxy carbonyl Derivatives.

The tertiary butyloxycarbonyl group has proved to be the most useful protection group under this heading. Anderson and McGregor¹²² prepared them from tertiary butyl *p*-nitrophenol carbonate which reacts with the free amino group to give the carbamate.



The tertiary butyloxycarbonyl group is removed by hydrogen chloride in acetic acid. Schwyzer et al.¹²³ have prepared a number of tertiary butyloxycarbonyl protected amino acids by using the stable *t*-butyl azidoformate (XXXVIII)



This protecting group is easily removed by the action of trifluoroacetic acid, but not acetic acid. It is unaffected by catalytic hydrogenation, or by sodium in liquid ammonia.

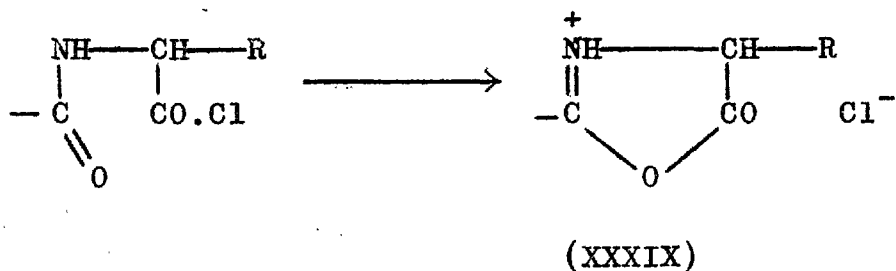
Other protecting groups.

There are a large number of protecting groups for the α -amino function of an amino acid which have not been mentioned above, e.g. the trifluoroacetyl, phthaloyl, etc. groups. For further information on these and the other protection groups the reader is directed to two excellent reviews.^{124,125}

COUPLING METHODS.

Acid Chlorides.

This is the oldest method for the production of the amide bond, but the method is falling into disuse because of the risk of racemization, due to the spontaneous formation of oxazolone hydrochlorides (XXXIX)

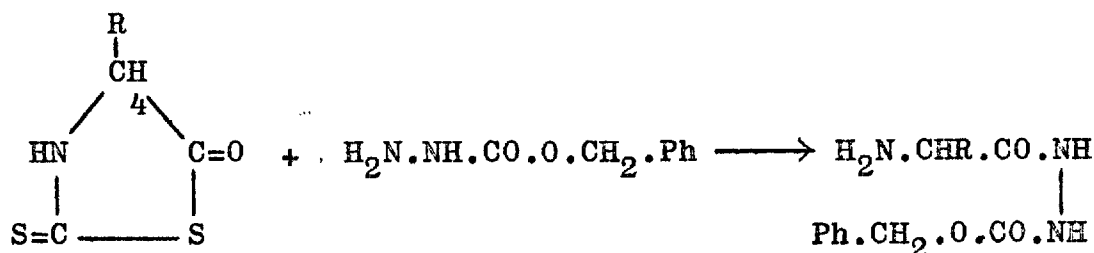


The oxazolone reacts with amines in the same way as do acid chlorides but they are easily racemized. Cyclization of benzyloxycarbonylamino acid chlorides is slower, and the oxazolone hydrochloride (XL) loses benzyl chloride forming a N-carboxyanhydride

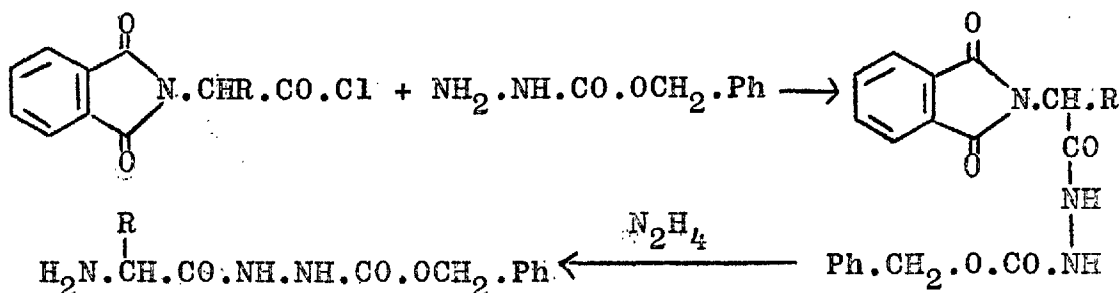
$$\text{Ph} \cdot \text{CH}_2 \cdot \text{O} - \text{C}(=\text{O}) - \text{NH}^+ - \text{CH}(\text{R}) - \text{C}(=\text{O}) - \text{O} \longrightarrow \text{Ph} \cdot \text{CH}_2 \cdot \text{Cl} + \text{O}=\text{C} - \text{NH} - \text{CH}(\text{R}) - \text{C}(=\text{O}) - \text{O}$$

(XL)

It is necessary during this preparation to work quickly because of the instability of the azide which decomposes to form the isocyanate (XLI) which will then form the urea (XLII) as a by-product. Modifications to the above method include the use of nitrosyl chloride and butyl nitrite in place of nitrous acid to form the azide; and to minimize urea formation.¹²⁸ The simple ester is not used but instead the terminal residue is made a benzyloxycarbonyl-¹²⁹ or a *N*-trityl-¹³⁰ or a *t*-butoxy-carbonyl-hydrazide.¹³¹ Hofmann et al.¹²⁹ used 4*R*-2-thio-5-thiazolidones, prepared from the amino acid and carbon disulphide, and condensed them with benzyloxycarbonylhydrazine, e.g.

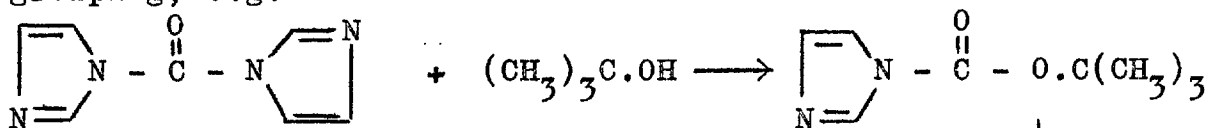


Hydrogenolysis gives the free hydrazino group which can be converted into the azide etc. Unfortunately the process for forming the *S*-thiazolidone causes racemization and so the phthaloyl compound was used instead.

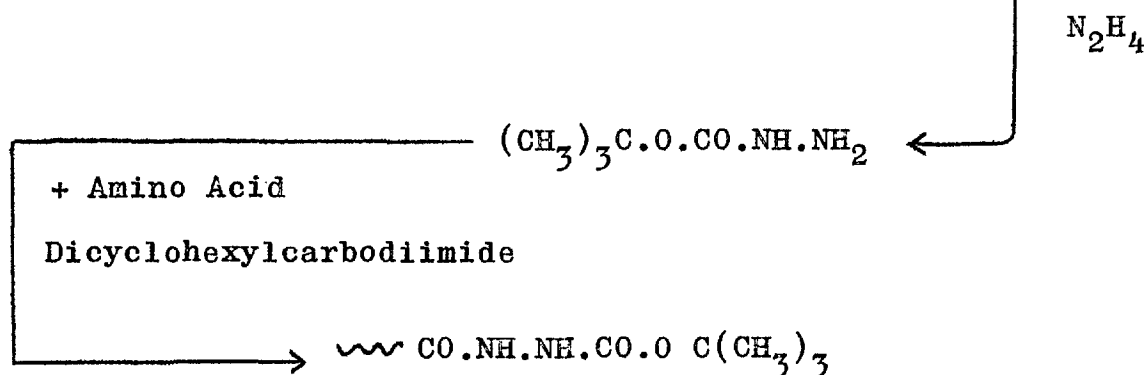


Weygand¹³⁰ prepared N-tritylhydrazine and coupled it with the α -carboxyl group of an amino acid by means of dicyclohexylcarbodiimide; thus removal of the trityl group exposed the free hydrazino group which would then be ready for the azide method of peptide synthesis.

Klee and Brenner¹³¹ used the t-butoxy-carbonyl-hydrazide grouping, e.g.



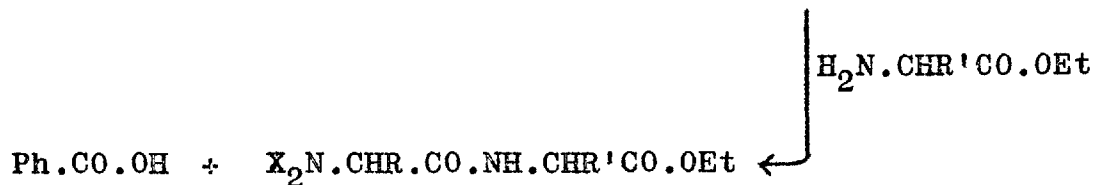
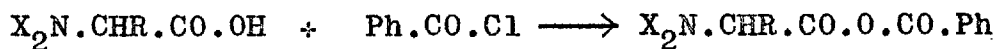
1,1carbonyldiimidazole



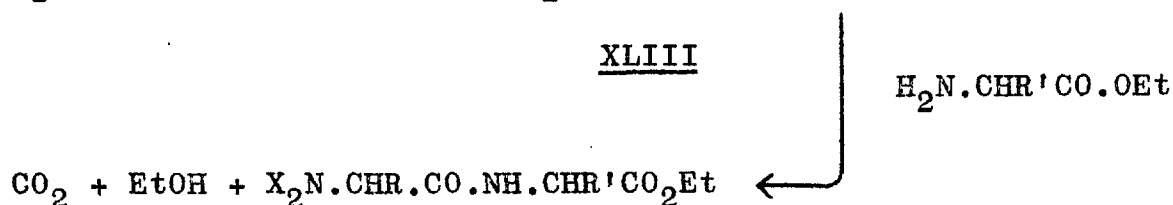
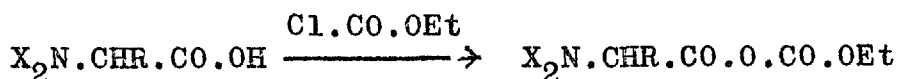
The tertiary butyloxycarbonyl group can be removed, by any of the methods mentioned above, to reveal the free hydrazine group ready for the azide coupling procedure.

Mixed Anhydrides derived from Carboxylic Acids.

This method of formation of the amide bond was pioneered by Wieland¹³² who prepared mixed anhydrides by the treatment of N-protected amino acids and benzoic acid, the former with benzoyl chloride and a tertiary amine in an inert solvent,



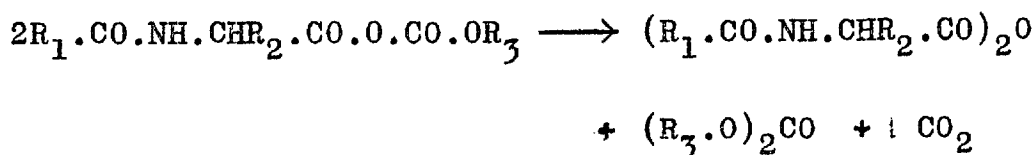
and showed that the mixed anhydride so formed reacted with amino acid esters to form the peptide. Boissonnas¹³³ found that mixed anhydrides (XLIII) derived from ethyl chloroformate also could be used to form the peptide.



It has been found¹³⁴ that isobutyl chloroformate is superior to the ethyl compound in a comparative study of the various mixed anhydrides derived from various alkyl chloroformates.

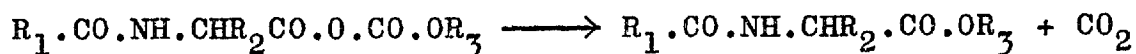
It is possible to use the free amino acids as their salts in aqueous solution as the amino component in the coupling reaction but the yields are poor. To increase the yield it is better to use the esters in a suitable inert solvent. One of the disadvantages of this method is the fact that racemization is quite prevalent. Racemization usually increases as the time and temperature for anhydride formation are increased, but the most important factor is

the nature of the solvent. Racemization is reduced in non polar solvents and in the absence of base; chloroform¹³⁵ and dimethylformamide are poor in this respect, since both of these solvents dissolve the triethylamine hydrochloride, formed during the preparation of the mixed anhydride, which may influence the rate of racemization. Another disadvantage of this method is that the anhydride can disproportionate e.g.

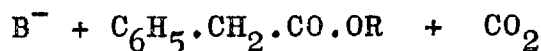
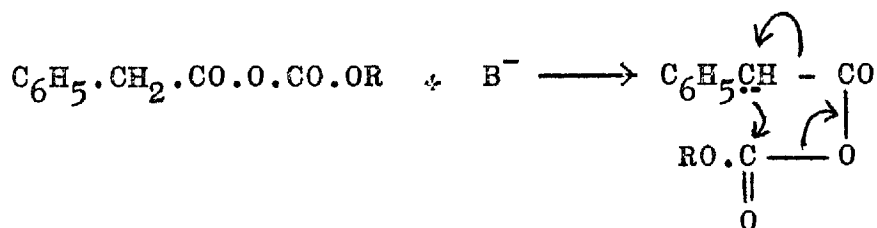


Disproportionation is favoured by long reaction times during the anhydride forming step, also low temperatures during this stage lowers disproportionation.

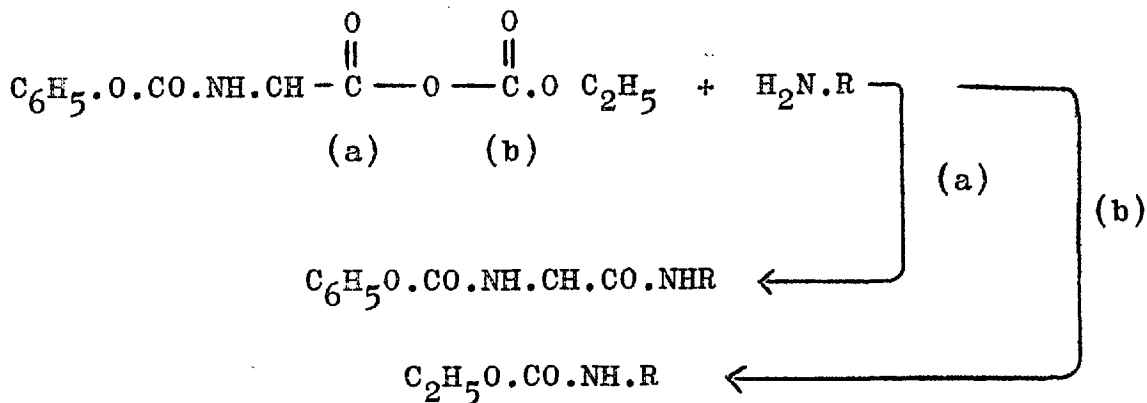
Ester formation is also found to occur and it has been suggested that this is due to the loss of carbon dioxide from the mixed anhydride.



An alternative explanation would be that the mixed anhydride reacts with the alcohol liberated during amide formation. Another explanation can be accommodated if there is some base present with the mixed anhydride, for then a modified Dakin-West reaction can occur, or by the intra-molecular mechanism shown.



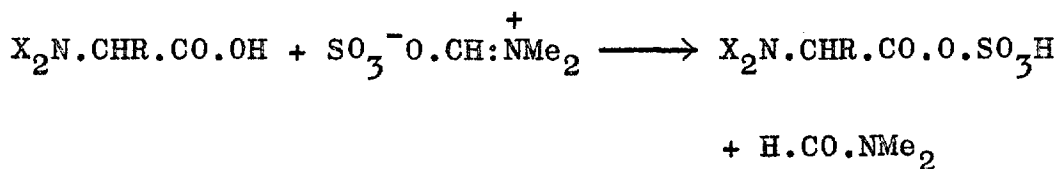
Urethanes are also sometimes formed, and this can be interpreted by having some unreacted chloroformate present which reacts with the amine, or by the amine attacking carbonyl (b) below:



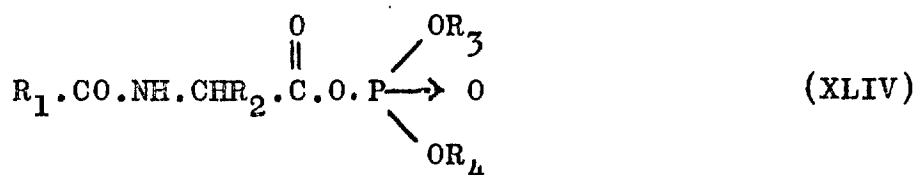
With this reaction it can be seen that various spurious side reactions can occur, but these can be kept to a minimum by the correct choice of alkyl chloroformate, time of formation of mixed anhydride, solvent, and temperature. All these factors play an important part in order to get an optically pure product in reasonable yield.

Mixed anhydrides derived from Inorganic Acids.

These include sulphuric acid anhydrides produced by the treatment of the lithium salt of N-protected amino acids with the dimethylformamide complex of sulphur trioxide.^{136,137}



Sulphonyl chlorides have been used, and the scope, and the yields obtained, appear to be comparable to those with the chloroformate procedure.¹³⁸ Other inorganic acid derivatives¹³⁹ which have been used are the α -acylaminoacyl phosphates of general formula (XLIV).

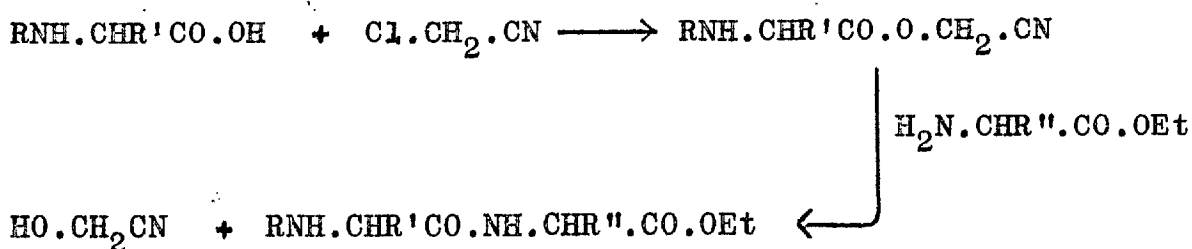


For fuller details of these methods and of others see Albertson.¹⁴⁰

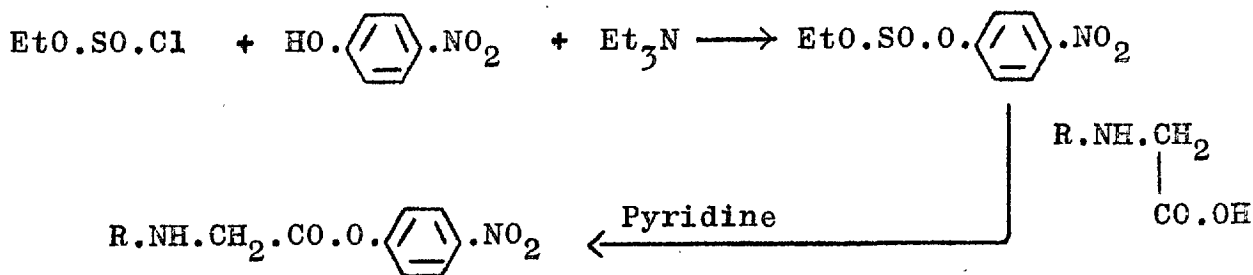
Activated Esters.

This method is based on the observation that some esters are unusually reactive towards amines. A large number of different types of ester have been used. Wieland¹⁴¹ used the phenyl thioesters $\text{X}_2\text{N}.\text{CHR}.\text{CO}.\text{SPh}$ of N-protected amino acids and showed that they coupled with the sodium salts of amino acids in aqueous solution. The analogous p-nitrophenyl thioesters are more reactive than the unsubstituted compounds. The method which has found extensive use is due to Schwyzer¹⁴² who first used the cyanomethyl esters. They are prepared from the N-protected

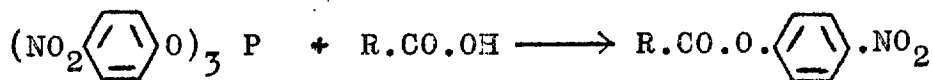
amino acids by the action of chloroacetonitrile in the presence of a tertiary amine. These esters couple readily with amino acid esters, the reaction being catalysed by acetic acid¹⁴³



However, the most popular activated esters used are those due to Bodansky,¹⁴⁴ namely the p-nitrophenol esters. These are formed from treatment of N-protected amino acids with p-nitrophenyl sulphite or phosphite.¹⁴⁵

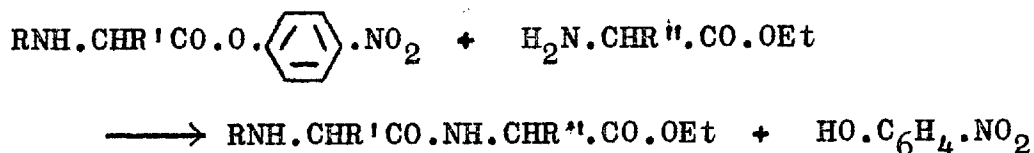


or

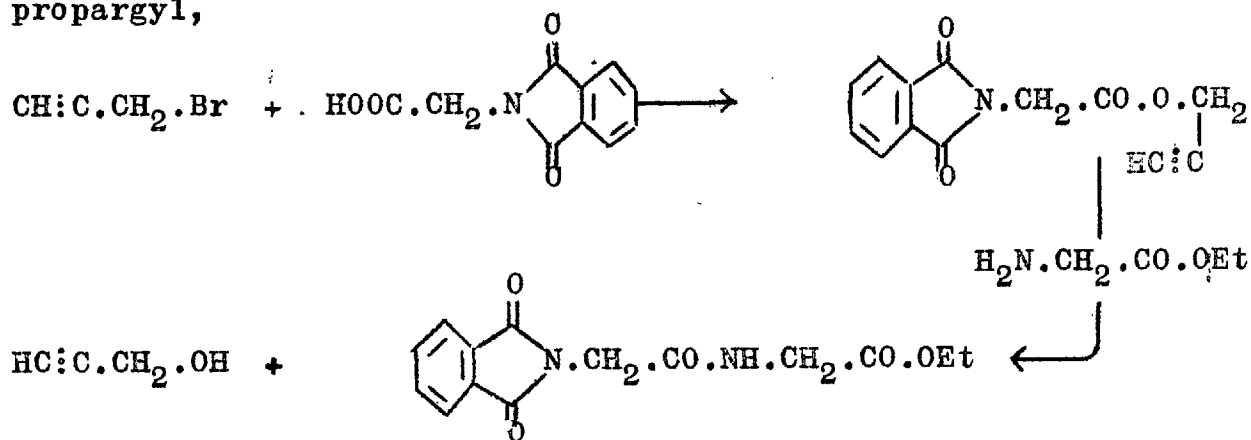


The p-nitrophenol esters are also formed by the condensation of p-nitrophenol and an N-acylamino acid using the mixed anhydride or dicyclohexylcarbodiimide procedures.¹⁴⁶ The esters, which are usually crystalline solids, couple easily :

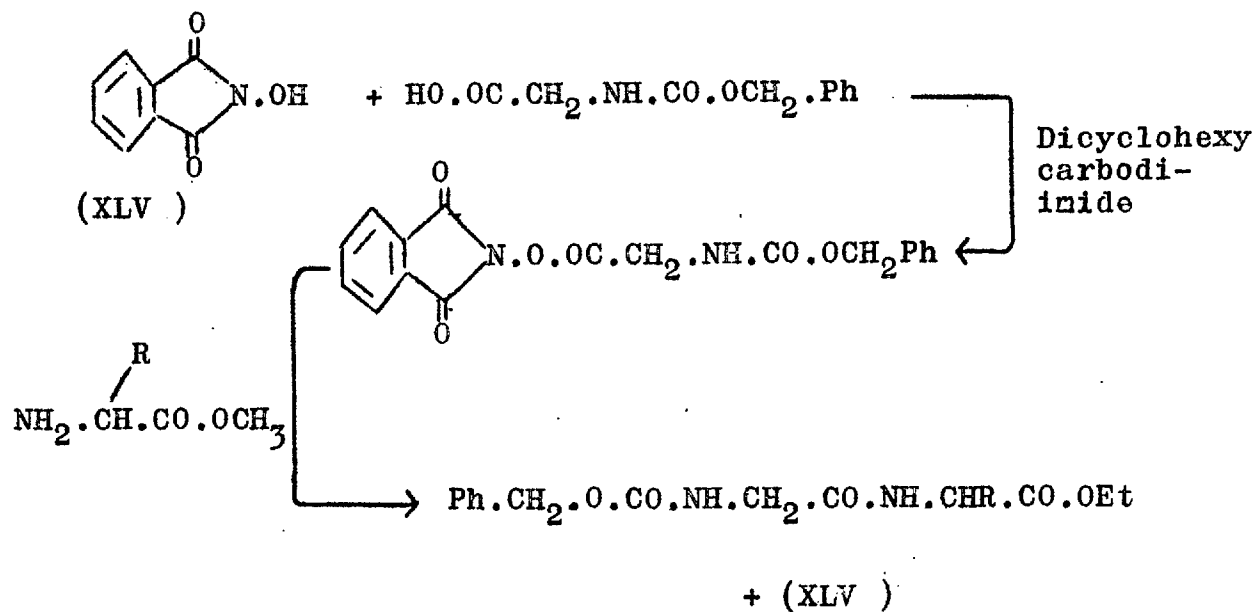
under mild conditions with amino acid esters. Some racemization has been reported but in spite of this they are a very popular method of preparing peptides.



Other activated compounds which come into this class are the propargyl,

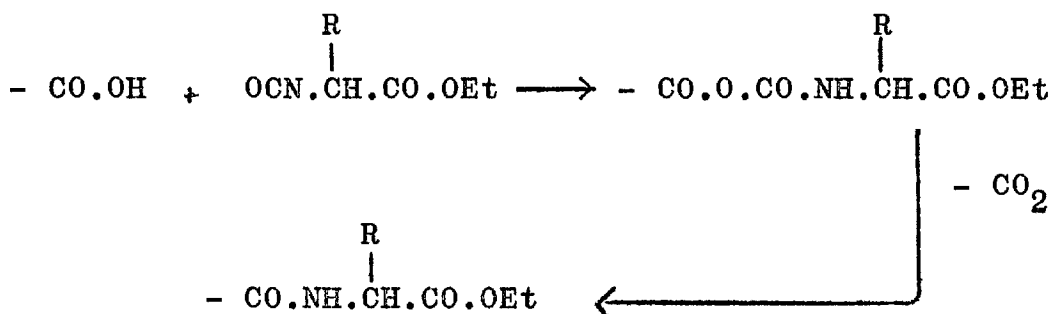


vinyl,¹⁴⁸ and the esters prepared from N-hydroxyphthalimide¹⁴⁹ (XLV), e.g.



Isocyanates.

The method uses the fact that reaction between a carboxylic acid and an isocyanate leads to a mixed anhydride of the carboxylic acid and a carbamic acid, and that the mixed anhydride decarboxylates when heated^{150,151}

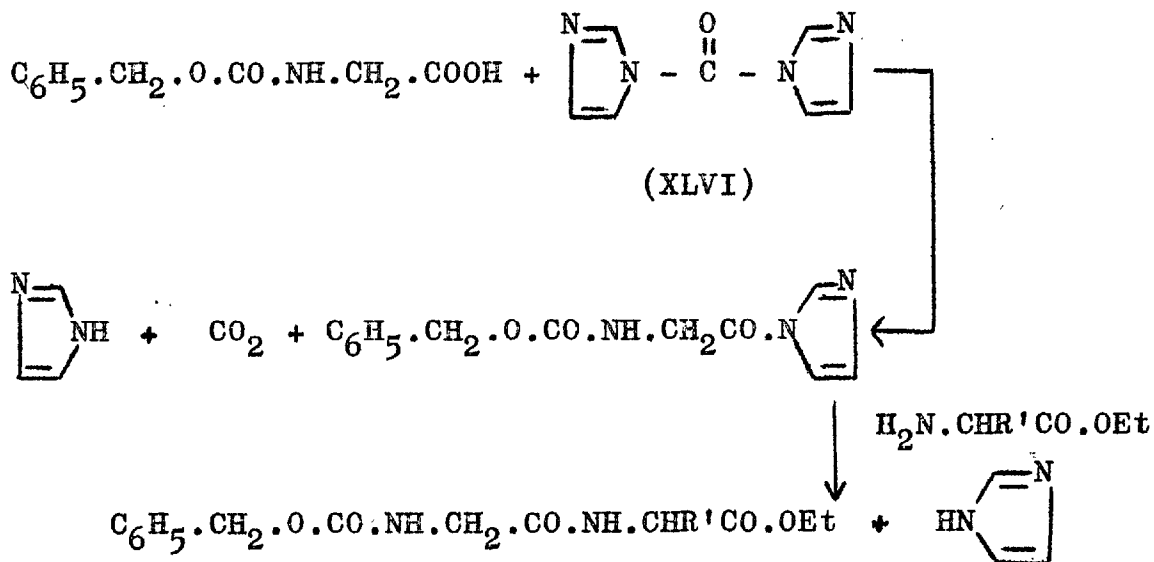


The isocyanates are prepared from the hydrochlorides of amino acid esters by means of phosgene. The method is not widely used but applications of the method can be found.^{152,153,154}

Acyylimidazoles.

NN'-Carbonyldiimidazole (XLVI) reacts readily with α -acylamino carboxylic acids to form α -acylaminoacylimidazoles (XLVII) and these have been used in peptide synthesis.¹⁵⁵

The NN'-carbonyldiimidazole is prepared from phosgene and imidazole in dry benzene. The reagent is added to an equimolecular amount of α -acylamino acid in an inert solvent at room temperature, sufficient time is allowed for the compound (XLVII) to form before the amino acid ester is added.

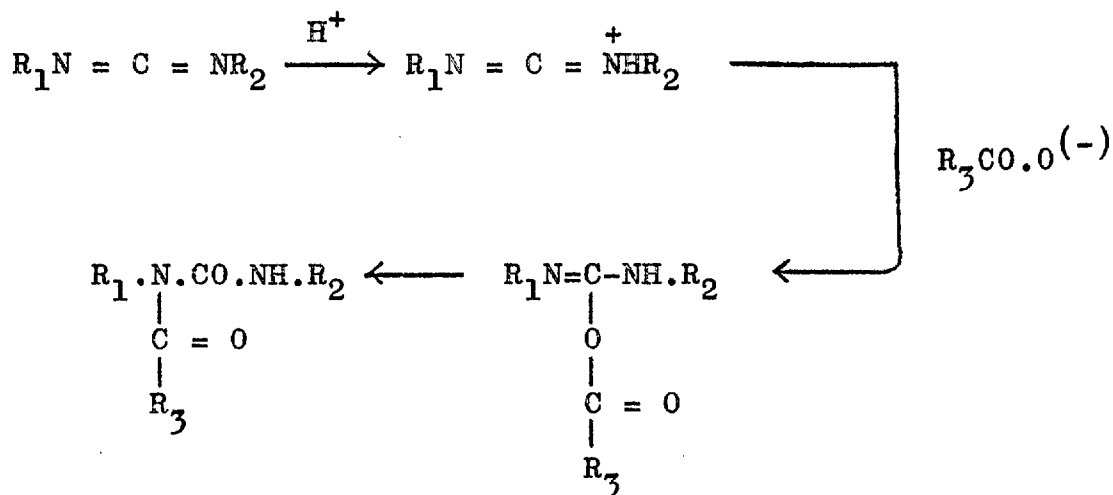


Racemization has been encountered with this method.

Carbodiimides.

This method^{156,157} must surely be the most popular coupling procedure used today. The coupling is performed in an inert solvent although it may be carried out in the presence of water. In general for one equivalent of α -acylamino acid there are employed 1 to 1.25 equivalents of the carbodiimide and 1 to 2 equivalents of amino acid ester. The three reactants are dissolved in a solvent (methylene chloride, acetonitrile are the best) and the reaction allowed to proceed at room temperature usually for 4 to 18 hours. The insoluble dicyclohexylurea crystallizes out. Although racemization has been encountered, it has been found that there is 40% to 100% retention of configuration. The other drawback of this method is the formation

of acylureas. It would appear that the solvent plays a part in the tendency to form acylureas. Khorana used dioxan, tetrahydrofuran, chloroform and ether as solvents and always obtained acylureas as by-products,¹⁵⁶ but not in methylene dichloride or acetonitrile.¹⁵⁷ Low temperatures, it would appear, suppress acylurea formation.¹⁵⁸



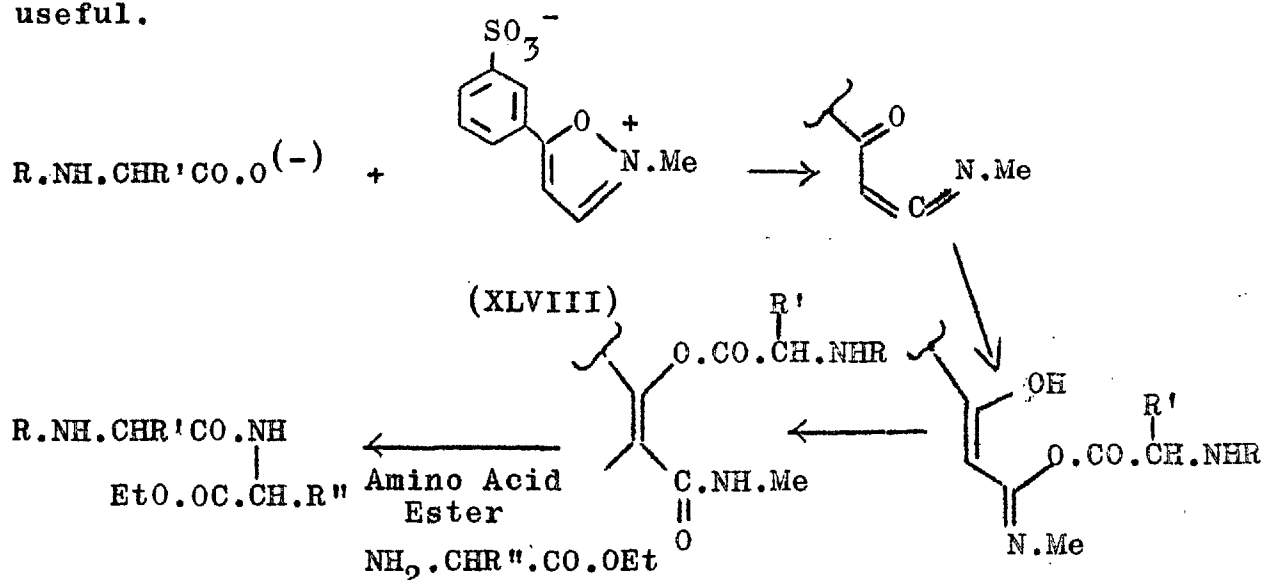
an acyl urea

The relevant literature of this method can be found with the following references^{156,159,160}.

Isooxazolium Salt method.

Woodward and his co-workers¹⁶¹ found that enol esters could be produced by the action of carboxylate ions with isoxazolium salts. This reaction has been used for peptide synthesis.¹⁶² N-Ethyl-S-phenyl-isoxazolium-3'-sulphonate (XLVIII) reacts with N-protected amino acids to give the enol esters, which are not isolated, but are reacted with the

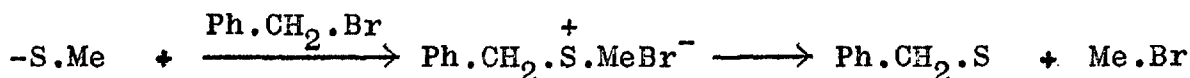
amino acid. The mild conditions and rapid reaction, little racemization and good yields could make this method very useful.



Protection of groups other than α -amino or α -carboxyl.

The mixed anhydride procedure for coupling interferes with the phenolic group of tyrosine, so the tosyl,¹⁶³ carbobenzyloxy,¹⁶⁴ and acetyl¹⁶⁸ derivatives have been used. With the dicyclohexylcarbodiimide method no protection is required. Alcoholic groups are usually insufficiently reactive to warrant protection when used in coupling reactions involving the carbodiimide, azide or activated ester method. However, in the case of serine, the O-acetyl compound¹⁵⁷ has frequently been prepared. Thiol groups must be protected and usually the benzyl group is chosen.¹⁶⁴ The benzyl group can be removed by using sodium in liquid ammonia.¹⁶⁵ Although, because of its acidity and

oxidizability the thiol group has to be protected, the S.Me group of methionine is stable. The only precaution that needs to be taken is when removing N-benzyloxycarbonyl groups by the action of hydrogen bromide, because of the possibility of alkyl exchange with the benzyl bromide liberated.¹⁶³ The



protection of amino groups is also essential. If both N-protecting groups can be removed simultaneously after coupling the α -carboxyl group, then the same group can be used to protect the two amino functions. However, if only one amino group is required to be protected then two ways are open, namely monosubstitution, or disubstitution and then removal of one of the protecting groups. It will have been noted that several of the N-protecting groups, given in a previous section, are stable in one medium while another was not; obviously the correct combination of protecting groups has to be worked out. Monoacylation of an amino group other than the α -amino group is effected by the use of the copper chelate; there are several examples of this method in the literature. The γ -carboxyl group of glutamic acid can also be preferentially protected by using the copper complex, with benzyl chloride.¹⁶⁶ The γ -methyl ester of glutamic acid has been prepared¹⁶⁷ using methanol and thionyl chloride at a low temperature.

The above methods for protecting groups other than the α -amino or α -carboxyl functions are only some of the methods available. The reader is directed to the reviews on this and the coupling reactions by Fruton,¹⁰⁸ Goodman et al.,¹²⁴ Rydon,¹²⁵ and Albertson.¹⁴⁰

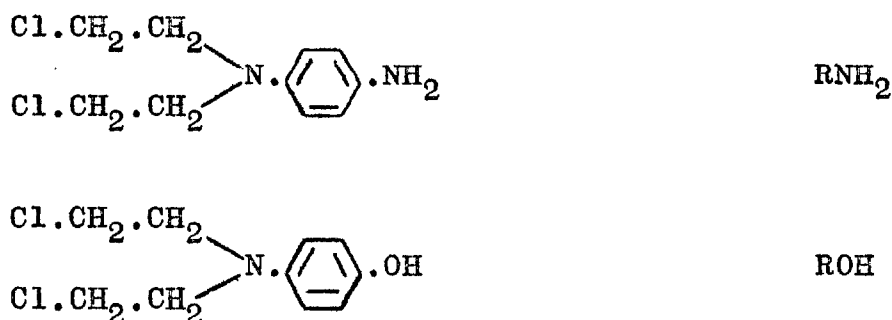
CHAPTER V

THE SYNTHESIS OF AMINO-ACID NITROGEN MUSTARD DERIVATIVES
DESIGNED TO BE POTENTIALLY SELECTIVE FOR TUMOURS

The experimental work described in this thesis is a continuation and development of the previous studies made in this Department by Owen et al.^{12,13,169} for the preparation of drugs with increased specificity towards tumours.

It has been noted in Chapter I that there are many biological and chemical variables which can be exploited in order to increase the specificity of a drug, one of which, facilitated diffusion, applies to amino acids, which are actively transported through cell membranes. Because a tumour usually loses its differentiation, and because its rate of growth is faster and less controlled than that of a normal cell, it would seem reasonable that the utilization of amino-acids would be greater by a tumour cell than that of a normal cell from which it had been derived. For these reasons amino-acids carrying a nitrogen mustard residue have been prepared, since they might be absorbed in greater quantities in the tumour than in the normal tissue in which it is found. As mentioned in Chapter II, amino-acid derivatives have already been described by other workers,^{169a} but those prepared by the present author were generally of a novel type.

It is already known that the nitrogen mustards RNH_2 and ROH^{x} have good anti-tumoural properties, but suffer from the disadvantage of being highly toxic, i.e. their C.I. is low.



It will be recalled, however, that groups which withdraw electrons from the tertiary nitrogen atom effectively reduce their toxicity. Thus amino-acids attached to these two nitrogen mustards via the peptide, ester, urea or carbamate linkage would be expected to detoxicate the nitrogen mustard and to be actively transported across cell membranes. At the tumour site, enzymes present or induced by potentiation might then split the linkage between the amino-acid and nitrogen mustard and release the free alkylating agent.

^xIn this and the following Chapter, the aromatic nitrogen mustard radical will be abbreviated to R, when it is the normal di-(2-chloroethyl) type. The analogous dibromo-compounds will carry the abbreviation R'.

Further specificity was hoped to be attained by using various protecting groups on the free α -amino function and so require utilization of other enzymes to remove them. Because carbamates are known to possess cytotoxic properties, formation of this particular type of protecting group could be an additional advantage. However, it was realized that any such increased specificity would most probably be at the expense of reducing the ability of the drug to be transported preferentially across a cell membrane.

It will be remembered that various amino-acid antagonists have anti-tumoural effects. Thus a compound containing both a nitrogen mustard and an amino-acid antagonist could prove useful, provided that the correct enzymes are present in the cell to release the two active portions. Preparation of several such compounds are described.

Derivatives of DL-Ethionine.

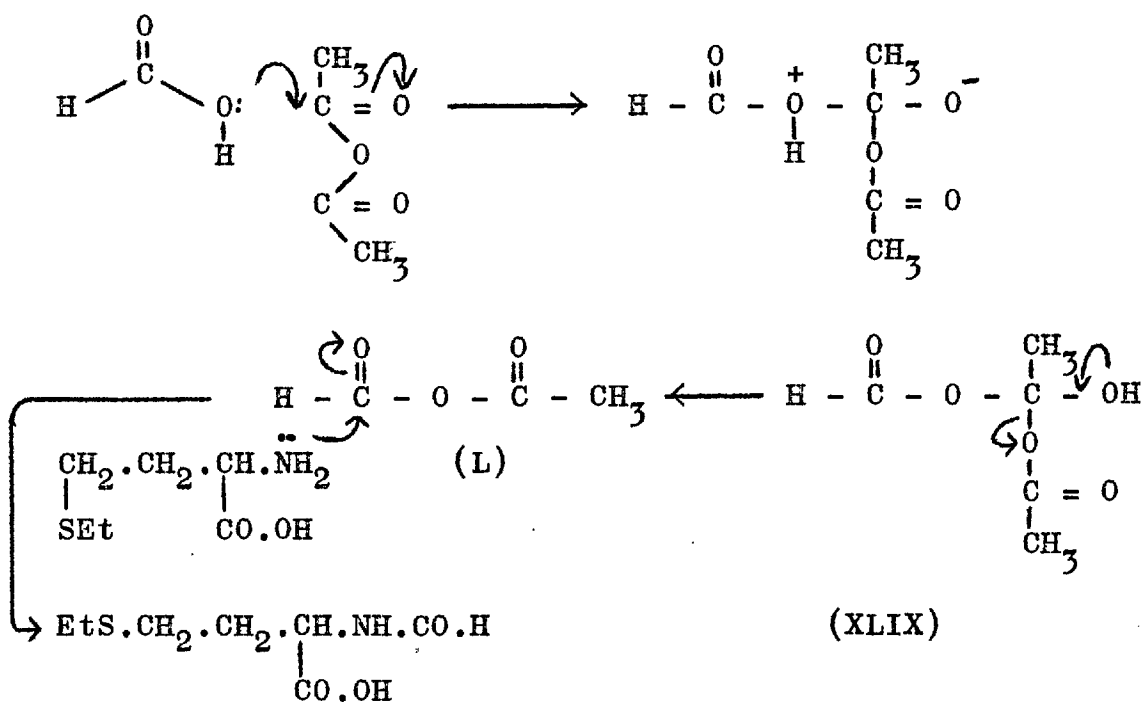
An amino acid is involved in a number of different metabolic functions, and it is expected that its analogues (e.g. ethionine is an analogue of methionine) are at least potentially capable of exerting effects at several sites, probably, however, with varying degrees of effectiveness, and so there is no reason to suppose that they are absolutely excluded from any pathways of amino acid metabolism.

Ethionine is known to be metabolized in the rat, and has been

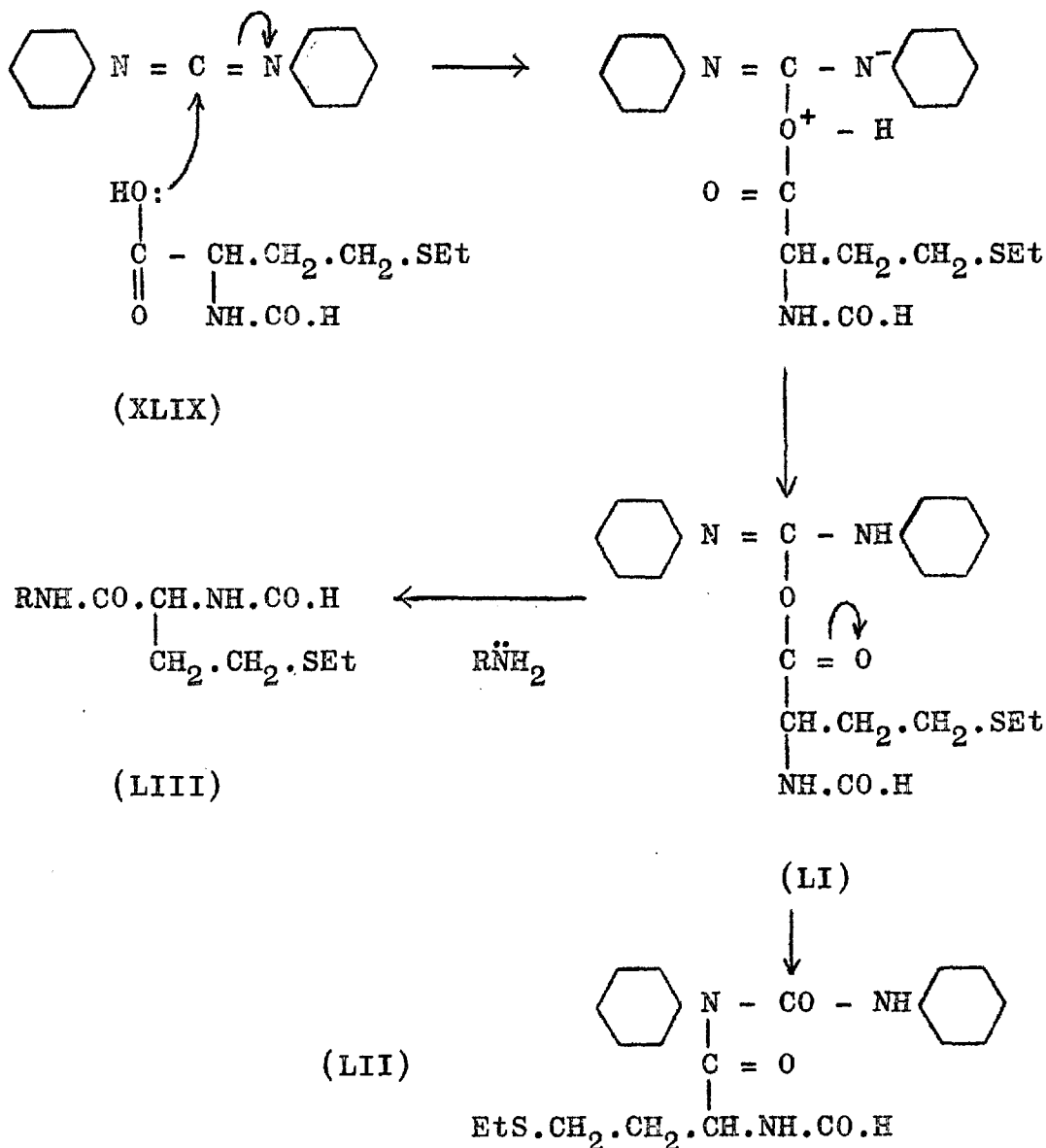
found to inhibit growth and/or protein synthesis, by taking the place of methionine in a number of reactions. It has, however, some toxic effects which are most probably due to the formation of ethyl analogues of essential metabolites, or may be due to synthesis of proteins containing ethionine and so interfering with the pattern of the proteins. For these reasons DL-ethionine was chosen to be one of the amino acid carriers of the nitrogen mustard RNH_2 .

As has been seen (Chapter IV) it is necessary to protect one of the functional groups of an amino acid in order to form the peptide bond with the other; here the N-formyl and N-methoxycarbonyl groups were used.

N-Formyl-DL-ethionine (XLIX) was prepared from 90% formic acid and acetic anhydride, the formylating agent probably being the mixed anhydride (L).

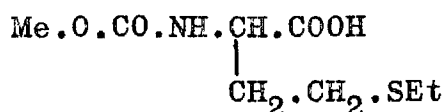


The formylated derivative (XLIX) was obtained as an oil and used directly in the coupling procedure. The mixed anhydride method of preparation was not examined because of the reported low yields,¹¹⁴ and so the NN'-dicyclohexylcarbodiimide method was used. The formyl derivative was dissolved in chloroform and had added to it the amino nitrogen mustard (RNH_2) which was in the form of its hydrochloride. The nitrogen mustard was freed from its salt in situ by the addition of 1 equivalent of triethylamine; heat was evolved and it was necessary to cool the reaction mixture to room temperature before addition of the NN'-dicyclohexylcarbodiimide in order to suppress the acylurea formation. Volumes were kept to a minimum in this and in all subsequent preparations in which the carbodiimide method was used. This is because the reaction of the free amino group with the N-formyl-DL-ethionine-carbodiimide adduct (LI) is intermolecular whereas the rearrangement of the adduct to the acylurea (LII) is intramolecular. Thus minimum volumes of the solvent should favour formation of the peptide intermediate.¹⁷⁰

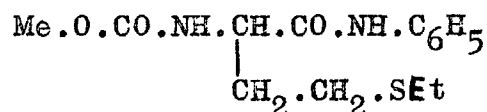


The crude product was chromatographed on alumina, a procedure always followed for all the compounds prepared by this method, in order to remove any acylurea formed as a by-product. In this way the pure compound (LIII) was obtained. The yield from this particular reaction was not very high, most probably due to the impurity of the N-formylated amino-acid.

The compound (LIV), containing the carbamate group, was prepared as an oil from DL-ethionine and methyl chloroformate, using standard Schotten-Baumann conditions. It was characterized as the anilide (LV) which was prepared by using the carbodiimide coupling procedure.

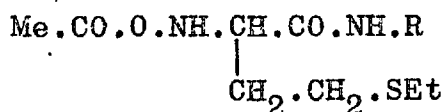


(LIV)



(LV)

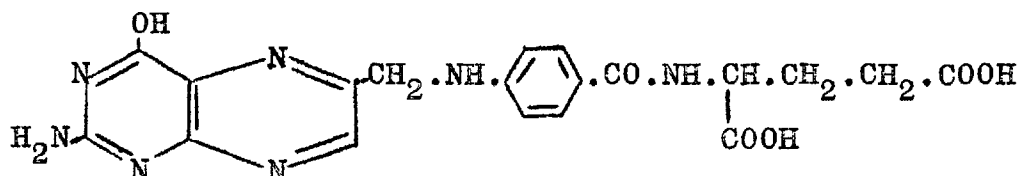
The nitrogen mustard derivative of (LIV) was also prepared using this coupling procedure for formation of the peptide bond, and gave (LVI) in 33% overall yield calculated from the starting quantity of DL-ethionine. This was higher than that for the formyl derivative (LIII).



(LVI)

Derivatives of L-Glutamic Acid.

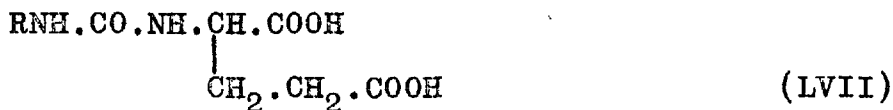
Folic acid (pteroylglutamic acid) is a vitamin which plays an essential part in the production and maturation of red blood cells.



Folic Acid (Vitamin Bc)

It has been found that replacement of glutamic acid by aspartic acid affords pteroylaspartic acid, which is a competitive antagonist of folic acid.¹⁷¹ Thus it can be seen that L-glutamic acid is an important precursor of Folic acid, and that if a nitrogen mustard residue was attached to the α -amino group of L-glutamic acid, sites which utilize the amino acid for the folic acid synthesis might release the free nitrogen mustard. It is, of course, realized that L-glutamic acid is found in other organs, e.g. brain, kidney and liver, but since the turnover of red blood cells is so high it would seem reasonable to suppose that the chances of administered L-glutamic acid or its derivatives of being incorporated into folic acid would be quite high.

It is a known fact that the urea linkage is relatively stable in the body, there being few naturally occurring enzymes for its hydrolysis; thus a nitrogen mustard linked to the L-glutamic acid moiety via such a linkage, e.g. (LVII) would probably have low activity, this perhaps being



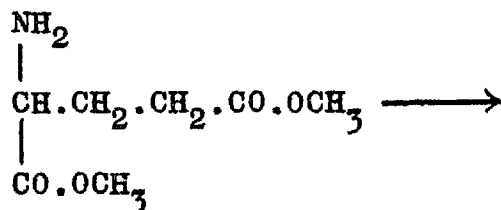
an advantage in that the drug could have a better chance of reaching its destination without premature hydrolysis occurring some distance away from the important site. For such a drug to be activated, however, it would probably be necessary to potentiate its action by prior induction of

a suitable enzyme at the tumour site.

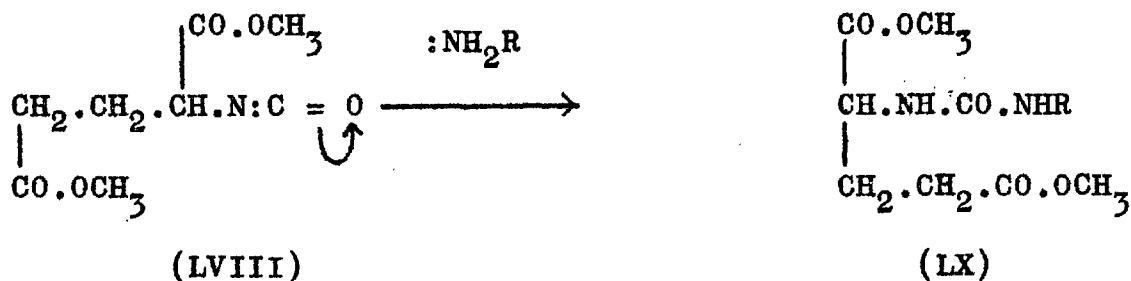
Another important aspect of this type of compound is that it has solubilizing groups available in the form of the α - and γ -carboxyl groups, because drugs which are soluble at physiological pH, or at the lower pH of neoplastic tissue, could have advantages over those which are not. Thus they could be rapidly transported to the site of action, although due to their ionized condition they may find difficulty ⁱⁿ ~~of~~ being transported through cell membranes. They would also be less likely to be precipitated in vivo, and they would also be more suitable for clinical administration.

Bearing these points in mind it was considered worthwhile that the drug (LVII) should be synthesized.

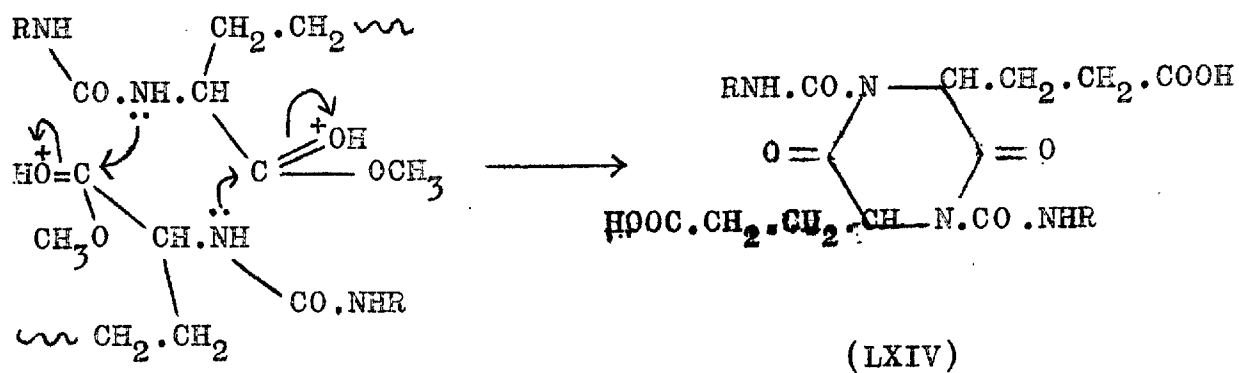
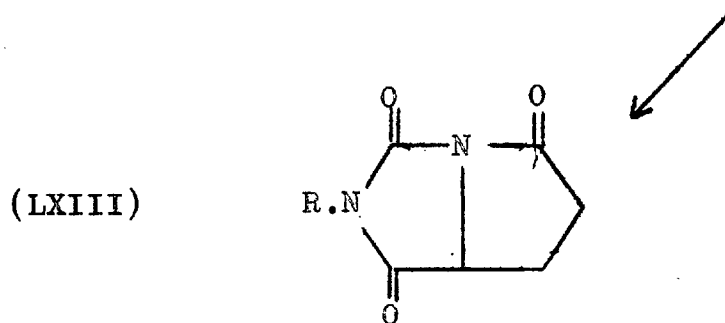
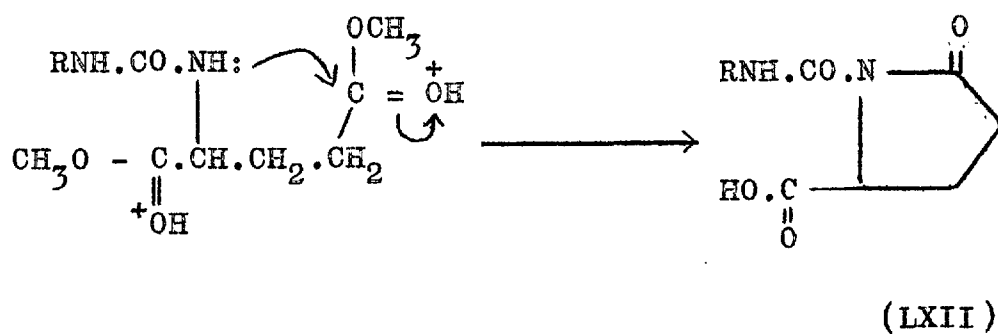
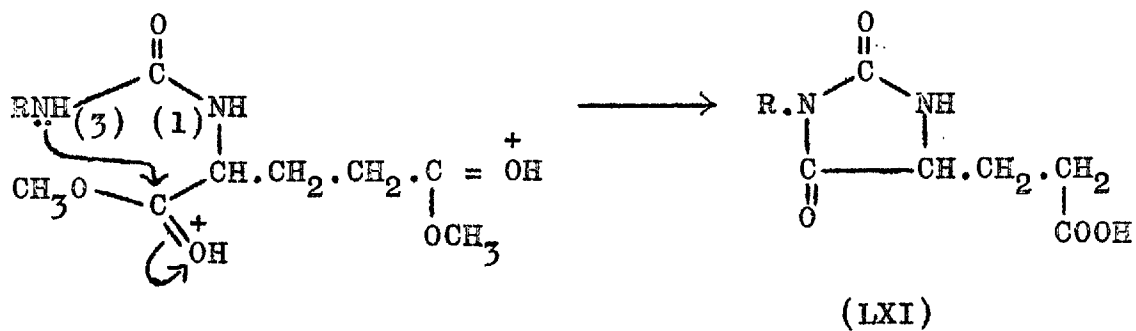
The dimethyl ester hydrochloride of L-glutamic acid was prepared by the method of Fischer¹⁷² and suspended in dry chloroform saturated with carbonyl chloride. The isocyanate¹⁷³ (LVIII) was obtained by boiling under reflux for 12 hours; the yield varied with the quantity of starting material, which could possibly be due to the formation of 5-methoxycarbonyl-2-pyrrolidone (LIX) the production of which appears to be quite easy.^{173,174} The isocyanate (LVIII) was characterized as its methylurethane derivative, by reaction with methanol.



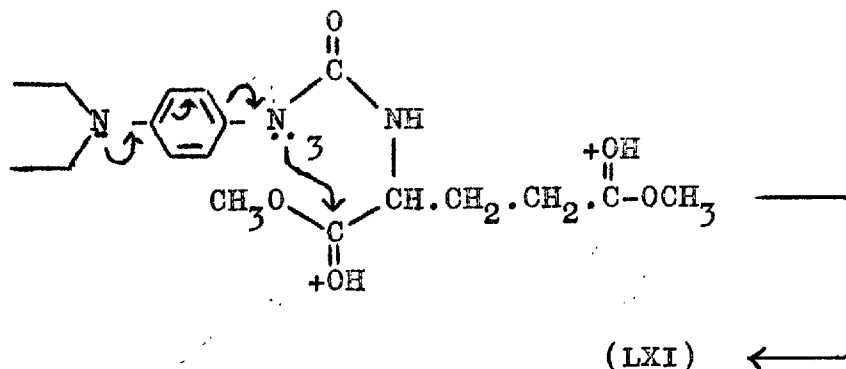
(LIX)



The isocyanate was reacted with the amino nitrogen mustard (RNH_2) in boiling ether-acetone (3.5:1) for 24 hours to give the urea (LX). Hydrolysis of this compound should have given the required product (LVII), but when it was boiled in a mixture of concentrated hydrochloric and glacial acetic acids for 25 minutes the product had an equivalent weight of 369 and was obviously not (LVII) which would require only half this value. Cyclization of some kind had obviously occurred.



The product was easily soluble in sodium bicarbonate solution and so the structure (LXIII) is excluded. A molecular weight determination gave a value which was half that required for the diketopiperazino compound (LXIV) so this was also excluded. However, this still leaves structures (LXI) and (LXII), both of which would fit the analytical figures. The infrared spectrum of the product showed bands at 1770 cm^{-1} ($-\text{CO}-\overset{\text{I}}{\text{N}}-\text{CO}-$) and 1713 cm^{-1} (COOH), but no bands in the region applicable to the secondary amide II band ($-\text{NH}-$) or the urea ($-\text{NH.CO.NH}-$), but which occurred in the dimethyl ester (1660 cm^{-1}). It is known that the amide II band of a secondary amide is not seen in cyclic structures (i.e. LXI) but is seen in open structures (i.e. LXII); also, comparison of the spectrum with hydantoin and substituted hydantoins confirms the structure as being of the form shown by the compound (LXI). This is quite an interesting result because there is no reason to think that the α -methyl ester is protonated before the γ -ester; thus it would appear that the nitrogen atom (3) is more electronegative than the nitrogen atom (1) due to the feed-back of electrons from the benzene ring. There is, of course, no complementary donation of electrons from the asymmetric carbon atom to the nitrogen atom (1).

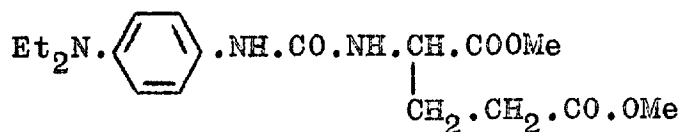


The compound was optically active and so no tautomerism had occurred under the conditions employed, before ring closure or afterwards, although Dakin¹⁷⁶ found that racemic hydantoins could be prepared under acid conditions

It has been found^{177,178} that substituted hydantoins can be converted by the body into their respective amino-acids. It is hoped that this will apply to (LXI).

It has been noted that dimethyl ester glutamate compounds have been easily hydrolysed, under alkaline conditions, without cyclization.¹⁷⁵ Acid conditions were used here because of the liability of the halogen atoms of the nitrogen mustard residue to hydrolyse under alkaline conditions, although there are exceptions to this general rule.

As has already been mentioned, the urea linkage is not very susceptible to hydrolysis in the body due to the lack of naturally occurring enzymes for this particular task. Thus, in order to produce an adaptive enzyme to potentiate the compound (LXI), the model (LXV) was produced by heating



(LXV)

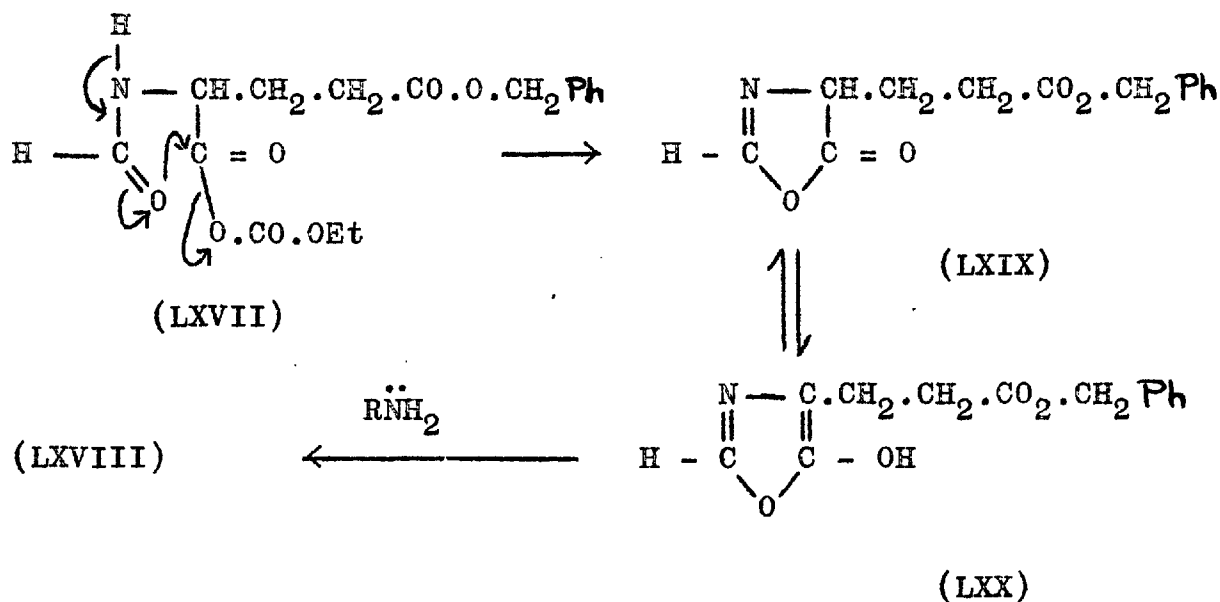
the isocyanate (LVIII) with NN-diethyl-p-phenylenediamine.

All the above compounds were optically active, but no attempts were made to find if they were optically pure; it would seem most unlikely that they were.

Another way of approaching this subject of utilizing folic acid synthesis for the release of, or the incorporation of, the cytotoxic agent one is using, would be to attach the nitrogen mustard residue to the α -carboxy group of glutamic acid. The α -amino group could be incorporated into the folic acid molecule, assuming of course that there is not any alkylation of the pteroyl system first, and so giving a nitrogen mustard with folic acid as its carrier.

To achieve this aim it is necessary to protect the γ -carboxyl and the α -amino functions of glutamic acid, and then couple the α -carboxyl group with the amino nitrogen mustard (RNH_2). The γ -benzyl ester of L-glutamic acid¹⁶⁶ was prepared using copper carbonate to form a chelate with the α -amino and carboxyl functions^{179,180,181} leaving the γ -carboxyl group free to be esterified with benzyl chloride. The copper complex was removed with hydrogen sulphide and the α -amino group formylated using 90% formic acid and

Racemization is very prevalent when the mixed anhydride method is used, when forming peptides protected by N-acyl group. Many examples can be found in the literature,^{184,185,186} and a review is given by Neuberger.¹⁸⁷ It has been found that racemization involves azlactonization, since Carter and Stevens¹⁸⁵ have managed to isolate the intermediate azlactone. Thus the mixed anhydride (LXVII) is envisaged as forming the azlactone (LXIX) which enolizes to (LXX) but which can still react with the amino nitrogen mustard RNH_2 to give (LXVIII)



The first step is the formation of the azlactone, the rate of which will depend on the activation of the carboxyl, as well as the acyl functions attached to the α -amino group. The use of mixed anhydrides derived from an N-formyl amino acid and another acid stronger than carbonic, such as

a carboxylic acid, would be expected to favour azlactonization. The azlactone will most probably be optically active when it is formed, but racemization will depend on the relative rates of ionization and of hydrolysis of the ring. Thus racemic products will be obtained if ionization is faster than hydrolysis of the ring.

It has been found by some workers that proteins obtained from malignant tumours contained a relatively large proportion of D-glutamic acid.^{188,189,190} If this is so, it would seem reasonable that the D-form of the racemic mixture of (LXVIII) would tend to find its way to the tumour site and be rapidly utilized, because normal cells use the unnatural isomer only slowly.

The γ -benzyl ester was used because of its reported¹⁷⁸ ease of removal by hydrogenolysis, but when this was attempted with the mustard derivative (LXVIII) over Adams' catalyst and 5% palladium-charcoal, the product was found to be starting material. This was concluded from comparison of the infra-red spectra of both starting and product materials. The product was also insoluble in saturated sodium bicarbonate solution.

Derivatives of Glycine.

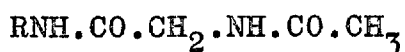
The use of small molecules in the biosynthesis of many of the complex substances found in living organisms is now

well known. Glycine is a precursor of a wide variety of nitrogenous compounds of great physiological importance; it is known to be converted into serine, glutathione, proteins, porphyrins, uric acid and nucleic acid purines.

In most animals the internal organs, with the exception of the heart, are relatively rich in glycine, the greatest concentration occurring in the liver, lungs and kidneys.

One of the metabolic pathways of glycine is its interconversion to serine by an active form of folic acid. Thus it may be postulated that if the active form of folic acid was alkylated then the interconversion of glycine to serine could be stopped. However, since glycine is a precursor for a large number of nitrogenous compounds, and because nitrogen mustard derivatives of it may be actively transported across cell membranes, a number of glycine derivatives have been prepared. Various N-substituents have been used on the α -amino group, in order to confer more specificity to the drugs.

N-Acetylglycine¹⁹¹ reacted with ethyl chloroformate to form the mixed anhydride. This in turn reacted with the amine nitrogen mustard (RNH_2) to form (LXXI). The solvent used was NN-dimethylformamide which generally gives impure products; the present case was no exception, although the yield was 64%.



(LXXI)

The reaction was repeated using NN'-dicyclohexylcarbodiimide as the condensing agent in dichloromethane but since N-acetylglycine is only sparingly soluble in this solvent, the heterogeneous reaction did not give such a good yield (50%), although the product was far cleaner. The normal precautions of cooling the mixture after the liberation of the free amino nitrogen mustard from its hydrochloride with triethylamine were taken, in order to reduce the amount of acylurea formed. Another reason for the low yield could be that the solvent volumes were comparatively large, and since this reaction involves a competition between an inter- and an intra-molecular process large volumes of solvents should be avoided. A mixed melting point of the peptides made by the two methods showed no depression.

N-Formylglycine was prepared by the method of Sheehan et al.¹¹⁴ and condensed with the amino nitrogen mustard (RNH_2) via the mixed anhydride formed with ethyl chloroformate in NN-dimethylformamide to give the peptide (LXXII). Racemization problems of course do not arise with glycine and so the length of time (20 minutes) for the preparation of the mixed anhydride does not matter from this point of view. However, it is obviously long enough for some reaction with the solvent to occur¹⁸³ and lower the yield and give an impure product (cf. the N-acetyl compound).

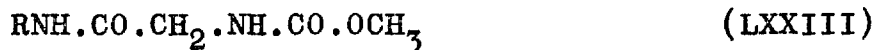


(LXXII)

The reaction was repeated using NN'-dicyclohexylcarbodiimide as the condensing reagent but due to the insolubility of N-formylglycine in dichloromethane a mixture of solvents was used, though the volume of reaction mixture was still large. Although one of the solvents in the mixture was NN-dimethylformamide, this method gave a far cleaner product and a yield of 65%. Mixed melting points of the samples of the peptide (LXXII) from both methods of preparation showed no depression.

As already mentioned, the urethane group is of special interest and it was considered worthwhile to include this moiety in one of the glycine nitrogen mustard derivatives.

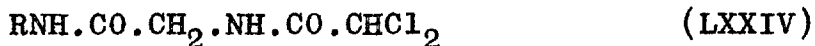
N-Methoxycarbonylglycine¹⁹² was prepared using normal Schotten-Baumann conditions. It was converted into the mixed anhydride with ethyl chloroformate in NN-dimethylformamide which was then reacted with the amino nitrogen mustard (RNH_2) to give the peptide (LXXIII). The yield (77%) was better than that for the N-acetyl and N-formyl derivatives prepared by this method, even though the conditions were the same.



It has been reported¹⁹⁴ that the N-dichloroacetyl derivative of serine has some anti-tumoural activity, and so it was thought interesting to produce the N-dichloroacetyl derivative of glycine and couple it to the amino nitrogen mustard RNH_2 , via the peptide bond. Thus hydrolysis of the

peptide bond (if this were to occur first) would release both the mustard and N-dichloroacetylglycine which may have comparable properties to that of the serine analogue. Or the dichloroacetyl group might be hydrolysed off first, leaving the free glycyl nitrogen mustard moiety to be actively transported to the sites which utilize glycine. It is interesting also to speculate whether dichloroacetic acid could be incorporated into systems which use acetyl groups as their precursors, and so cause the production of anti-metabolites.

N-Dichloroacetylglycine was prepared by direct acylation of the amino-acid with dichloroacetyl chloride in ethyl acetate.¹⁹³ This was then coupled to the amino nitrogen mustard (RNH_2) by the use of NN'-dicyclohexylcarbodiimide as the condensing agent, to give (LXXIV).

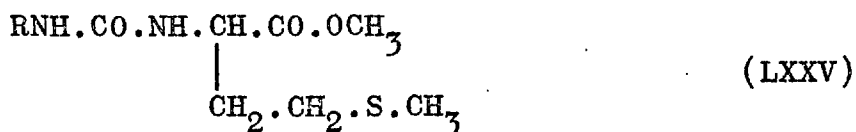


Derivatives of DL-Methionine.

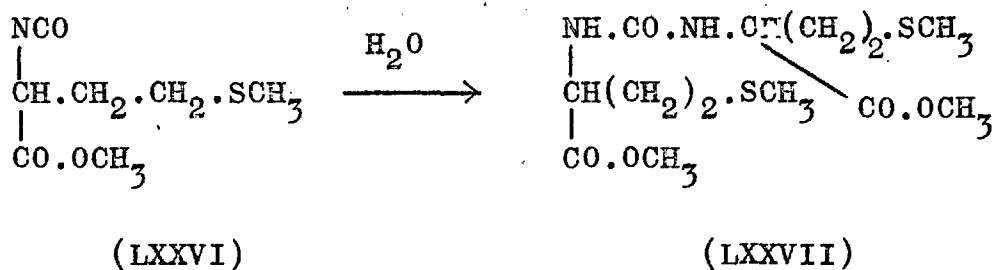
Of the various amino acids which have been used in this work for carrying the nitrogen mustard moiety only methionine is an 'essential' amino acid. Not only is it indispensable but both the D- and the L-form can be utilized by the rat. Thus it would seem reasonable that if a nitrogen mustard attached to DL-methionine was given to a tumour-bearing

rat which had been fed on a diet deficient in DL-methionine then the chances of the drug being utilized and of reaching the required sites should be greater.

As already noted, the urea linkage is not easily hydrolysed in the body, and so it was considered profitable to prepare a nitrogen mustard derivative of methionine containing this linkage and attempt to potentiate it biologically with a suitable precursor. Thus the compound (LXXV) was synthesized.



The methyl ester hydrochloride of DL-methionine was prepared, using methanol saturated with hydrogen chloride. The crude product was suspended in dry benzene saturated with carbonyl chloride and boiled under reflux to give the oily isocyanate (LXXVI) which was characterized as the urea (LXXVII).



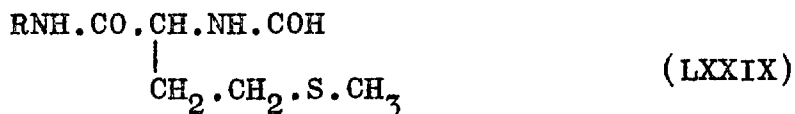
The isocyanate (LXVI) was then reacted with the amino nitrogen mustard (RNH₂) in ether to give the urea (LXXV).

The amino nitrogen mustard (RNH_2) was also linked to various N-substituted DL-methionine derivatives via the peptide linkage in order to confer more specificity to the resulting drug. Both the mixed anhydride and the carbodiimide procedures were used to perform the condensation.

N-Acetyl-DL-methionine¹⁹⁴ was prepared and used to form the mixed anhydride with methyl chloroformate in NN-dimethylformamide, 15 minutes being allowed for this. The nitrogen mustard was then added, and the crude product was chromatographed on alumina to give a 60% yield of the peptide (LXXVIII).

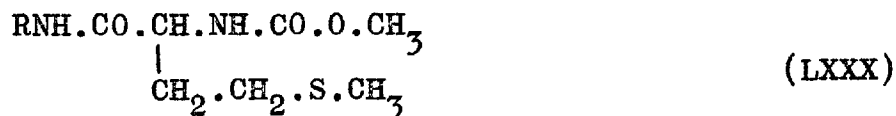
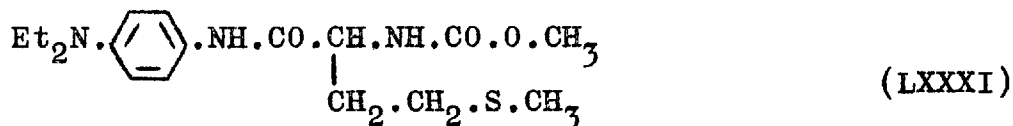


N-Formyl-DL-methionine was prepared by the method of Sheehan et al.¹¹⁴ and condensed, by formation of the mixed anhydride with ethyl chloroformate, with amino nitrogen mustard. The yield, as noted before for formyl derivatives was poor, but analysis confirmed that the correct product, i.e. (LXXIX) had been prepared.



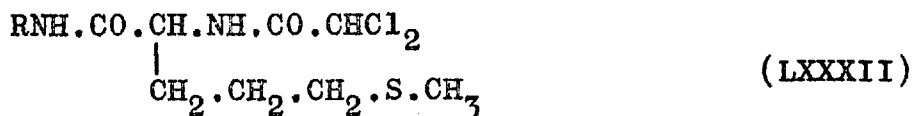
N-Methoxycarbonyl-DL-methionine was prepared using normal Schotten-Baumann conditions from methyl chloroformate and the amino-acid. The amino-acid now contains the

important carbamate linkage. Further to enhance the action of the nitrogen mustard derivative (LXXX) to be prepared from this compound it was thought to be of advantage to make the model compound (LXXXI) for potentiation of (LXXX).



Both compounds were synthesized from the unpurified N-protected DL-methionine, using the carbodiimide method to form the peptide linkage. The model compound was prepared in chloroform, the volume of which was kept small, but the yield was only 40%, probably because crude starting materials were used. The nitrogen mustard derivative was prepared in dichloromethane and the normal precautions were taken but the yield was also low (39%).

The dichloroacetyl derivative of DL-methionine was prepared¹⁹³ because it may have properties similar to those of N-dichloroacetylserine (see p.94). It was coupled with the amino nitrogen mustard using NN'-dicyclohexylcarbodiimide in nitromethane. The normal precautions were taken, and yielded the product (LXXXII).



It seems likely that tumour tissue is composed of a diversity of cell types each of which may require a specific kind of chemotherapeutic attack. Danielli¹⁹⁵ used a combination of two aromatic nitrogen mustards and found that very effective action on the tumours was obtained, and since the toxic effect on the host was distributed the overall damage was reduced. One of the compounds used was the monofluoroacetate of RNH_2 which caused fluoro-acetate poisoning involving the central nervous system, and so it was thought that the N-fluoroacetyl derivative of DL-methionine may be useful in combination with other drugs.

N-Monofluoroacetyl-DL-methionine was prepared by the action of monofluoroacetyl chloride on DL-methionine under Schotten-Baumann conditions, to give an oil, the infrared spectrum of which showed a band at 1680 cm^{-1} which is characteristic of the amide group. It was sufficiently pure to be coupled to the amino nitrogen mustard via the peptide linkage, which was formed by the use of NN'-dicyclohexylcarbodiimide, to give the required product (LXXXIII) in 63% yield.



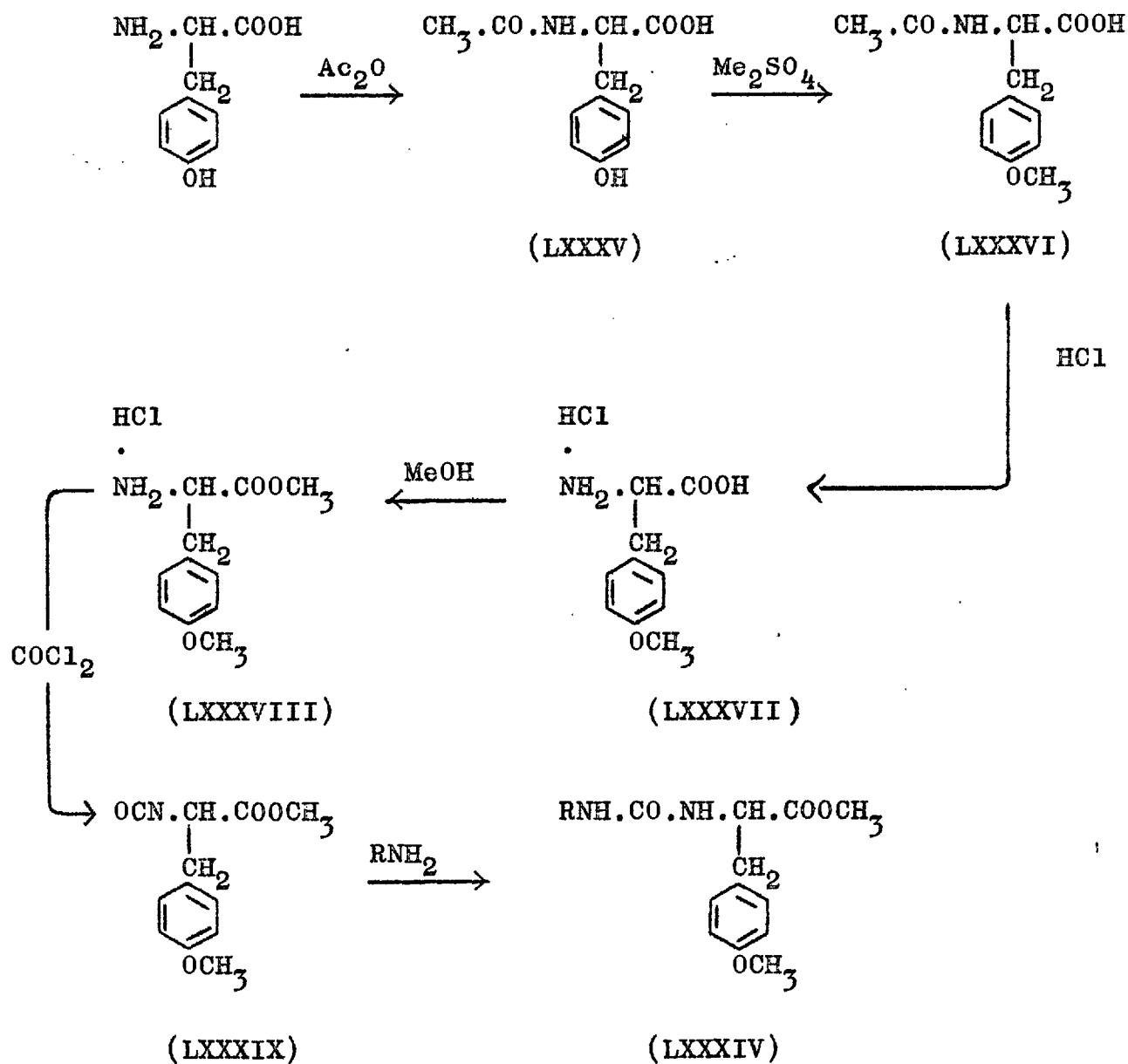
Derivatives of L-Tyrosine.

In the rat, phenylalanine is oxidized to tyrosine; the reverse does not occur even in phenylalanine deficient rats, and so tyrosine is not 'essential' if the phenylalanine intake is adequate. Thus it would seem reasonable to suppose that if a tumour bearing rat was fed on a diet deficient in phenylalanine it would most probably be deficient in tyrosine as well. If then a nitrogen mustard derivative carrying a tyrosine moiety was administered to the rat, then one might obtain greater specificity.

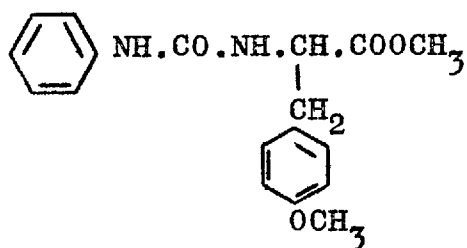
Several groups^{39,197,198,199} of workers have prepared methylated forms of amino-acids in the search for amino-acid antagonists in the metabolism of tumour cells. It would seem reasonable that if some common amino-acids were modified to contain a portion of a natural antibiotic, antimetabolites useful for cancer chemotherapy may result. O-Methyltyrosine is a constituent of the antibiotic puromycin and so it was considered worthwhile to use this moiety attached to a nitrogen mustard via the urea, carbamate, peptide, and ester linkages. The amino nitrogen mustard was also linked to N-protected tyrosine derivatives with free phenolic groups, via the peptide linkage.

As mentioned before the urea linkage is relatively stable in the body and so a L-tyrosine moiety linked to the amino nitrogen mustard via such a linkage would probably have

a low activity, but this could be overcome by potentiation with the correct drug. Thus (LXXXIV) was prepared by the scheme shown.



L-Tyrosine was selectively acetylated at the α -amino position by the conditions used, which were to warm the amino acid in the acetylating mixture on a steam bath, a few drops of detergent being used to aid solubility. The product (LXXXV) was obtained as a green gum, which was dissolved in sodium hydroxide, and reacted with dimethyl sulphate. The product (LXXXVI) was obtained by extraction into chloroform. It was deacetylated with 6N hydrochloric acid to give (LXXXVII)²⁰⁰ which was esterified by boiling under reflux with methanol saturated with hydrogen chloride. The crude product (LXXXVIII) was suspended in toluene, saturated with carbonyl chloride and boiled to give the isocyanate (LXXXIX), which was characterized by reaction with aniline to yield (XC).

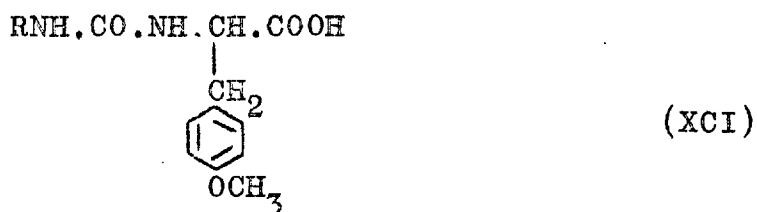


(XC)

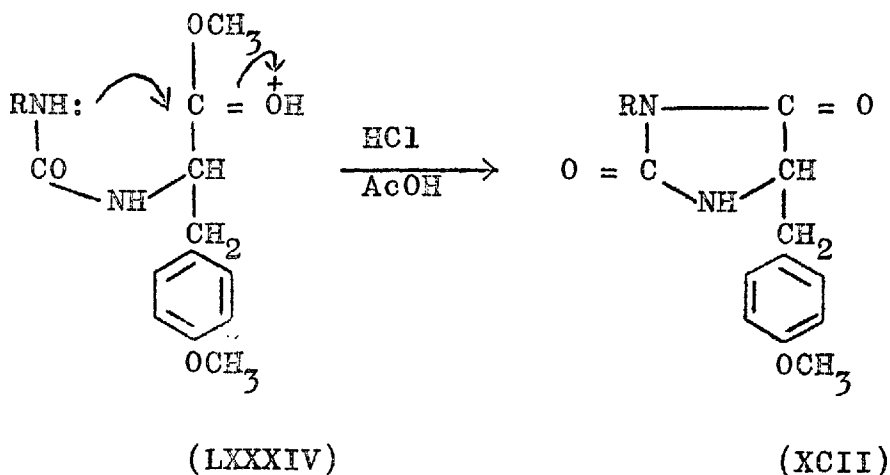
The amino nitrogen mustard (RNH_2) was reacted with (LXXXIX) by boiling under reflux for 1 hour in ether to give the required product (LXXXIV). This was optically inactive in acetone, although the intermediate (LXXXVII) could not have been completely racemized because other optically

active products were obtained from it (see p.106).

As mentioned before, solubilizing groups attached to a nitrogen mustard derivative confer certain advantages upon it (see p.83). Therefore, an attempt was made to hydrolyse the methyl ester, (LXXXIV), under acid conditions, to the free acid (XCI).

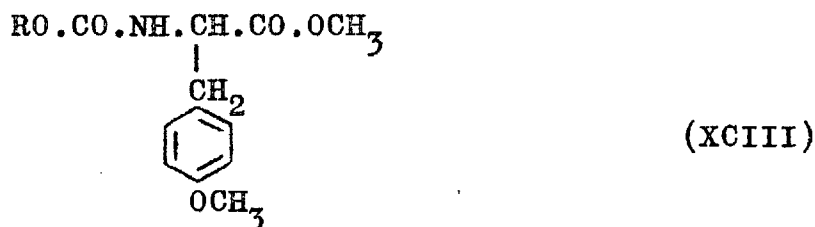


However, the product obtained was insoluble in saturated sodium bicarbonate solution and had an infrared spectrum which showed a band at 1770 cm^{-1} ($-\text{CO}-\overset{|}{\text{N}}-\text{CO}-$) and no secondary amide II band in the region $1500-1600 \text{ cm}^{-1}$. In fact the spectrum between 1500 to 1800 cm^{-1} was very similar to that of the hydantoin (LXI) p.86. Thus the hydrolysis product of (LXXXIV) was assigned the structure (XCII)



Because hydantoins are usually hydrolysed in the body to their respective amino-acids¹⁷⁷ it is hoped that this will apply to (XCII).

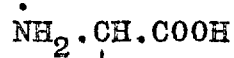
A nitrogen mustard derivative containing the carbamato linkage can sometimes be potentiated should the drug prove to be of low activity ^{and so} the nitrogen derivative (XCIII) could be interesting.



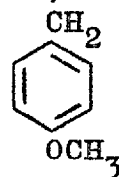
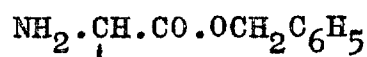
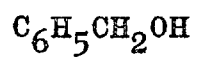
It was prepared by reacting the isocyanate (LXXXIX) with the phenolic nitrogen mustard (ROH) for 3 hours at 130-150° in a sealed flask. The resulting glass was very difficult to crystallize, but finally was obtained as a solid from benzene. Both this and the urea (LXXXIV) were found to contain benzene of crystallization and so whenever possible the succeeding derivatives of tyrosine were crystallized from solvents other than benzene.

Because of the optical inactivity of (LXXXIV) and (XCIII) and the ease with which the methyl ester of (LXXXIV) cyclizes to the hydantoin upon hydrolysis, it was decided to prepare the benzyl ester of (LXXXVIII) and synthesize nitrogen mustard derivatives from it. Hydrogenolysis as the final stage should then remove the benzyl ester.

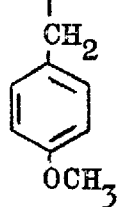
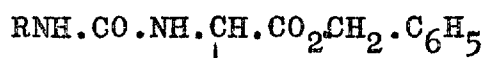
HCl



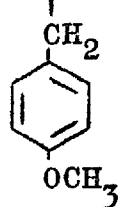
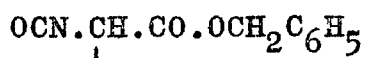
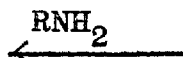
(LXXXVII)



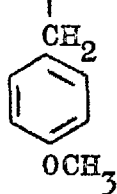
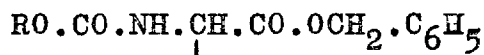
(XCIV)



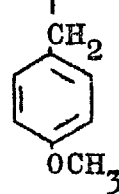
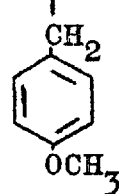
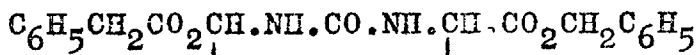
(XCVII)



(XCV)



(XCVIII)



(XCVI)

The reaction sequence followed is shown on p.105.

p-Methoxy-L-phenylalanine was released from its hydrochloride (LXXXVII) with ammonia, taken up in benzene and reacted with benzyl alcohol using p-toluenesulphonic acid as catalyst, to produce (XCIV) as the p-toluenesulphonic acid salt. The water formed during the esterification was removed using a Dean-Stark separator and the last traces by anhydrous silica gel in a Soxhlet tube. The p-toluenesulphonic acid salt was decomposed by washing with saturated sodium bicarbonate solution to give an oil which was taken up in chloroform and treated with hydrogen chloride gas to give the hydrochloride. This was suspended in toluene, saturated with carbonyl chloride and boiled under reflux, to give the isocyanate (XCV), which was characterized as the urea (XCVI) by reaction with water. The isocyanate was optically active.

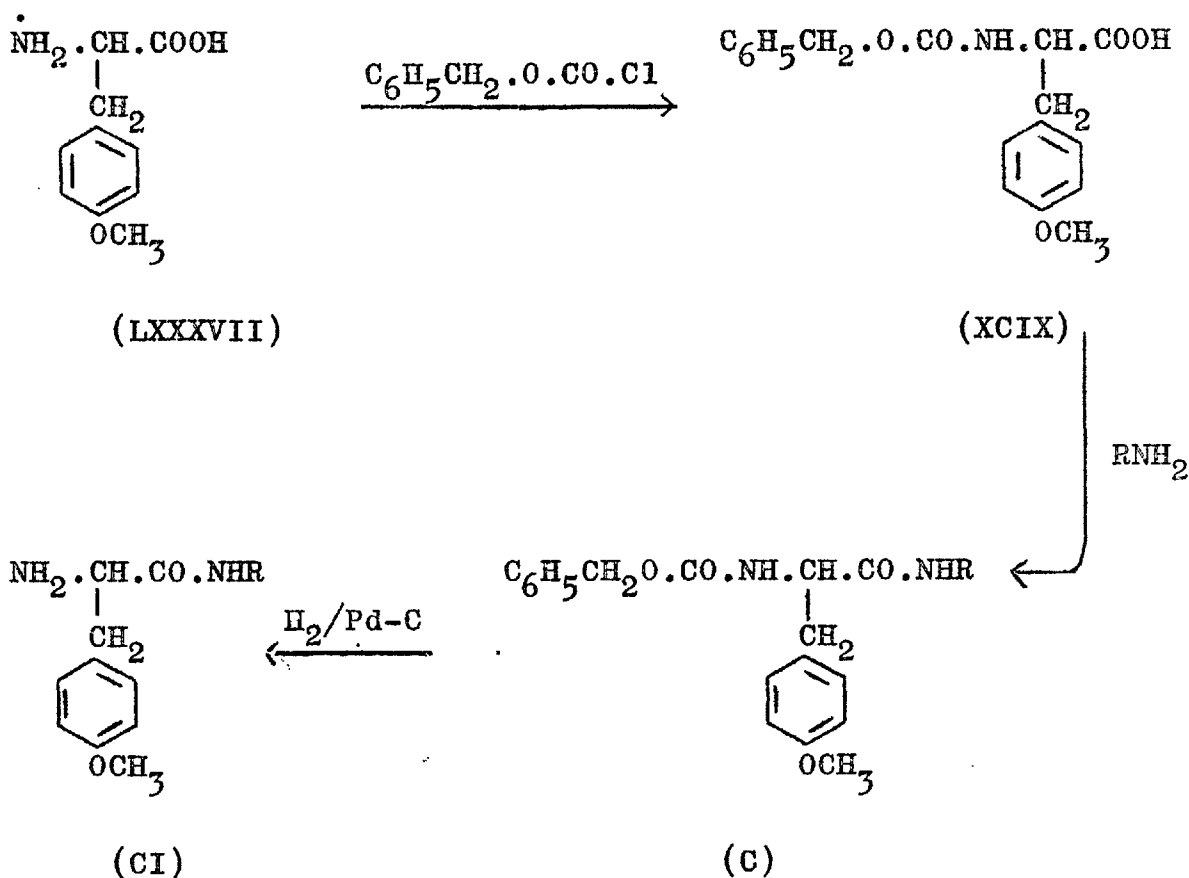
Reaction of the isocyanate (XCV) with the phenol nitrogen mustard (ROH) in boiling chloroform gave the carbamate (XCVIII). It was optically active and so the preceding compounds must also have been so (e.g. (LXXXVII); see p.103).

The urea (XCVII) was prepared by the action of the amino nitrogen mustard (RNH_2) with the isocyanate (XCV) in boiling ether.

Neither of these two nitrogen mustard derivatives (XCVII) nor (XCVIII) was obtained in sufficient quantity to proceed to the final stage, namely hydrogenolysis of the benzyl ester.

The nitrogen mustard moiety can also be attached to p-methoxy-L-phenylalanine via the peptide or ester linkage by first protecting the α-amino group with a function which could be later removed without breaking the nitrogen mustard linkage. The following reaction scheme was followed.

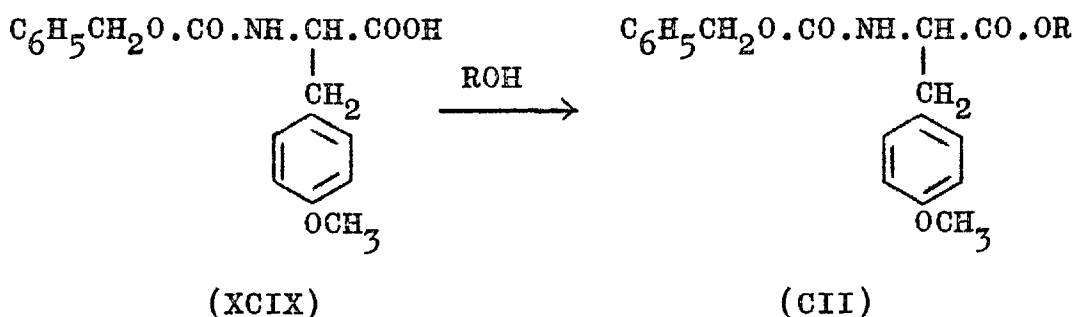
HCl



p-Methoxy-L-phenylalanine was obtained from its hydrochloride (LXXXVII) by the action of ammonia and reacted with benzyl chloroformate, under normal Schotten-Baumann conditions, to give the N-protected amino acid derivative (XCIX).²⁰¹ This reacted with ethyl chloroformate in NN-dimethylformamide to

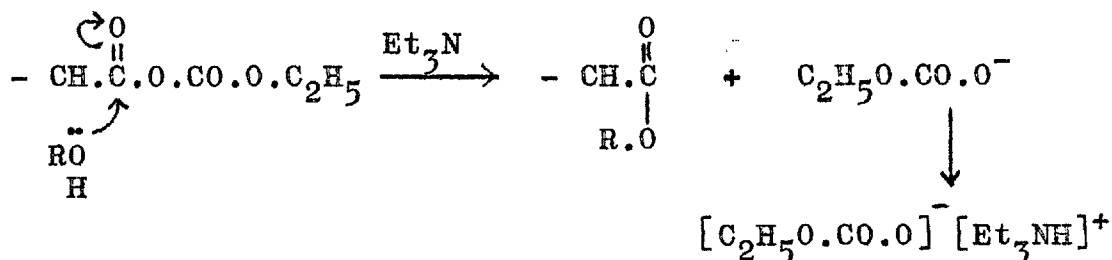
give the mixed anhydride (15 minutes reaction time) which was used to form (C) by reaction with the amino nitrogen mustard (RNH_2). Although the product was impure and the yield low (48%), some optical activity had been maintained, because when it was hydrogenolysed over 5% palladium charcoal catalyst in ethyl acetate the product (CI) showed optical activity. Unfortunately it was crystallized from benzene; and as has been mentioned before, these compounds tend to crystallize with benzene of crystallization.

No nitrogen mustards linked to the amino-acid moiety via the ester linkage have been described so far; the opportunity was taken to correct this omission.



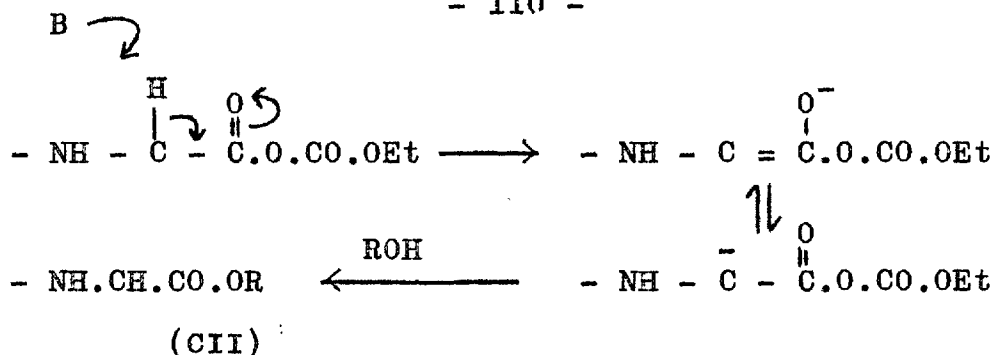
The N-protected derivative (XCIX) was reacted with ethyl chloroformate to form the mixed anhydride in NN-dimethylformamide, 20 minutes being allowed for this to occur. It was then used to produce the compound (CII) by reaction with the phenol nitrogen mustard (ROH). An extra equivalent of triethylamine was used in this reaction in order to restrain

the ethyl carbonate ion from breaking down to ethanol, which would then react with the mixed anhydride, e.g.



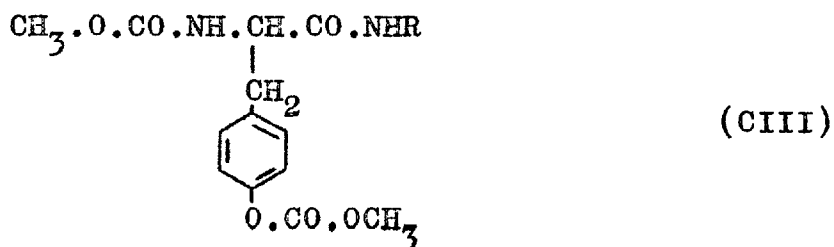
This procedure of reducing unwanted side reactions has been used by Johnson.²⁰²

The compound (CII) was found to be optically inactive, which is not usual when the amino function is protected by the N-benzyloxycarbonyl group,^{203,204} because of its reluctance to form azlactones. Thus we have a situation where the same mixed anhydride has been formed and it has been reacted with RNH_2 to form an optically active product (C) and also reacted with ROH to produce an optically inactive product (CII), the latter condensing in a medium which is only different from the former by the addition of one extra equivalent of triethylamine, and being left for a further five minutes at the stage at which the mixed anhydride is formed. The mechanism by which the optical activity is lost could be visualized as follows:



B denotes a base. The solvent was NN-dimethylformamide which should be sufficiently polar to allow some form of ionization. The base is most probably the excess triethylamine which, although not a good nucleophile, is a good extractor of protons provided that they are sufficiently acidic, as it is thought to be in this case.

A nitrogen mustard derivative of L-tyrosine where the α -amino and the phenolic functions were both protected by groups which would require specific enzymes to remove them, (and thereby increase the specificity of the drug) was thought worthwhile to prepare. Thus the compound (CIII) was synthesized, the carbamato linkage being chosen for reasons which have been expounded previously in this thesis.

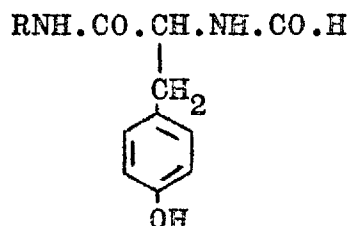


Bis-N,O-dimethoxycarbonyl-L-tyrosine²⁰⁶ was prepared by the action of methyl chloroformate on the amino-acid under

Schotten-Baumann conditions. The product was obtained as an oil which was condensed with the amino nitrogen mustard (RNH_2) by the use of NN'-dicyclohexylcarbodiimide to produce the compound (CIII) which was optically active.

In order to have tyrosine derivatives with a free phenol group, which may aid facilitated diffusion, the production of the peptide bond must be done using a method which does not interfere with this group. The carbodiimide method is ideal for this.

N-Formyl-L-tyrosine was prepared by Fischer's method²⁰⁷ using 98% formic acid which gave the derivative containing 1 mole of water of crystallization. Because production of the peptide bond goes in better yield in anhydrous conditions the compound was dehydrated by heating under vacuum. However, the resulting product was very sparingly soluble in the usual solvents employed for this coupling procedure, so the peptide bond was formed in a heterogeneous system using NN-dimethylformamide as solvent. The yield of (CIV) was low, but optical activity had been maintained.



(CIV)

The overall conclusions which can be drawn from this work on amino-acid derivatives of nitrogen mustards containing the peptide linkage confirm the general points already noted in the literature, namely that the mixed anhydride method can lead to racemization, and that the use of the N-formyl derivative as the amino protecting group gives low yields and racemic products. NN-Dimethylformamide has been found to be a solvent tending to give impure products.

The NN'-dicyclohexylcarbodiimide method has been found to be far better for ease of synthesis, and cleanness of products, although care has to be taken over the removal of acylureas which may have been formed. With the optically active N-acylamino-acids which were used to be coupled to the amino nitrogen mustard (RNH_2) by this method, optically active products were obtained every time.

EXPERIMENTAL SECTION

(NN-Di-2-chloroethyl)-p-phenylenediamine hydrochloride RNH_2, HCl will be designated as nitrogen mustard A, and p(NN-di-2-chloroethylamino)phenol hydrochloride ROH, HCl as nitrogen mustard B. Two general methods were used on releasing the free amine or phenol from their salts:

I. A suspension of the nitrogen mustard A or B in a solvent, which will be specified in the following text, was washed thoroughly with excess saturated sodium bicarbonate solution, and then water, separated and dried (MgSO_4).

II. The free amine or phenol was released in situ by the addition of dry triethylamine (1 eq.).

The pertinent method used in each experiment will be indicated by the numeral I or II, e.g. 'the nitrogen mustard A(I) in ether' etc.

When 'dry NN-dimethylformamide' is used in the text, it means NN-dimethylformamide dried with molecular sieves B.D.H. type 4a.

All concentrations of solutions were performed by distillation under reduced pressure.

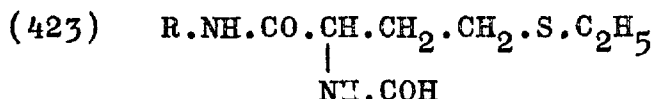
Numerals in brackets following sub-headings refer to page numbers in the original laboratory notebooks.

DERIVATIVES OF DL-ETHIONINE

N-Formyl-DL-ethionine (421)

Acetic anhydride (83 ml.) was added dropwise to ethionine (3.26 g.) in 90% formic acid (50 ml.) at such a rate as to maintain the temperature at 50-60°. After addition, the mixture was stirred for one hour, and then concentrated to give a yellow viscous gum (2.64 g.) which was used in the next experiment.

N'-Formyl-DL-ethionyl-(NN-di-2-chloroethyl)-p-phenylenediamine



N-Formyl-DL-ethionine (2.64 g.) was dissolved in chloroform (14 ml.) to which was added the nitrogen mustard A(II) (3.73 g.). The reaction mixture was cooled to room temperature before adding NN'-dicyclohexylcarbodiimide (3 g., 10% excess) in chloroform (5 ml.). It was shaken for 12 hours and then glacial acetic acid (0.5 ml.) was added, and the mixture was left for a further 2 hours. The precipitated urea and triethylamine were filtered off, and the filtrate was washed with dilute hydrochloric acid (2 x 50 ml.), water (3 x 50 ml.), dried (MgSO₄), and chromatographed (alumina, chloroform) to give the amide (2.1 g., 26% overall yield calculated from starting quantity of ethionine), which

recrystallized from chloroform-petroleum (b.p. 40-60°), and had m.p. 143° (Found: N, 9.55; O, 16.19; S, 10.49.

C₁₄H₂₀N₂O₃S requires N, 9.45; O, 16.19; S, 10.82%).

N'-Methoxycarbonyl-DL-ethionyl-(NN-di-2-chloroethyl)-p-phenylenediamine (426)

$$\begin{array}{c} \text{R.NH.CO.CH.CH}_2\text{.CH}_2\text{.S.C}_2\text{H}_5 \\ | \\ \text{NH.CO.OCH}_3 \end{array}$$

N-Methoxycarbonyl-DL-ethionine (2.86 g.) was dissolved in dichloromethane (15 ml.) to which was added the nitrogen mustard A(II) (3.49 g.) and the mixture was cooled to 21° before NN'-dicyclohexylcarbodiimide (2.7 g.) in dichloromethane (10 ml.) was added. It was shaken for 12 hours, the precipitated urea and triethylamine hydrochloride were filtered off, and glacial acetic acid (0.5 ml.) was added to the filtrate which was left for a further 2 hours. The solution was washed with dilute hydrochloric acid (2 x 40 ml.) and water (3 x 40 ml.), dried (MgSO₄) and chromatographed (alumina, chloroform) to give the amide (2.5 g., 33% overall yield calculated on starting quantity of ethionine) which was recrystallized from chloroform-petroleum (b.p. 30-40°) it had m.p. 109-110° (Found: C, 48.95; H, 5.99; Cl, 16.36; N, 10.07. C₁₈H₂₇Cl₂N₃O₃S requires C, 49.54; H, 6.23; Cl, 16.24; N, 9.66%).

L-Glutamic acid was esterified with boiling methanol saturated with dry hydrogen chloride gas.

$$\text{CH}_3 \cdot \text{O} \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \underset{\substack{| \\ \text{CO} \cdot \text{OCH}_3}}{\text{CH}} \cdot \text{NCO}$$

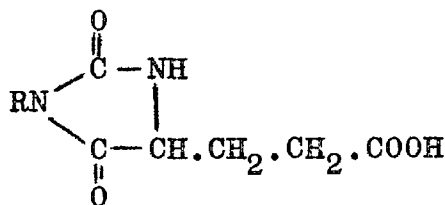
Dimethyl L-glutamate hydrochloride (14.4 g.) was suspended in dried chloroform (170 ml.), stirred and cooled to 0°, through which was passed carbonyl chloride, and then boiled under reflux for 12 hours, cooled, concentrated, and distilled (11 g., 80% overall yield calculated on starting quantity of L-glutamic acid), b.p. 96-97°/9 x 10⁻⁴ mm., n_D¹⁷ 1.4502, [α]_D²² -41.6° (c, 7.5 in acetone). The isocyanate was characterized as the methyl carbamate, b.p. 109°/3 x 10⁻³ mm., (Found: C, 46.07; H, 6.60; N, 5.91. C₉H₁₅NO₆ requires C, 46.34; H, 6.48; N, 6.01%).

$$(292) \quad \text{RNH} \cdot \text{CO} \cdot \text{NH} \cdot \underset{\text{CO} \cdot \text{OCH}_3}{\text{CH}} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{OCH}_3$$

The nitrogen mustard (A(I) (2.1 g.) in ether (25 ml.) to which dimethyl 2-isocyanatoglutarate (1.73 g.) in acetone (7 ml.) was added, was boiled under reflux for 24 hours,

the last half hour with charcoal. The mixture was cooled, filtered, and concentrated to an oil, which was triturated with ether, and crystallized from acetone-carbon tetrachloride to give the urea (2.74 g., 85%), m.p. 121°, $[\alpha]_D^{19} -18.7^\circ$ (c, 1.2 in CHCl_3). The infrared spectrum showed a broad band at 1660 cm^{-1} (- NH.CO.NH -) which had a well defined shoulder which was interpreted as being the secondary amide II band. (Found: C, 50.08; H, 6.30; Cl, 16.25; N, 9.85. $\text{C}_{18}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_5$ requires C, 49.77; H, 5.80; Cl, 16.32; N, 9.68%).

3-[p-(NN-Di-2-chloroethylamino)phenyl]-5-carboxyethylhydantoin
(430,431)

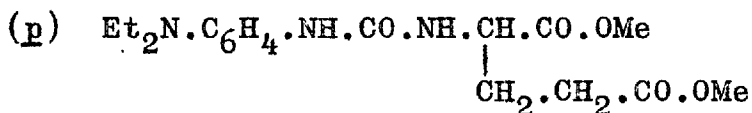


Dimethyl 2-p-(NN-di-2-chloroethylamino)-phenylureidoglutamate (1.44 g.) was dissolved in glacial acetic acid (11.5 ml.) to which was added concentrated hydrochloric acid (8.6 ml.). The solution was boiled under reflux for 25 minutes, cooled to 0° and diluted with water. The oily solid which was thrown out was extracted into chloroform (3 x 25 ml.), dried (MgSO_4), and concentrated down to give a white solid which was crystallized from acetone-petroleum (b.p. 30-40°) to give a product (0.88 g., 68%) m.p. 162-163°, $[\alpha]_D^{19} -32.4^\circ$ (c, 1.2 in chloroform). The infrared spectrum showed a band at

1770 cm^{-1} (- CO - $\overset{|}{\text{N}}$ - CO -) and at 1713 cm^{-1} (- COOH).

There were no bands applicable to the secondary amide II band region. The carboxylic acid was confirmed by the compound being soluble in sodium bicarbonate solution. (Found: C, 49.17; H, 4.92; Cl, 17.97; N, 10.60; Eq.wt., 368.9; M.wt., 403.9. $\text{C}_{16}\text{H}_{19}\text{Cl}_2\text{N}_3\text{O}_4$ requires C, 49.49; H, 4.93; Cl, 18.27; N, 10.82%; Eq.wt., 388.3; M.wt., 388.3).

Dimethyl 2-p-(NN-diethylamino)phenylureidoglutarate (272)



NN-Diethyl-p-phenylenediamine (0.82 g.) and dimethyl 2-isocyanatoglutarate (1.0 g.), with pyridine (0.1 ml.) as catalyst, were warmed for 90 minutes at 100° in a sealed flask. The compound was decolourized with charcoal in acetone (15 ml.), and finally chromatographed (alumina, chloroform) to give the urea (1.2 g., 66%), m.p. 109-110°, $[\alpha]_D^{19} +15^\circ$ (c, 0.6 in chloroform). (Found: C, 59.13; H, 7.03; N, 11.47. $\text{C}_{18}\text{H}_{27}\text{N}_3\text{O}_5$ requires C, 59.17; H, 7.40; N, 11.50%).

γ -Benzyl L-glutamate.¹⁶⁶ (351)

γ -Benzyl L-glutamate was prepared by the method of Hanby, Waley and Watson using copper carbonate as a chelating agent and benzyl chloride for the esterification of the γ -carboxyl group. It had m.p. 167-168°, $[\alpha]_D^{22} +20.1^\circ$ (c, 2.7 in glacial acetic acid). Lit, 169-170°.

γ -Benzyl N-formyl-L-glutamate¹⁸² (362)

This was prepared by the method of Borek and Waelsch using 90% formic acid and acetic anhydride. It had m.p. 132-134°, (Lit, 132-134°), $[\alpha]_D^{21} +10^\circ$ (c, 1.0 in acetone).

γ -Benzyl N'-formyl-DL-glutamyl-(NN-di-2-chloroethyl)-p-phenylenediamine. (366)
$$\text{RNH.CO.CH} \begin{array}{c} \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CO.OCH}_2 \cdot \text{C}_6\text{H}_5 \\ | \\ \text{NH.CO.H} \end{array}$$

γ -Benzyl N-formyl-L-glutamate (5.5g.) cooled in an ice bath was taken up in dry NN-dimethylformamide (20 ml.) to which was added triethylamine (2.86 ml.). Ethyl chloroformate (1.98 ml.) was then added and the mixture left for 20 minutes at 4°. The nitrogen mustard A(II) (5.38 g) in NN-dimethylformamide (20 ml.) was cooled to ice temperature and then added to the mixed anhydride solution. The mixture was kept at 4° for 48 hours. The triethylamine hydrochloride was filtered off, and the filtrate was concentrated to give an oil. This was dissolved in chloroform and washed with dilute hydrochloric acid (2 x 50 ml.), water (3 x 50 ml.), dried (MgSO₄) and chromatographed (alumina, chloroform) to give the amide, m.p. 151°. The compound showed no optical rotation (c, 0.4 in chloroform) (Found: C, 57.77; H, 5.82; Cl, 14.54; N, 8.76.

C₂₃H₂₇Cl₂N₃O₄ requires C, 57.49; H, 5.67; Cl, 14.74; N, 8.76%).

Attempted preparation of $\underline{N}'$ -formyl-DL-glutamyl-($\underline{NN}$ -di-2-chloroethyl)- $\underline{p}$ -phenylenediamine. (391)
$$\text{RNH.CO.CH} \begin{array}{c} \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COO} \cdot \\ | \\ \text{NH.CO.H} \end{array}$$

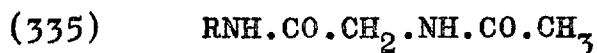
γ -Benzyl $\underline{N}'$ -formyl-DL-glutamyl-($\underline{NN}$ -di-2-chloroethyl)- $\underline{p}$ -phenylenediamine (0.63 g.) was dissolved in chloroform (100 ml.) to which was added Adams' catalyst (0.0228 g.) and 5% palladium-charcoal (1 g.) and hydrogenolysed at atmospheric pressure (85 ml. of H_2). The product had the same infrared spectra as the starting material, also it was insoluble in sodium bicarbonate solution, m.p. 150-151 $^{\circ}$.

DERIVATIVES OF GLYCINE

N-Acetylglycine.¹⁹¹ (332)

This was prepared from glycine and acetic anhydride by the method according to Herbst and Shemin. It had m.p. 206-7°. Lit: 207-8°.

N'-Acetylglycyl(NN-di-2-chloroethyl)-p-phenylenediamine.



a) Ethyl chloroformate (1.98 ml.) was added to an ice cold solution of N-acetylglycine (2.34 g.) in dry NN-dimethylformamide (15 ml.) and triethylamine (2.86 ml.) and left for 20 minutes at 4°. A cooled mixture of the nitrogen mustard A(II) (5.38 g.) in NN-dimethylformamide (30 ml.) was added to the mixed anhydride solution and then left for 4 days at 4° before filtering off the precipitated triethylamine hydrochloride. The filtrate was concentrated to give an oil which was taken up in chloroform and washed with dilute hydrochloric acid (2 x 50 ml.), water (3 x 50 ml.), dried (MgSO_4), and chromatographed (alumina, chloroform) to give the anide (4.2 g., 64%) which was recrystallized from acetone and had m.p. 171-172° (Found: C, 50.65; H, 5.75; N, 12.74. $\text{C}_{14}\text{H}_{19}\text{Cl}_2\text{N}_3\text{O}_2$ requires C, 50.60; H, 5.76; N, 12.65%).

b) N-Acetylglycine (0.585 g.) was suspended in dichloromethane (20 ml.) to which was added a dichloromethane

solution (15 ml.) of nitrogen mustard A(II) (1.35 g.). The mixture was cooled to 22° before adding a solution of NN'-dicyclohexylcarbodiimide (1.1 g.) in dichloromethane (5 ml.), and shaken for 16 hours. The precipitated urea and triethylamine hydrochloride were filtered off, and the filtrate treated with glacial acetic acid (0.5 ml.) and left for 2 hours at 22° before washing with dilute hydrochloric acid (2 x 15 ml.) and water (3 x 15 ml.). It was dried (MgSO_4), concentrated and then chromatographed (alumina, chloroform) to give the amide (0.83 g., 50%), m.p. 172°. There was no depression of the melting point when mixed with a sample of the material made by method (a).

N-Formylglycine¹¹⁴ (333)

This was prepared by the action of 90% formic acid and acetic anhydride on glycine by the method of Cheehan et al. It had m.p. 160°. Lit: 160°.

N'-Formylglycyl-(NN-di-2-chloroethyl)-p-phenylenediamine.
(340, 406) $\text{RNH.CO.CH}_2\text{.NH.CO.H.}$

a) N-Formylglycine (1.00 g.) was dissolved in dry NN-dimethylformamide (10 ml.) and triethylamine (1.43 ml.), cooled to 4°, and ethyl chloroformate (0.99 ml.) was added. After 20 mins. the cooled solution of nitrogen mustard A(II) (2.7 g.) in dry NN-dimethylformamide was introduced and the mixture left at 4° for 24 hours. The precipitated

triethylamine hydrochloride was filtered off, and the filtrate concentrated to give an oil which was taken up in chloroform, washed with dilute hydrochloric acid (2 x 30 ml.) and then with water (3 x 30 ml.), dried (MgSO_4) and chromatographed (alumina, chloroform) to give the amide (1.95 g., 61%) which was crystallized from chloroform-acetone, it had m.p. 163° . (Found: Cl, 22.46; N, 12.91. $\text{C}_{13}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_2$ requires Cl, 22.39; N, 13.08%).

b) N-Formylglycine (0.515 g.) was dissolved in NN-dimethylformamide-dichloromethane (20 ml., 1:1) to which was added a solution (10 ml.) of the nitrogen mustard A(II) (1.35 g.), using the same solvents in the same proportions. To this mixture was added NN'-dicyclohexylcarbodiimide (1.3 g.) and the solution cooled and shaken for 14 hours at 22° . The precipitate was filtered off, and the filtrate concentrated to give an oil which was dissolved in chloroform, washed quickly with dilute hydrochloric acid (2 x 10 ml.) and then with water (3 x 10 ml.), dried (MgSO_4) and chromatographed (alumina, chloroform) to give the amide (1.04 g., 65%), m.p. 163° . No depression of the melting point was observed when mixed with an analytically pure sample from (a).

N-Methoxycarbonylglycine.¹⁹² (342)

This was prepared from methyl chloroformate and glycine according to the method of Leuchs. It had m.p. $95-96^\circ$, Lit, $95-96^\circ$.

N'-Methoxycarbonylglycyl-(NN-di-2-chloroethyl)-p-phenylene-
diamine. (352) $\text{RNH.CO.CH}_2\text{.NH.CO.OCH}_3$

N-Methoxycarbonylglycine (2.66 g.) was dissolved in dry NN-dimethylformamide (20 ml.) and triethylamine (2.86 ml.), cooled to 4°, and ethyl chloroformate (1.98 ml.) was added. After 20 minutes the cooled solution of nitrogen mustard A(II) (5.38 g.) in NN-dimethylformamide (30 ml.) was added and the mixture was kept for 48 hours at 4°. The precipitated triethylamine hydrochloride was filtered off and the filtrate concentrated to give an oil which was taken up in chloroform, washed with dilute hydrochloric acid (2 x 50 ml.), water (30 x 50 ml.) and dried (MgSO_4). It was chromatographed (alumina, chloroform) to give the amide (5.4 g., 77%) which was crystallized from chloroform-petroleum (b.p. 60-80°) and had m.p. 169°. (Found: C, 48.32; H, 5.46; Cl, 20.29; N, 12.07. $\text{C}_{14}\text{H}_{19}\text{Cl}_2\text{N}_3\text{O}_3$ requires C, 48.27; H, 5.50; Cl, 20.36; N, 12.07%).

N-Dichloroacetylglycine¹⁹³ (449)

This was prepared from dichloroacetyl chloride and glycine by the method of Ronwin.

N'-Dichloroacetylglycyl(NN-di-2-chloroethyl)-p-phenylene-diamine. (438) $\text{RNH.CO.CH}_2\text{.NH.CO.CHCl}_2$

N-Dichloroacetylglycine (2.21 g.) was dissolved in acetonitrile (25 ml.) to which was added the nitrogen

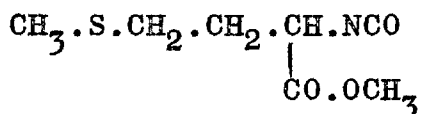
mustard A(II) (3.21 g.), cooled to 22°C, before NN'-dicyclohexylcarbodiimide (2.45 g.) in acetonitrile (5 ml.) was added. The reaction mixture was shaken for 16 hours, after which the precipitated urea and triethylamine hydrochloride were filtered off. Glacial acetic acid (0.5 ml.) was added to the filtrate which was allowed to stand for a further 2 hours. It was then washed quickly with dilute hydrochloric acid (2 x 15 ml.) and water (3 x 15 ml). The precipitated solid was filtered off and dissolved in chloroform, dried (MgSO_4) and concentrated down to give a solid, m.p. 165-167°. The filtrate was dried (MgSO_4), and chromatographed (alumina, chloroform) to give a solid, m.p. 170-171°. A mixture of both fractions showed no depression of the melting point and so the two crops were combined and crystallized from chloroform-petroleum 40-60° to yield the amide (3.1 g., 65%), m.p. 171°. (Found: C, 42.43; H, 4.49; Cl, 35.12; N, 10.71. $\text{C}_{14}\text{H}_{17}\text{Cl}_4\text{N}_3\text{O}_2$ requires C, 41.91; H, 4.27; Cl, 35.34; N, 10.48%).

DERIVATIVES OF DL-METHIONINE

Methyl 2-amino-4-methylthio-n-butyrate hydrochloride.¹⁷²
(300)

This was prepared from DL-methionine (10 g.) and methanol saturated with dry hydrogen chloride gas, and boiled under reflux for 24 hrs. This was not purified but used in the next experiment.

Methyl 2-isocyanato-4-methylthio-n-butyrate. (304)



Methyl 2-amino-4-methylthio-n-butyrate hydrochloride was suspended in dry benzene (400 ml.) and saturated with carbonyl chloride before being boiled under reflux for 24 hours. The benzene was distilled off under reduced pressure and the resulting oil distilled (7.7 g., 66.5% overall calculated from starting quantity of DL-methionine), b.p. $86^\circ / 1.35 \times 10^{-2}$ mm. (Found: C, 44.60; H, 5.89; N, 7.57. $\text{C}_7\text{H}_{11}\text{NO}_3\text{S}$ requires C, 44.41; H, 5.86; N, 7.40%). The isocyanate was characterized as the urea, (which crystallized from methanol-water) m.p. 92° . (Found: N, 8.11; O, 22.78; S, 18.28. $\text{C}_{13}\text{H}_{24}\text{N}_2\text{O}_5\text{S}_2$ requires N, 7.96; O, 22.70; S, 18.20%).

Methyl 2-[p-(NN-di-2-chloroethylamino)phenylureido]-4

methylthio-n-butyrate. (309)
$$\text{RNH.CO.NH.}\underset{\text{CO.OCH}_3}{\text{CH}}.\text{CH}_2\text{CH}_2\text{SCH}_3$$

The nitrogen mustard A(I) (2.70 g.) in ether (25 ml.) was reacted with methyl 2-isocyanato-4-methylthio-n-butyrate (1.89 g.), with pyridine (0.2 ml.) added as catalyst, by boiling under reflux for 3 hours. The reaction mixture was concentrated to give the urea which recrystallized from acetone-petroleum (b.p. 60-80°) and had m.p. 138°. (Found: Cl, 16.93; N, 10.13. $\text{C}_{17}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_3\text{S}$ requires Cl, 16.79; N, 9.95%).

N-Acetyl-DL-methionine.¹⁹⁴ (329)

This was prepared from acetic anhydride and DL-methionine according to the method of Birnbaum et al. It had m.p. 114-115°. Lit, 112°.

N'-Acetyl-DL-methionyl-(NN-di-2-chloroethyl)-p-phenylene-

diamine. (330)
$$\text{RNH.CO.CH.}\underset{\text{CH}_2.\text{CH}_2.\text{SCH}_3}{\text{NH}}.\text{CO.CH}_3$$

N-Acetyl-DL-methionine was dissolved in dry NN-dimethylformamide (10 ml.) to which was added triethylamine (1.43 ml.) cooled to 4°, and then methyl chloroformate (0.8 ml.) was introduced. After 15 minutes a cooled solution of the nitrogen mustard A(II) (2.70 g.) in dry NN-dimethylformamide

(30 ml.) was added. The mixture was left for 3 days at 4°, after which the precipitated triethylamine hydrochloride was filtered off, and the filtrate concentrated to give an oil, which was dissolved in chloroform, and washed with dilute hydrochloric acid (2 x 30 ml.), water (3 x 30 ml.), dried (MgSO₄), and then chromatographed (alumina, chloroform) to give the amide (2.4 g., 60%) which was recrystallized from chloroform-petroleum (b.p. 60-80°), and had m.p. 181°. (Found: Cl, 17.75; N, 10.37. C₁₇H₂₅Cl₂N₃O₂S requires Cl, 17.49; N, 10.34%).

N-Formyl-DL-methionine¹¹⁴ (339)

Was prepared from DL-methionine, 90% formic acid and acetic anhydride according to the method of Sheehan et al. It had m.p. 103-104°. Lit, 103-104°.

N'-Formyl-DL-methionyl-(NN-di-2-chloroethyl)-p-phenylene-diamine. (343)

$$\begin{array}{c} \text{RNH.CO.CH.CH}_2\text{.CH}_2\text{.SCH}_3 \\ | \\ \text{NH.CO.H} \end{array}$$

N-Formyl-DL-methionine (3.45 g.) was dissolved in dry NN-dimethylformamide (40 ml.) and triethylamine (2.86 ml.) cooled to 4° before adding ethyl chloroformate (1.98 ml.). After 20 minutes a cooled solution of the nitrogen mustard A(II) (5.38 g.) in dry NN-dimethylformamide (30 ml.) was added to the mixed anhydride solution and kept for 48 hours at 4°. The NN-dimethylformamide was distilled off after

added and the mixture was shaken for 16 hours after which the urea was filtered off, and glacial acetic acid (0.5 ml.) added to the filtrate, which was left for a further 2 hours. The mixture was washed with dilute hydrochloric acid (2 x 20 ml.), water (3 x 20 ml.), dried (MgSO_4), and concentrated to an oil, which was chromatographed (alumina, chloroform) to give the amide (1.4 g., 40%), m.p. 122° . (Found: N, 12.19; O, 13.31. $\text{C}_{17}\text{H}_{27}\text{N}_3\text{O}_3\text{S}$ requires N, 11.89; O, 13.58%).

N'-Methoxycarbonyl-DL-methionyl-(NN-di-2-chloroethyl)-p-phenylenediamine. (396)
$$\begin{array}{c} \text{RNH.CO.CH.CH}_2\text{.CH}_2\text{.SCH}_3 \\ | \\ \text{NH.CO.OCH}_3 \end{array}$$

N-Methoxycarbonyl-DL-methionine (1.04 g.) was dissolved in dichloromethane (5 ml.), to which was added the nitrogen mustard A(II) (1.36 g.) in dichloromethane (10 ml.). The mixture was cooled to 20° before adding NN'-dicyclohexylcarbodiimide (1.3 g.) and the temperature maintained whilst vigorous shaking was performed. The mixture was left for 16 hours at this temperature before the precipitated urea and triethylamine hydrochloride were filtered off. Glacial acetic acid (0.5 ml.) was added to the filtrate, which was left for 2 hours, after which it was washed with dilute hydrochloric acid (2 x 20 ml.), water (3 x 20 ml.), dried (MgSO_4) and concentrated. The buff coloured solid was

crystallized from acetone-petroleum (b.p. 40-60°) to give the amide (0.81 g., 39%), m.p. 141°. (Found: Cl, 16.67; N, 9.77. $C_{17}H_{25}Cl_2N_3O_3S$ requires Cl, 16.79; N, 9.94%).

N-Dichloroacetyl-DL-methionine¹⁹³ (413)

This was prepared by the method according to Ronwin from dichloroacetyl chloride and DL-methionine.

N'-Dichloroacetyl-DL-methionyl-(NN-di-2-chloroethyl)-p-phenylenediamine. (413)
$$\begin{array}{c} \text{RNH.CO.CH.CH}_2\text{CH}_2\text{SCH}_3 \\ | \\ \text{NH.CO.CHCl}_2 \end{array}$$

N-Dichloroacetyl-DL-methionine (1.3 g.) was dissolved in nitromethane (15 ml.) and the nitrogen mustard A(II) (1.35 g.) was introduced, all the while maintaining the temperature at 21°. The solution was shaken for 16 hours before filtering off the precipitated urea and triethylamine hydrochloride. Glacial acetic acid (0.5 ml.) was added to the filtrate, which was left for 2 hours before washing with dilute hydrochloric acid (2 x 15 ml.), water (3 x 15 ml.), drying ($MgSO_4$) and concentrated. The compound so obtained was taken up in chloroform and chromatographed on alumina to give the amide, (which was crystallized from acetone-petroleum (b.p. 30-40°)) m.p. 172°. (Found: C, 43.42; H, 4.99; Cl, 29.80; N, 8.73. $C_{17}H_{23}Cl_4N_3O_2S$ requires C, 42.96; H, 4.88; Cl, 29.83; N, 8.84%).

N-Monofluoroacetyl-DL-methionine. (434)

DL-Methionine (3 g.) was dissolved in 8N NaOH (5 ml.) cooled to 1° in ice and the monofluoroacetyl chloride (3 ml.) was added dropwise, with stirring, at such a rate that the temperature was maintained at 1 - 5° for 2 hours. The solution was left for a further 12 hours before acidification with dilute hydrochloric acid. The compound was extracted into chloroform (3 x 30 ml., dried MgSO₄) and concentrated down to produce an oil. The infrared absorption curve showed the presence of amide (- CONH -) (1680 cm⁻¹). The oil (2.08 g., 50%) was considered to be sufficiently pure to be used in the next experiment.

N'-Monofluoroacetyl-DL-methionyl-(NN-di-2-chloroethyl)-p-phenylenediamine. (435)

$$\begin{array}{c} \text{RNH.CO.CH.CH}_2\text{.CH}_2\text{.SCH}_3 \\ | \\ \text{NH.CO.CH}_2\text{F} \end{array}$$

N-Monofluoroacetyl-DL-methionine (2.08g.) and the nitrogen mustard A(II) (2.7 g.) were dissolved in dichloromethane (35 ml.). To this solution, with cooling to 20°, was added NN'-dicyclohexylcarbodiimide (2.06 g.) and left at 22° for 48 hours with shaking. The precipitated urea, and triethylamine hydrochloride were filtered off and glacial acetic acid (0.7 ml.) added to the filtrate. After 2 hours the solution was washed with dilute hydrochloric

acid (2 x 30 ml.), water (3 x 30 ml.), dried (MgSO_4), and concentrated to give a solid, which was chromatographed (alumina, chloroform) to give the anide (1.8 g., 63%) which was recrystallized from acetone-petroleum (b.p. $30-40^\circ$) or chloroform-petroleum (b.p. $30-40^\circ$) and had m.p. 160° .

(Found: C, 48.08; H, 6.10; N, 9.91; M.wt., 412.7.

$\text{C}_{17}\text{H}_{24}\text{FCl}_2\text{N}_3\text{O}_2\text{S}$ requires C, 48.12; H, 5.70; N, 9.90%; M.wt., 424.4).

SOME DERIVATIVES OF L-TYROSINE

N-Acetyl-p-methoxy-L-phenylalanine.²⁰⁰ (142, 175, 177)

This was prepared by the method of B. R. Baker using L-tyrosine and preferentially acetylating the amino group with acetic anhydride. Although the N-acetyl-tyrosine so produced is a gum (due to the detergent added) it is sufficiently pure to be used for the methylation of the phenol group with dimethyl sulphate to give the product required (29 g., 50%), m.p. 144-145°. Lit, 144-145°.

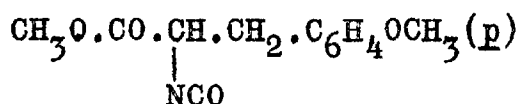
p-Methoxy-L-phenylalanine.²⁰⁰ (144, 145, 178, 179)

This was prepared by the deacetylation of the compound described in the previous section using 6N hydrochloric acid. The hydrochloride so produced was treated with ammonia (d. 0.880) to give the free amino compound (16 g., 69%), m.p. 250-252°. Lit, 250-252°.

Methyl 2-amino-3-(p-methoxyphenyl)propionate hydrochloride.
(128)

Was prepared from p-methoxy-L-phenylalanine and methanol saturated with hydrogen chloride gas.

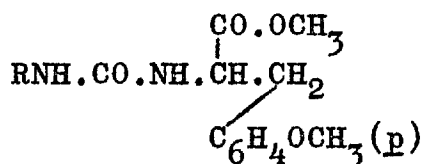
Methyl 2-isocyanato-3-(p-methoxyphenyl)propionate. (146, 147)



Methyl 2-amino-3-(p-methoxyphenyl)propionate hydrochloride (10.0 g.) was suspended in dry toluene (200 ml.), cooled to

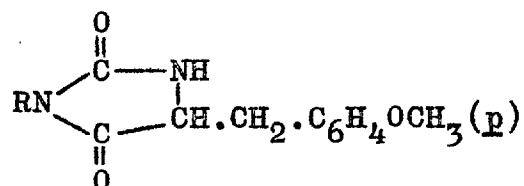
0° and saturated with carbonyl chloride. The temperature of the solution was allowed to rise and it was boiled under reflux for 7 hours. Any excess phosgene was blown off with dry nitrogen, and the solution concentrated to give an oil which was distilled (5.1 g., 53%) b.p. 126-128°/0.2 mm. (Found: C, 60.57; H, 6.05; N, 5.85. $C_{12}H_{13}NO_4$ requires C, 61.09; H, 5.58; N, 5.95%). The isocyanate was characterized as its anilide (which recrystallized from methanol-water) m.p. 120°. (Found: C, 65.67; H, 6.26; N, 8.68. $C_{18}H_{20}N_2O_4$ requires C, 65.82; H, 6.13; N, 8.53%).

Methyl 2-[p-(NN-di-2-chloroethyl-amino)phenylureido]-3-(p-methoxyphenyl)propionate. (148)



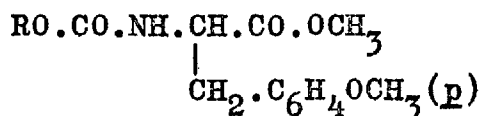
The nitrogen mustard A(I) (1.0 g.) in ether (5 ml.) was added to methyl 2-isocyanato-3-(p-methoxyphenyl)-propionate (0.85 g.) and boiled under reflux, with (0.1 ml.) of pyridine as catalyst, for 1 hour. The white solid which had been thrown out of solution was recrystallized from benzene to give the urea (1.21 g., 70%), m.p. 147°. (Found: C, 57.57; H, 6.43; Cl, 14.90. $C_{22}H_{27}Cl_2N_3O_4$ plus $\frac{1}{4}$ mole of benzene of crystallisation requires C, 57.84; H, 5.89; Cl, 14.57%). No optical rotation was observed (c, 1.4 g. in acetone).

3-[p-(NN-Di-2-chloroethylamino)phenyl]-5-p'-methoxybenzyl-hydantoin. (447)



Methyl 2-[p-(NN-di-2-chloroethylamino)phenylureido]-3-(p-methoxyphenol)propionate (100 mg.) was dissolved in glacial acetic acid (3 ml.) and concentrated hydrochloric acid (3 ml.) and boiled under reflux for 25 minutes. The solution was cooled, and diluted with ice water (20 ml.), the white solid so produced was extracted into chloroform (3 x 10 ml.), dried (MgSO₄) and concentrated. The solid was chromatographed (silica gel, chloroform) to give the product (0.06 g., 64%). The infrared spectrum showed a strong absorption at 1770 cm⁻¹ (- CO - $\overset{\cdot}{\text{N}}$ - CO -) with no bands in the region which is usual for secondary amide II bands. No optical rotation was observed (c, 1.3 g. in acetone). It had m.p. 75°. (Found: C, 57.57; H, 5.18; O, 10.83. C₂₁H₂₃Cl₂N₃O₄ requires C, 57.79; H, 5.31; O, 11.00%).

Methyl 2-[N'-p-(NN-di-2-chloroethylamino)phenoxy-carbonylamino]-3-(p-methoxyphenyl)propionate. (152, 153)

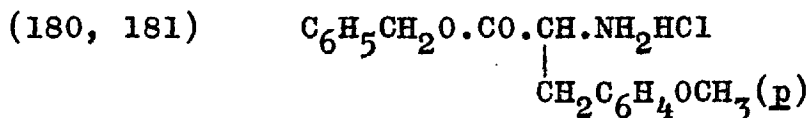


The nitrogen mustard B(I) (1.5 g.) in chloroform was

concentrated to an oil, to this was added methyl 2-isocyanato-3-(p-methoxyphenyl)propionate (1.3 g.). Pyridine (0.1 ml.) was added as catalyst, and the mixture was heated for 3 hours at 130-150°. The resulting glass was extracted with petroleum (b.p. 60-80°) which deposited an oil when concentrated. The oil crystallized from benzene after being kept at 4° for 7 weeks to give the carbamate, m.p. 67-70°. No optical rotation was observed (c, 2.7 g. in acetone).

(Found: C, 56.71; H, 5.91; Cl, 14.29. $C_{22}H_{26}Cl_2N_2O_5$ plus $\frac{1}{4}$ mole of benzene of crystallization requires C, 57.02; H, 5.60; Cl, 14.33%).

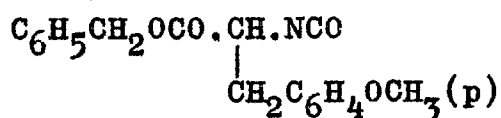
Benzyl 2-amino-3-(p-methoxyphenyl)propionate hydrochloride.



p-Methoxy-L-phenylalanine (12 g.) and p-toluenesulphonic acid (17.5 g.) were boiled under reflux with benzyl alcohol (50 ml.) and benzene (400 ml.). The water produced during the esterification was removed by using a Dean-Stark separator, and later a Soxhlet tube containing anhydrous silica gel. On cooling, a solid appeared, which was filtered off, washed with saturated sodium bicarbonate to give an oil. The oil was extracted into chloroform, dried (MgSO_4) and treated with anhydrous hydrogen chloride in order to precipitate the hydrochloride. The hydrochloride

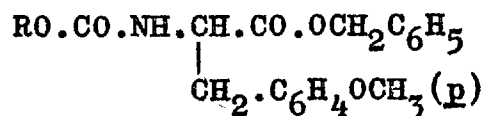
was filtered off and washed with chloroform to give the product (13.5 g., 68%), m.p. 200°.

Benzyl 2-isocyanato-3-(p-methoxyphenyl)propionate. (181,202)



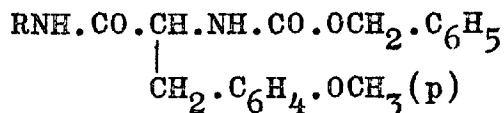
Benzyl 2-amino-3-(p-methoxyphenyl)propionate hydrochloride (13.5 g.) was suspended in dry toluene (200 ml.) and cooled to 0° before being saturated with carbonyl chloride. The suspension was boiled under reflux for 90 minutes concentrated to give an oil which distilled (9.05 g., 76%), b.p. 170°/0.193 mm. m.p. 36°, $[\alpha]_D^{22}$ -88° (c, 9.5 g. in acetone), n_D^{19} 1.5540. (Found: C, 69.10; H, 5.56; N, 4.52. $\text{C}_{18}\text{H}_{17}\text{NO}_4$ requires C, 69.42; H, 5.51; N, 4.50%). The isocyanate was characterized as its urea, (which crystallized from alcohol) m.p. 148-149°. (Found: C, 70.96; H, 6.25; N, 4.71. $\text{C}_{35}\text{H}_{36}\text{N}_2\text{O}_7$ requires C, 70.44; H, 6.08; N, 4.70%).

Benzyl 2-[N'-p-(NN-di-2-chloroethylamino)phenoxy-carbonylamino]-3-(p-methoxyphenyl)propionate. (204)



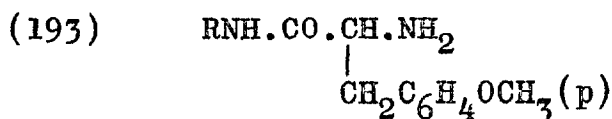
The nitrogen mustard B(I) (1.16 g.) was dissolved in chloroform (15 ml) and benzyl 2-isocyanato-3-(p-methoxyphenyl)propionate (1.30 g.) was added. The solution was boiled

N'-Benzyloxycarbonyl-O-methyl-L-tyrosyl-(NN-di-2-chloroethyl)-p-phenylenediamine. (169)



N-Benzylcarbamato-p-methoxy-phenylalanine (3.3 g.) was dissolved in dry NN-dimethylformamide (22 ml.) and dry triethylamine (1.43 ml.) cooled to 4° before ethyl chloroformate (0.99 ml.) was added. After 15 minutes the formation of the mixed anhydride was adjudged completed, so to this solution was added the nitrogen mustard A(II) (2.69 g.) in dry NN-dimethylformamide (17 ml.). The temperature of the reaction mixture was maintained at 4°, during the addition and for the next 22 hours. The precipitated triethylamine hydrochloride was filtered off, and the filtrate stripped down to half its original volume. It was then washed with dilute hydrochloric acid which threw out a buff coloured solid, which was dried, and crystallized from benzene-petroleum (b.p. 80-100°) to give the amide (2.5 g., 48%), m.p. 150-151°. (Found: C, 61.28; H, 5.90; Cl, 13.01. C₂₈H₃₁Cl₂N₃O₄ requires C, 61.76; H, 5.74; Cl, 12.85%).

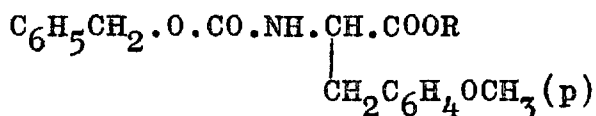
O-Methyl-L-tyrosyl(NN-di-2-chloroethyl)p-phenylenediamine.



N'-Benzylcarbamato-O-methyl-L-tyrosyl-(NN-di-2-chloroethyl)-p-phenylenediamine (0.95 g.) was taken up in ethyl

acetate (50 ml.) and hydrogenolized (40 ml. of H_2) using 5% palladium-charcoal catalyst (2.5 g.). The ethyl acetate solution was filtered, and concentrated leaving a gum, which was soluble in benzene. The free amino group was shown by a small portion being subjected to a little hydrogen chloride gas which precipitated the hydrochloride. The other portion was crystallized from benzene to give the free amino compound m.p. 104-105°, $[\alpha]_D^{22} +9.6^\circ$ (c, 1.6 g. in glacial acetic acid). (Found: Cl, 14.81; N, 8.74. $C_{26}H_{31}Cl_2N_3O_2$ plus 1 mole of benzene of crystallization requires Cl, 14.54; N, 8.60%).

p-(NN-Di-2-chloroethylamino)phenyl 2-N'-benzyloxycarbonylamino-3-(p-methoxyphenyl)propionate. (186)



N-Benzyloxycarbonyl-p-methoxy-L-phenylalanine (3.3 g.) was dissolved in NN-dimethylformamide (22 ml.). To this solution, cooled in an ice bath, was added triethylamine (1.43 ml.) and ethyl chloroformate (0.99 ml.). The solution was kept for 20 minutes at 4° before the nitrogen mustard B(II) (2.70 g.) dissolved in NN-dimethylformamide (30 ml.) and 1 equivalent, in excess, of triethylamine was added. The mixture was kept at 4° for 4 days. The triethylamine hydrochloride was filtered off and the filtrate was concentrated to half its original volume, diluted with chloroform,

washed with water (3 x 50 ml.), dried (MgSO_4), and concentrated to give an oil. It was chromatographed (alumina, benzene) to give the ester, (which was crystallized from chloroform-petroleum (b.p. $60-80^\circ$)) m.p. $72-75^\circ$. (Found: Cl, 13.18; N, 4.98. $\text{C}_{28}\text{H}_{30}\text{Cl}_2\text{N}_2\text{O}_5$ requires Cl, 13.00; N, 5.13%). No optical rotation was observed (c, 1.73 g. in acetone).

Bis-N,O-Dimethoxycarbonyl-L-tyrosine²⁰⁶ (402)

Was prepared from L-tyrosine dissolved in 4N sodium hydroxide and treated with methyl chloroformate according to the method of Havestadt et al., $[\alpha]_{19}^{22} +25^\circ$ (c, 19.4 g. in chloroform).

Bis-N,O-Dimethoxycarbonyl-L-tyrosyl-(NN-di-2-chloroethyl)-p-phenylenediamine. (405)

$$\text{RNE.CO.CH.NH.CO.OCH}_3$$

$$\quad \quad \quad |$$

$$\quad \quad \quad \text{CH}_2.\text{C}_6\text{H}_4.\text{O.CO.OCH}_3(\text{p})$$

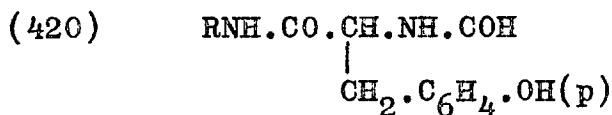
Bis-N,O-Dimethoxycarbonyl-L-tyrosine (1.48 g.) was dissolved in dichloromethane (10 ml.) to which was added the nitrogen mustard A(II) (1.35 g.) in dichloromethane (15 ml.). The temperature of the solution was kept at 22° while NN'-dicyclohexylcarbodiimide (1.3 g.) was added. The mixture was shaken for 16 hours, before the precipitated urea and triethylamine hydrochloride were filtered off. Glacial acetic acid (0.5 ml.) was added to the filtrate, which was left for a further 2 hours, and then washed with dilute

hydrochloric acid (2 x 25 ml.), water (3 x 25 ml.), dried (MgSO_4), and concentrated to give an oil. It was chromatographed to give the amide (1.25 g., 50%) (which was recrystallized from chloroform-petroleum (b.p. 30-40°)), m.p. 168°, $[\alpha]_D^{22} + 15.6^\circ$ (c, 1.3 in CHCl_3). (Found: Cl, 13.93; N, 8.27. $\text{C}_{23}\text{H}_{27}\text{Cl}_2\text{N}_3\text{O}_6$ requires Cl, 13.84; N, 8.20%).

N-Formyl-L-tyrosine.²⁰⁷ (420)

This was prepared from L-tyrosine and 98% formic acid according to the method of E. Fischer to give the formyl derivative monohydrate, m.p. 106°. The anhydrous compound was obtained by heating and drying in vacuo. It had m.p. 173-175°. Lit, 171-4°.

N'-Formyl-L-tyrosyl-(NN-di-2-chloroethyl)-p-phenylenediamine.



N-Formyl-L-tyrosine (1.94 g.) was suspended in dry NN-dimethylformamide (30 ml.) to which was added the nitrogen mustard A(I) (2.51 g.) in NN-dimethylformamide (20 ml.). NN'-Dicyclohexylcarbodiimide (2.1 g.) was added and the resulting heterogeneous mixture was shaken vigorously for 10 days. Unchanged N-formyl-L-tyrosine, urea and triethylamine hydrochloride were filtered off. The filtrate was concentrated to give a buff coloured solid, which was

dissolved, not without difficulty, in acetone and boiled under reflux with animal charcoal. The clean solution produced the amide, m.p. 191-192°, $[\alpha]_D^{22} +23.2^\circ$ (c, 1.5 g. in glacial acetic acid). (Found: C, 56.65; H, 5.60; N, 10.08. $C_{20}H_{23}Cl_2N_3O_3$ requires C, 56.61; H, 5.46; N, 9.90%).

BIOLOGICAL RESULTS

BIOLOGICAL RESULTS

The biological results given in this chapter were performed, under the direction of Professor J. F. Danielli, by his co-workers at the University of Buffalo, U.S.A.

The toxicity is expressed as LD₅₀ administered as a single subcutaneous dose in Swiss mice, which were observed for 14 days. As a number of the results are only preliminary, no inferences will be drawn from them.

COMPOUND	LD ₅₀ mice mg./Kg.	Dose mg./Kg./day	Route	No. of Rats	Effect on Tumour	Effect on Rat % wt. loss	Anti- tumour activity
$\begin{array}{c} \text{CH}_3\text{CO.NH.CH.CH}_2.\text{SCH}_3 \\ \\ \text{CO.NHR} \end{array}$	>1000	400, 200 x 2	im	3	Nil	0	- [ⓧ]
$\begin{array}{c} \text{Me.O.CO.NH.CH.CH}_2.\text{SMe} \\ \\ \text{CO.NHR} \end{array}$	< 500	200 x 3	im	2	P.R.	0 - 5	++ [ⓧ]
$\text{RNHCOCH}_2\text{NHCOCH}_3$	30	18 x 5 12 x 5 6 x 5	im im im	2 1 2	CR PR PR(1)GI(1)	15 10 - 15 10 - 15	+++

[ⓧ] Preliminary Result.

CR = Complete Regression; PR = Partial Regression; GI = Growth Inhibition.

im = intra-muscular.

CHAPTER VI

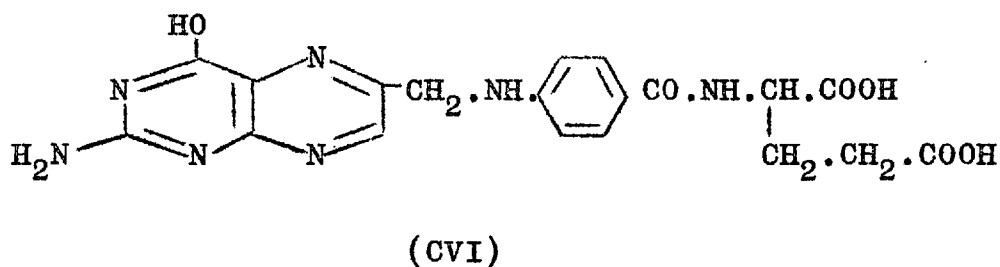
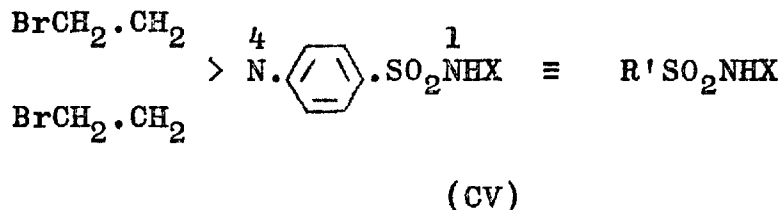
SOME SYNTHESSES OF NITROGEN MUSTARDS OF THE SULPHONAMIDE,

SULPHONHYDRAZIDE, SULPHONYLOXY AND SCHIFF BASE TYPE

One of the themes running through Chapter V was that it was hoped to achieve extra activity by the use of nitrogen mustards linked to known or potential antimetabolites. This theme is to be amplified in this Chapter.

It has been found that sulphonamides are antimetabolites²⁰⁵ effectively inhibiting utilization of *p*-aminobenzoic acid in the synthesis of folic acid. The nature of inhibition appears to involve competition between the sulphonamide and the *p*-aminobenzoate for the active site on the enzyme.²⁰⁸ However, once the sulphonamide competes favourably for the active site it can apparently react with the pteridine component to yield products which are inactive. Thus it was decided to use substituted sulphonamides to carry the nitrogen mustard moiety (see fig. CV), in order to exploit cellular active transport mechanisms. Because a tumour is rapidly dividing, active transport mechanisms are likely to be more active in the tumour than in normal cells. The sulphonamides are somewhat analogous to *p*-aminobenzoic acid, and as noted above they compete with this metabolite for enzyme

sites and so it would seem reasonable to postulate that sulphonamides would also be taken up at an increased rate by tumour cells.



Thus if compounds of the type (CV) were competitively adsorbed on sites which are usually occupied by the p-amino-benzoic acid or p-aminobenzoylglutamic acid moieties (see fig. CVI), then the β -bromoethyl residue (CV) would be in a position to alkylate the pteridine component, most probably at the ring nitrogen positions rather than at the free amino group (the pK_a value of which could be too high and so be protonated at physiological or neoplastic pH).

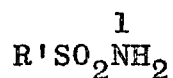
It has been found that for antibacterial action it is necessary to have the p-amino group free, i.e. N(4) (see fig. CV), or to have it protected by a group which can be removed easily by the body, e.g. acetyl, methyl or benzyl.²⁰⁹ Thus

compounds of the type (CV) would not be expected to have sulphonamide activity unless the N(4) atom was dealkylated first.

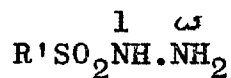
Bell and Roblin²¹¹ have put forward a hypothesis correlating chemical constitution with the in vitro biological activity of the sulphonamide drugs. These authors base their arguments on considerations of how closely a sulphonamide molecule approaches p-aminobenzoic acid in shape and general activity. As regards reactivity it was found that the dissociation constants of the N(4) amino group did not vary widely within a large group of sulphonamides studied, but the dissociation constants of the - SO₂NH - moiety did. Comparison of the - SO₂NH - and COOH moieties shows that the latter group is more acidic than the former. However, substitution on the N(1) atom can make the acidity of the - SO₂NH - moiety approach that of the COOH group, and, according to the hypothesis, the greater will be the bacteriostatic activity of the drug.

The postulated action of the nitrogen mustard sulphonamides, given above, requires that the sulphonamide portion must be actively transported, and must be able to compete effectively for the enzyme site. Creighton²¹⁰ prepared the nitrogen mustard sulphonamide (CVII) and the sulphonylhydrazide (CVIII), both of which were found to have good

anti-tumoural activity,^{212, 214} but were also toxic, showing



(CVII)



(CVIII)

symptoms resembling those of folic acid deficiency.²¹³

However, administration of folic acid proved to be of no value. Thus these compounds were believed to be working by a process which was different from that of the normal sulphonamide action.²¹³

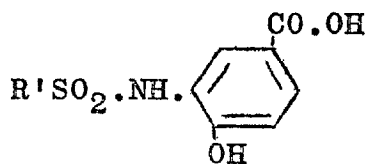
Thus if the compounds (CVII) and (CVIII) are compared with respect to the Bell and Roblin theory then (CVIII) would be expected to have a weaker sulphonamide action than (CVII) because the ω -amino group would not withdraw electrons from N(1) and therefore would not increase the acidity of the proton on this atom. In fact the ω -amino group is basic and most probably would have the reverse effect to that required by the Bell and Roblin theory. However, both these compounds have good anti-tumoural activity, although (CVII) was slightly better, and so it was decided to prepare a number of nitrogen mustard sulphonamides with suitable variations in X in order to discover any indications of a correlation between structure and activity, and to throw light on the mechanism by which these drugs work.

The general method of preparation of these nitrogen mustard derivatives was to add the nitrogen mustard sulphonyl chloride ($R'SO_2Cl$)²¹⁰ to the amino or hydrazino compound using pyridine as solvent for both reactants. The temperature was not allowed to rise too appreciably otherwise an increased formation of coloured products was obtained. It has been claimed that formation of coloured products was diminished by using pyridine which had been dried over potassium hydroxide pellets and distilled.²¹⁵ This, however, was not noticed to any appreciable extent by the author, and normal commercial pyridine was used in the majority of the syntheses. After time had elapsed for reaction to occur (usually 12 hours), the reaction mixture was slowly poured into an excess of 2N hydrobromic acid with cooling. The product was filtered off quickly, so that hydrolysis of the labile halogen atoms of the nitrogen mustard would be kept to a minimum. The majority of the compounds were chromatographed on activated alumina, though this method of purification of the crude product probably accounts to some extent for the low yields sometimes obtained. It was found for several compounds that they crystallized with benzene of crystallization when this particular solvent was used. Benzene was therefore not used for recrystallization whenever other solvents could be employed.

Creighton prepared the derivative (CIX) which showed slight anti-tumoural activity so it was decided to prepare a



number of derivatives containing substituents on the benzene ring in order to find out if there was any enhancement of the activity; these were:



(CX)



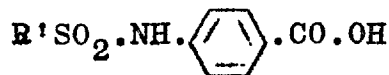
(CXI)



(CXII)



(CXIII)



(CXIV)

Compound (CX) was soluble in sodium bicarbonate solution and so would be advantageous, when compared to the insoluble derivatives, for clinical administration. The ortho-phenolic substituent on the benzene ring would be expected to donate electrons to it as would the para-phenolic group of the derivative (CXI). The compound (CXII) would be expected to show some specificity due to the requirement of a

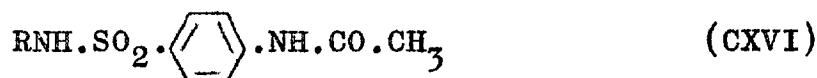
deacetylating enzyme, thereby releasing the free amino group, which would then be expected to donate electrons to the benzene ring. The derivatives (CXIII) and (CXIV) both have electron withdrawing groups on the benzene ring, (CXIV) having the advantage of being soluble in sodium bicarbonate.

It was thought that the electronic effects of these substituents on the benzene ring may be translated to the - SO₂NH - group, and so affect the ability of the proton to be held or released. According to the Bell and Roblin theory, the effectiveness of the sulphonamide depends, to some extent, on this ability of the - SO₂NH - group to lose its proton (see page 151). A compound which would be interesting from this point of view would be (CXV), in which two powerfully electron attracting groups are present. An attempt was made

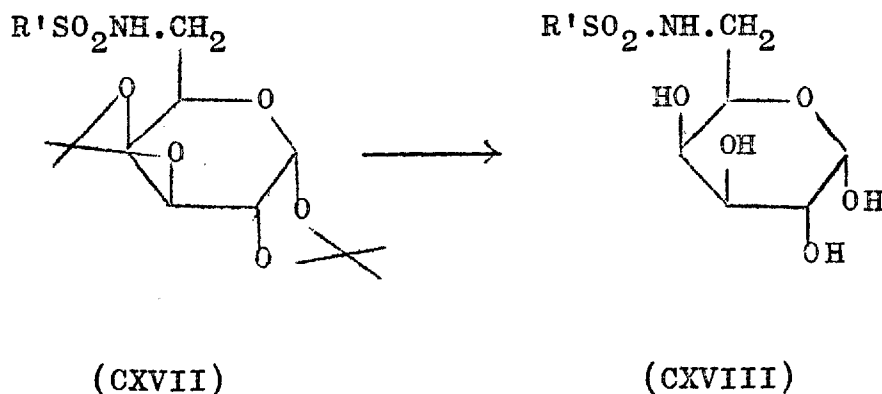


to prepare it by reaction of the sulphonyl chloride with 2,4-dinitroaniline, but this failed probably because the 2,4- dinitro-substituents make the amino group very unreactive.

The 'reverse sulphonamide' (CXVI) was prepared from the amino nitrogen mustard (RNH₂) and p-acetylamidobenzenesulphonyl chloride. This might well show some anti p-aminobenzoic

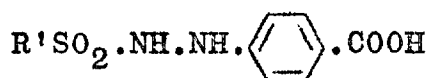


acid activity once the acetyl group had been hydrolysed off leaving the free N(4) amino group.

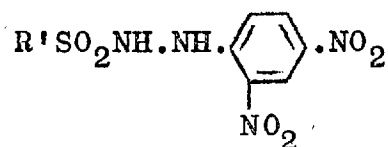


The compounds (CXVII) and (CXVIII) were prepared to exploit the facilitated diffusion shown by sugar moieties. 6-Amino-6-deoxy-1,2,-3,4-di-O-isopropylidene-D-galactose was prepared from the 6-mesyl compound²¹⁶ and reacted in the normal way with the nitrogen mustard sulphonyl chloride to give (CXVII). The protecting isopropylidene groups were removed by the action of 5% hydrobromic acid in dioxan. The reaction was followed by observing the change in optical rotation; when a constant rotation was observed the reaction mixture was worked up. The conditions may seem a little severe, but the 'hydrolysis rate' for these sulphonamides was found to be only 2-10%. It now appears that the precautions taken to keep the previous compounds from contact with wet solvents were unnecessary.

Derivatives of the compound (CVIII) were also made, i.e.:-



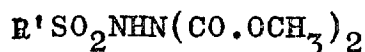
(CXIX)



(CXX)

Both of these were prepared in exactly the same way as the derivatives of (CVII), by adding the nitrogen mustard sulphonyl chloride to the hydrazino derivative. The derivative (CXIX) was soluble in sodium bicarbonate solution, due to the carboxyl group which should deactivate the benzene ring. The 2,4-dinitro derivative (CXX) has an additional feature of interest when one remembers that 2,4 dinitro compounds are toxic. Again the benzene ring would be expected to be highly deactivated due to the electron withdrawing power of nitro groups.

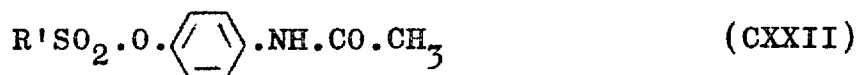
In view of the interest in carbamato compounds, the derivative (CXXI) was prepared.



(CXXI)

The parent sulphonhydrazide (CVIII) was reacted with methyl chloroformate using a suspension of potassium carbonate to remove the hydrogen chloride produced. Normal Schotten-Baumann conditions could not be used because of the liability of the bromine atoms to hydrolyse. Although the intention was to produce a monosubstituted sulphonhydrazide the resulting derivative could be very interesting.

A few nitrogen mustard sulphonic acid esters were prepared. It was hoped that hydrolysis of the ester linkage would occur in vivo and that, if suitably chosen, the non-mustard moiety would itself have some biological activity; this would therefore be additional to any effect produced by the nitrogen mustard sulphonic acid. These compounds were prepared from the nitrogen mustard sulphonyl chloride ($R'SO_2Cl$) and a substituted phenol, using the same conditions as for the substituted sulphonamides and sulphonhydrazides.



This compound (CXXII) was prepared because once it had been deacetylated and the ester hydrolysed, the free aminophenol may undergo oxidative deamination to give quinone, a type of compound which has some toxic properties.



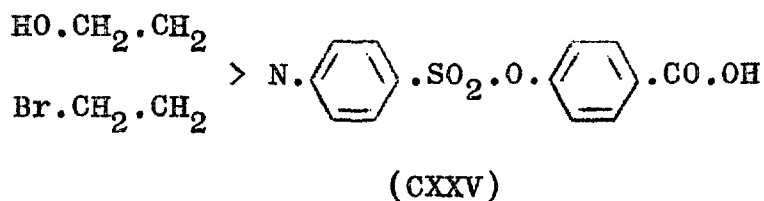
The methyl ester (CXXIII) was prepared because impure products were obtained when reaction was carried out between the sulphonyl chloride and free p-hydroxybenzoic acid. Hydrolysis of this ester should have given the free acid (CXXIV), which would be of interest because its pk_a value would be higher than that of the sulphonic acid $R'SO_2.OH$



The hydrolysis was attempted using a mixture of concentrated hydrobromic acid and glacial acetic acid; however, this was unsuccessful. Therefore the benzyl ester of p-hydroxybenzoic was prepared²¹⁷ and reacted with the sulphonyl chloride ($R'SO_2Cl$) to produce (CXXV).

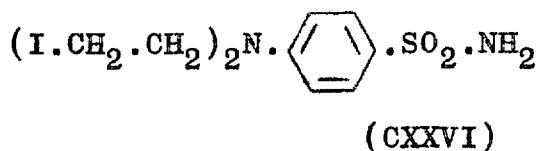


Hydrogenolysis over 10% Palladium-charcoal catalyst should have given the free acid (CXXIV). The product was soluble in sodium bicarbonate solution, but analysis showed it to be (CXXV).

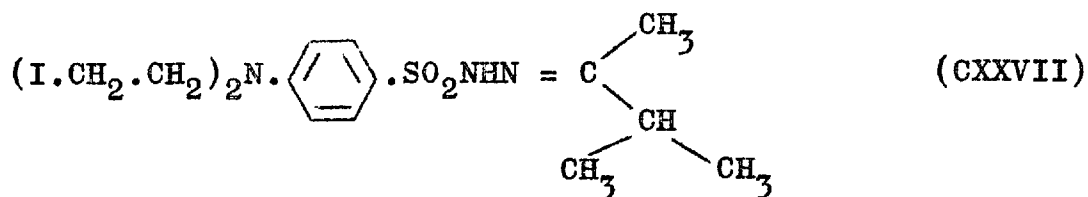


It has been noted (see page 19) that variation of the β halogen atoms of nitrogen mustards can alter the reactivity of the nitrogen mustard moiety. This has been found to be the case with the sulphonamides. The chlorine analogues²⁴ of (CVII) and (CVIII) are biologically less effective than the bromo compounds. The iodo derivatives usually show a decrease in activity. However, it was decided to prepare two iodo-derivatives to see if there was any advantage to be gained from the use of this halogen with this particular type of nitrogen mustard derivative.

The compound (CXXVI) was prepared from the bromo analogue by the use of sodium iodide.

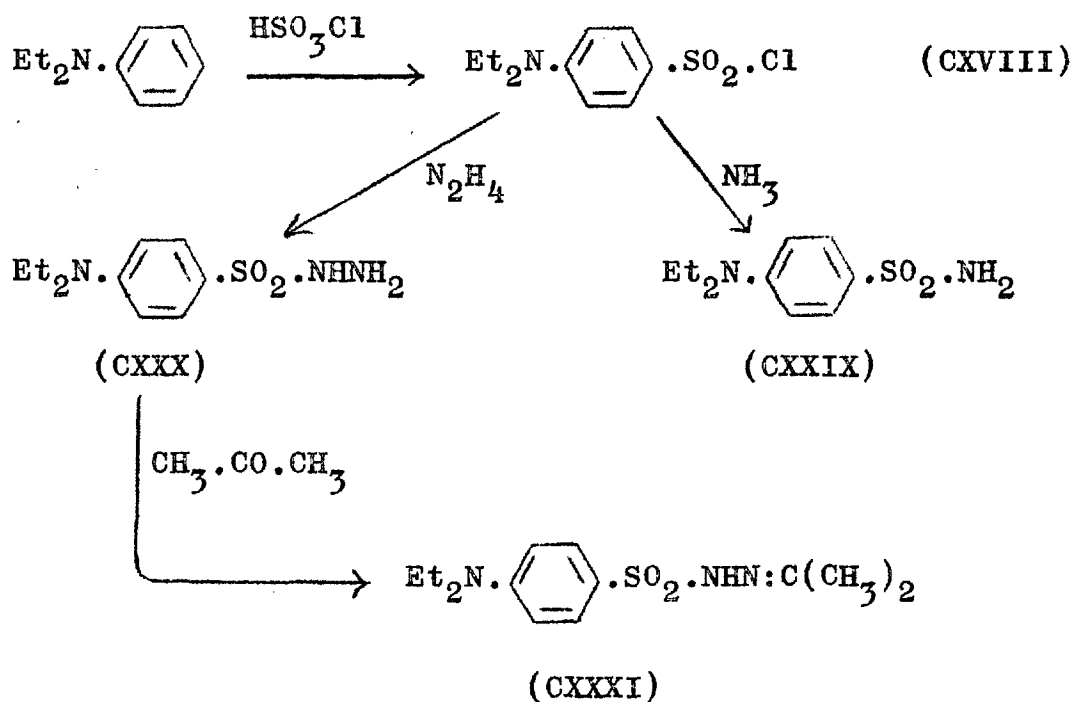


The other iodo derivative made was an ω -substituted derivative of the iodo nitrogen mustard sulphonhydrazide, i.e. (CXXVII).



This was prepared from the bromo nitrogen mustard hydrazide (CVIII) by the action of excess sodium iodide in methyl isopropyl ketone.

It was hoped that potentiation of the nitrogen mustard sulphonamides would increase their activity, and for this reason the following compounds were made:-

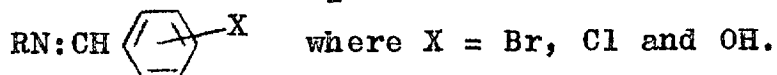


NN-Diethylaniline was reacted with chlorosulphonic acid in carbon tetrachloride to give, in good yield, the sulphonyl chloride (CXVIII) which was characterized as its anilide. The sulphonyl chloride reacted easily with ammonia, to give (CXXIX) and hydrazine hydrate to give (CXXX). The sulphonhydrazide was further reacted with acetone to give (CXXXI), which may be a potentiator for (CXXVII).

SOME SCHIFF'S BASES

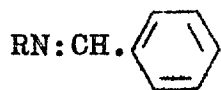
⁴⁶
Grice made a series of substituted benzyl halides one of which, *p*-aldehydebenzyl chloride, had some anti-tumoural activity. It was decided to couple this with the amino nitrogen mustard via the Schiff base. With this in mind it was decided to prepare a short series of these Schiff bases, using various substituents on the benzene ring of the aldehyde moiety in the hope of varying the activity of the drugs.

Popp²¹⁸ has prepared a few Schiff bases of the amino nitrogen mustard (RNH₂), of the general formula

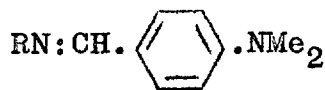


He has reported that they are very toxic, although no comparative figures are given between the Schiff bases and the free amino nitrogen mustard. However, all of his substituted Schiff bases have electron donating groups on the benzene ring of the aldehyde moiety and this may explain the high toxicity. Although this was not the reason for preparing the Schiff bases described in this thesis, the series does include substituents of both the electron withdrawing and donating type, so it could be interesting to compare the toxicities of these compounds with the unsubstituted one (CXXXII).

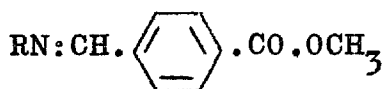
The general method of preparation was to add the substituted benzaldehyde to the amino nitrogen mustard, which had been previously liberated from its hydrochloride salt, using chloroform for both reactants. The mixture was left at room temperature for a period of days, or, when it was heterogeneous, shaken for several weeks, and then concentrated and chromatograph^{ed} on activated alumina. The compounds produced are shown below:-



(CXXXII)



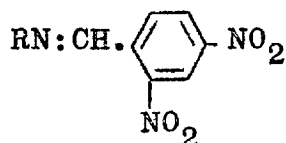
(CXXXIII)



(CXXXIV)



(CXXXV)



(CXXXVI)

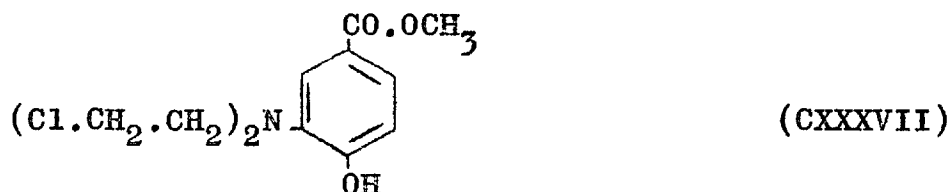
The unsubstituted Schiff base (CXXXII) was prepared as a reference standard in order to compare any changes in activity of the substituted derivatives. If there is any correlation between the type of substituent and the toxicity then compound (CXXXIII) should be more toxic than (CXXXII) due to the feed in of electrons to the benzene ring. The

derivatives (CXXXIV) and (CXXXV) should be, by virtue of the substituents withdrawing electrons from the benzene ring, the reverse of this. The compound (CXXXV) was also produced in order to increase the solubility of the drug. Because the - N = C - linkage is easily hydrolysed¹⁰ it was decided not to attempt to hydrolyse the methyl ester derivative (CXXXIV) to obtain the acid (CXXXV), but first to hydrolyse the methyl ester of *p*-aldehydobenzoic acid and then condense the free aldehydo-acid with the amino nitrogen mustard (RNH₂). This produced (CXXXV). The 2,4-dinitro compound (CXXXVI) should be a very deactivated compound, by virtue of the strong electron withdrawing effects of these substituents on the benzene ring. But because this moiety is in its own right a toxic substance the derivative (CXXXVI) could well be highly toxic.

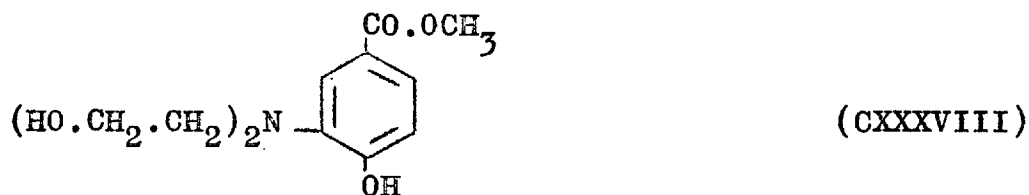
SOME DIFUNCTIONAL AROMATIC NITROGEN MUSTARDS

In order to confer more specificity to the aromatic nitrogen mustard moiety it was decided to prepare a number of difunctional nitrogen mustards, such that several groups could be attached to the various functions in order that a number of biological variables could be accommodated in one drug. At the time of writing only one of these difunctional nitrogen mustards has been completely synthesized; two others, which will be mentioned, are in the penultimate stage of their synthesis.

The completed one (CXXXVII) was prepared from orthocaine



(methyl 3-amino-4-hydroxybenzoate), by the action of ethylene oxide in aqueous acetic acid, to give (CXXXVIII).

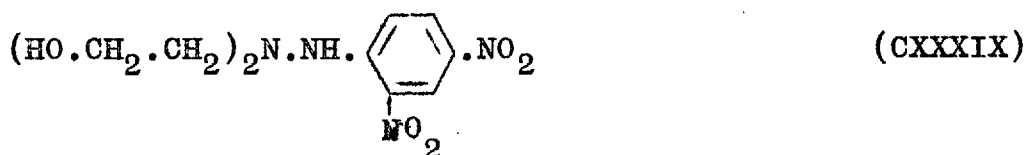


This was then halogenated using thionyl chloride in benzene, by boiling under reflux for 20 minutes to give (CXXXVII).

The methyl ester was found to be untouched by this treatment (cf.¹⁰) because both (CXXXVII) and (CXXXVIII) showed strong

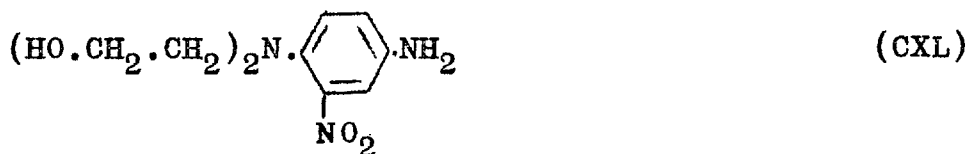
infrared absorption peaks in identical positions, i.e. 1700 cm^{-1} and also the product (CXXXVII) was insoluble in saturated sodium bicarbonate solution.

One of the difunctional compounds which represents the penultimate stage of a synthesis is (CXXXIX).

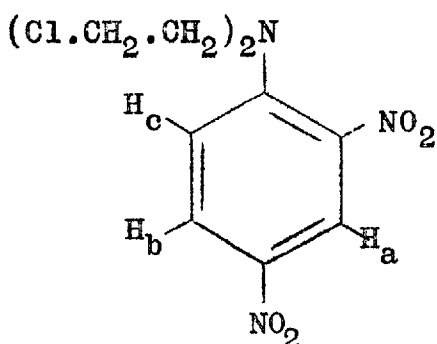


This was prepared from NN-diethanolhydrazine⁶¹ and 2,4-dinitrochlorobenzene in methanol. The reaction mixture was boiled under reflux; in the middle of this process alcoholic potash was added in order to neutralize the hydrogen chloride produced by the reaction.

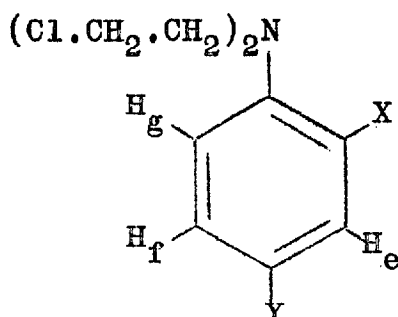
The other nitrogen mustard precursor produced was (CXL)



This was prepared from 2,4-dinitro-p-(NN-di-2-hydroxyethylamino)benzene⁵³ (CXLI) by the action of sodium polysulphide which is known to preferentially reduce only one of the two nitro groups of dinitrobenzene. The p-amino function was thought to be the product, as shown in (CXL); conclusive proof of this structure was obtained by consideration of the N.M.R. spectrum.



(CXLI)



(CXL)

(CXL) a) $\text{X} = \text{NO}_2$, $\text{Y} = \text{NH}_2$

(CXL) b) $\text{X} = \text{NH}_2$, $\text{Y} = \text{NO}_2$

The lettered protons of (CXLI) and (CXL) can be unambiguously identified from the N.M.R. spectra of the compounds (measured on solutions in acetone). The chemical shifts of these protons (as tau values relative to t.m.s.,) are

Dinitro-compound	Amino-nitro-compound
$\text{H}_a = 1.50 \text{ p.p.m.}$	$\text{H}_e = 2.45 \text{ p.p.m.}$
$\text{H}_b = 1.82 \text{ p.p.m.}$	$\text{H}_f = 2.52 \text{ p.p.m.}$
$\text{H}_c = 2.50 \text{ p.p.m.}$	$\text{H}_g = 2.84 \text{ p.p.m.}$

The correct structure will be found by comparison of the shifts of H_e and H_f with those of H_a and H_b . If group X had been reduced then the ortho proton (H_e) would have

shifted far greater than that (H_f) of the para position²¹⁹ when compared with H_a and H_b of (CXLI). However, if group Y was reduced then both the ortho protons (H_e and H_f) would have been shifted by approximately the same amount when compared with H_a and H_b . It will be noticed from the table above that the observed shifts were 0.95 and 0.70 p.p.m. respectively. This difference is not too large and most certainly not as large as would be required if X had been reduced. Thus the structure (CXL)a was assigned to the reduction product.

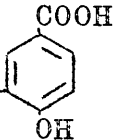
EXPERIMENTAL SECTION

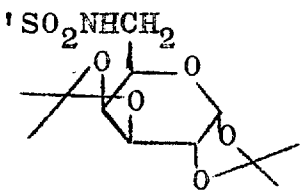
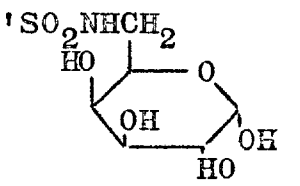
The figures in brackets directly after the name of the substance indicates the page number in the original laboratory note books. .

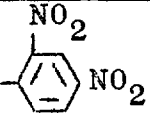
The abbreviations R' and R" stand for the bromo and iodo analogue of p-(NN-di-2-haloethylamino)benzene respectively.

In the following tables the solvents employed for recrystallization are denoted by letters. The key is as follows:-

A	=	Acetone
B	=	Benzene
C	=	Chloroform
CT	=	Carbon tetrachloride
E	=	Ether
Pet	=	Petroleum

I.C. No.	COMPOUND	M.Pt.	Recrystal- lization solvent	FOUND			MOLECULAR FORMULAE	% yield	THEORY			PAGE NO.
				C	H	Br			C	H	Br	
146	$R'SO_2NH$  OH	160-161°	A-CT (1:1)	40.19	3.79	33.40	$C_{16}H_{18}Br_2N_2O_3S$	56	40.83	4.33	33.57	25
147	$R'SO_2NH$  SO_2NH_2	195-196°	A-CT			29.28	$C_{16}H_{19}Br_2N_3O_4S_2$	53			29.44	24, 30.
148	$R'SO_2NH$  $NHCOCH_3$	222-223°	A	42.33	4.30	30.80	$C_{18}H_{21}Br_2N_3O_3S$	63	41.64	4.08	30.78	47
149	$R'SO_2NH$  $COOH$	216°	C-Pet (b.p. 60- 80°) (1:1)			31.18	$C_{17}H_{18}Br_2N_2O_4S$	35			31.54	65, 73, 363.
150	$R'SO_2NH$ 	232°	A-CT (1:1)	39.68	3.99	30.53	$C_{17}H_{18}Br_2N_2O_5S$	34	39.10	3.47	30.60	63, 75.

I.C. No.	COMPOUND	M.Pt.	Recrystal- ization Solvent	FOUND				MOLECULAR FORMULAE	% yield	THEORY				Page No.
				C	H	Br	N			C	H	Br	N	
182		173° 174°	A-Pet (b.p. 40-60°)			25.80	4.49	C ₂₂ H ₃₂ Br ₂ N ₂ O ₇ S	35			25.66	4.36	374, 376.
183		205°	C-Pet (b.p. 40-60°)			28.89		C ₁₆ H ₂₄ Br ₂ N ₂ O ₇ S	6			29.15		385
155	RNHSO ₂  NHCOCH ₃	203°	A-B	50.32	5.02	<u>Cl</u> 16.55		C ₁₈ H ₂₁ Cl ₂ N ₃ O ₃ S	30	50.13	5.11	<u>Cl</u> 16.44		94
154	R'SO ₂ NHNH  COOH	194°	B	41.79	4.01	29.51		C ₁₇ H ₁₉ Br ₂ N ₃ O ₄ S + $\frac{1}{4}$ mole of benzene.	65	41.12	3.91	29.44		102 115

I.C No.	COMPOUND	M.Pt.	Recrystal- lization Solvent	FOUND				MOLECULAR FORMULAE	% yield	THEORY				Page No.
				C	H	Br	N			C	H	Br	N	
170	$R'SO_2NHNH-$ 	182°	B	35.70	3.32	27.61		$C_{16}H_{17}Br_2N_5O_6S$ + $\frac{1}{4}$ mole of benzene	50	35.73	3.52	27.26		101
173 [⊗]	$R'SO_2NHN(COOCH_3)_2$	171°	CT-A-Pet (b.p.60- 80°) (1:1:1)	32.93	3.77	31.16	8.26	$C_{14}H_{19}Br_2N_3O_6S$	40	32.50	3.70	30.96	8.13	327
153	$R'SO_2O$  $NHCOCH_3$	150°	A			30.51	5.38	$C_{18}H_{20}Br_2N_2O_4S$	58			30.71	5.39	57, 78, 275, 328.
152	$R'SO_2O$  $COOCH_3$	122- 123°	A			30.56		$C_{18}H_{19}Br_2NO_5S$	40			30.66		34
185	$R'SO_2O$  $COOCH_2Ph$	105- 106°	B	50.25	3.98	25.52	2.43	$C_{24}H_{23}Br_2NO_5$ + $\frac{1}{4}$ mole of benzene	43	49.64	4.00	25.88	2.27	259, 265, 276.

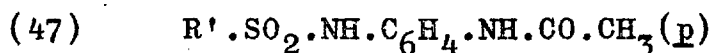
[⊗]For method of preparation see p.175.

SOME DERIVATIVES OF THE NITROGEN MUSTARD SULPHONYL CHLORIDE

The following method is one example of the general procedure adopted for some of the compounds described in this thesis. For a list of compounds prepared by this method with their physical constants and analysis, see the Tables on p. 171-173

General Method of preparation

p[p-(NN-Di-2-bromoethylamino)benzenesulphonamido]acetanilide.



To a solution of p-aminoacetanilide (3.75 g.) in pyridine (25 ml.) was added dropwise with stirring a solution of p-(NN-di-2-bromoethylamino)benzenesulphonyl chloride (10.14 g.) in pyridine (20 ml.). The mixture was left at room temperature for 24 hours, before being added dropwise, with stirring, to an excess of ice-cooled 2N hydrobromic acid. The precipitate was collected and dried, and the filtrate, after being saturated with salt, was extracted with chloroform (3 x 20 ml.). The solid was dissolved in this extract which was then dried ($MgSO_4$), concentrated to small bulk and chromatographed (alumina, acetone). The eluate was evaporated from acetone to give the sulphonamide (8.20 g., 63%) m.p. 222-223°. Analysis: see Table.

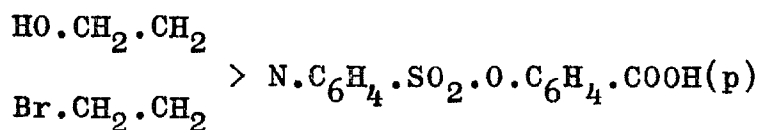
Other methods of preparation

The following compounds were not prepared by the general method described above.

ω -NN-Bismethoxycarbonyl-p-(NN-di-2-bromoethylamino)benzenesulphonhydrazide. (327) $R'.SO_2.NH.N(CO.OCH_3)_2$

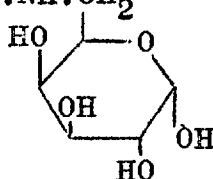
(p-NN-Di-2-bromoethylamino)benzenesulphonhydrazide (4.01 g.) was dissolved in chloroform (40 ml.) to which was added, with stirring, finely divided potassium carbonate (0.69 g.). Methyl chloroformate (0.8 ml.) in chloroform (10 ml.) was then introduced dropwise at such a rate that the temperature did not rise above 30°. The mixture was stirred for 2 days, and left at room temperature for a further 3 weeks, and then filtered. The filtrate was concentrated and chromatographed (alumina, chloroform). The infrared absorption spectrum of the major fraction had a band at 1738 cm⁻¹ (ester) and its melting point (169-170°) was depressed on addition of the original hydrazide. Recrystallization from a mixture of carbon tetrachloride, acetone, and petroleum (b.p. 60-80°) (in equal proportions) gave the ω -disubstituted sulphonhydrazide (1.85 g., 40%) m.p. 171°. Analysis: see Table.

p-[p-(N-2-Bromoethylamino-N-2-hydroxyethylamino)benzenesulphonyloxy]benzoic acid. (345)



Benzyl p-[p-(NN-di-2-bromoethylamino)benzenesulphonyloxy] benzoate (1.37 g.) was dissolved in dry ethyl acetate (20 ml.) with 10% palladium-charcoal catalyst (1 g.) and hydrogenolysed at atmospheric pressure. After $2\frac{1}{2}$ hours the uptake of hydrogen (54 ml.) ceased; the reaction mixture was filtered and concentrated, to give a solid, which was soluble in sodium bicarbonate solution. It was crystallized from a mixture of acetone and petroleum (b.p. $60-80^{\circ}$), or from chloroform, to give the sulphonyloxy compound m.p. 196° . (Found: C, 48.04; H, 4.25; N, 3.17; O, 18.56; S, 7.37. $C_{17}H_{18}BrNO_5S$ requires C, 47.68; H, 4.24; N, 3.27; O, 18.56; S, 7.37%).

6-p-(NN-Di-2-bromoethylamino)benzenesulphonamido-6-deoxy-
D-glactose. (385) $R'.SO_2.NH.CH_2$



6-p-(NN-Di-2-bromoethylamino)benzenesulphonamido-6-deoxy-1,2-3,4-di-O-isopropylidene-D-galactose (0.2633 g.) was dissolved in dioxan (7 ml.) and 5% hydrobromic acid (3 ml.) and the optical rotation observed $[\alpha]_D^{22} -18.5^{\circ}$. After six hours at room temperature no change in rotation had been found, so the solution was kept for 3 hours at

60°, the rotation changing to a constant reading of $[\alpha]_D^{22} +26.6$ calculated on starting material. The solution was saturated with salt and extracted with ethyl acetate (3 x 10 ml.). The extracts were dried (MgSO_4), and concentrated to a gum, which crystallized from a mixture of chloroform and petroleum (b.p. 40-60°) to give the sulphonamide (15 mg., 6%) m.p. 204-205°. Analysis: see Table.

p-(NN-Di-2-bromoethylamino)benzenesulphonamide.²¹⁰ (290)

$\text{R}' \cdot \text{SO}_2 \cdot \text{NH}_2$

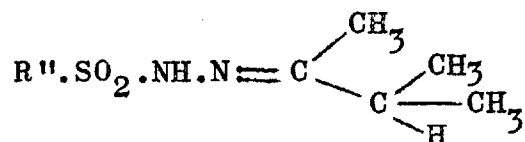
This was prepared by the method of Creighton in 74% yield, and had m.p. 118-119°. Lit., 74% yield, m.p. 117.5-118°.

p-(NN-Di-2-iodoethylamine)benzenesulphonamide. (414, 427)

$\text{R}'' \cdot \text{SO}_2 \cdot \text{NH}_2$

Sodium iodide (20 g.) was dissolved in boiling methyl isopropyl ketone (210 ml.), to which was added p-(NN-di-2-bromoethylamino)benzenesulphonamide (5.0 g.) in methyl isopropyl ketone (50 ml.) and boiled under reflux for 8½ hours. On cooling the precipitated sodium salts were filtered off and the filtrate concentrated to give a yellow solid. It was dissolved in acetone and passed through an ion exchange column (Zeo-Karb 225), to give the iodosulphonamide (3.2 g., 51%) m.p. 165°. (Found: C, 25.28; H, 2.89; I, 52.58; N, 5.85. $\text{C}_{10}\text{H}_{14}\text{I}_2\text{N}_2\text{O}_2\text{S}$ requires C, 25.01; H, 2.94; I, 52.84; N, 5.83%).

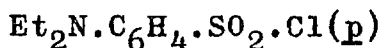
p-(NN-di-2-iodoethylamino)benzenesulphonhydrazone of methyl isopropyl ketone. (417)



Sodium iodide (8 g.) was dissolved in boiling methyl isopropyl ketone (80 ml.) to which was added p-(NN-di-2-bromoethylamino)benzenesulphonhydrazide (2 g.) in methyl isopropyl ketone (20 ml.), and boiled under reflux for 90 minutes. On cooling the precipitated sodium salts were filtered off, and the filtrate concentrated to give a brown solid, which was dissolved in chloroform and the insoluble material filtered off. The filtrate was chromatographed (alumina, chloroform) to give the hydrazone, (which crystallized from chloroform-petroleum (b.p. 40-60°)) m.p. 151°. (Found: C, 32.40; H, 4.43; I, 45.14; N, 7.61. $C_{15}H_{23}I_2N_3O_2S$ requires C, 32.00; H, 4.11; I, 45.06; N, 7.46%).

SOME MODEL SULPHONAMIDES

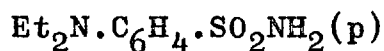
p-(NN-Diethylamino)benzenesulphonyl chloride. (312)



Chlorosulphonic acid (60 ml.) was added dropwise to a vigorously stirred, ice cooled solution of NN-diethylaniline in carbon tetrachloride (20 ml.). When the addition was

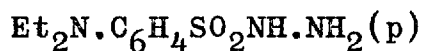
complete, the reaction mixture was heated to 130° for 4 hours, cooled, poured into ice, and extracted into chloroform (total 1 litre). It was dried (MgSO_4) and concentrated to give a solid, which was dissolved in benzene and shaken with alumina. The yellow solution was filtered and concentrated to give the sulphonyl chloride (36.7 g., 70%). It was recrystallized from benzene-petroleum (b.p. $60-80^{\circ}$) and had m.p. 86° . (Found: C, 48.63; H, 5.77; N, 5.74. $\text{C}_{10}\text{H}_{14}\text{ClNO}_2\text{S}$ requires C, 48.49; H, 5.70; N, 5.66%). The acid chloride was characterized as the sulphonanilide, (which was recrystallized from chloroform-petroleum (b.p. $60-80^{\circ}$)) m.p. 176° . (Found: C, 63.05; H, 6.42; N, 8.97. $\text{C}_{10}\text{H}_{20}\text{N}_2\text{SO}_2$ requires C, 63.13; H, 6.62; N, 9.20%).

p-(NN-Diethylamino)benzenesulphonamide. (314)



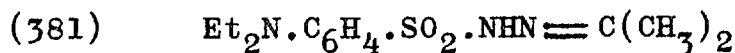
p-(NN-Diethylamino)benzenesulphonyl chloride (6 g.) was dissolved in a stirred solution of acetone (30 ml.), to which was added ammonia (d. 0.880) (20 ml.). The mixture was allowed to stand for 2 hours, before being poured into water (500 ml.). The precipitate was collected, dried and recrystallized from chloroform-petroleum (b.p. $60-80^{\circ}$) to yield the sulphonamide (4.5 g., 81%), m.p. 141° . (Found: C, 52.46; H, 6.98; N, 12.19. $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_2\text{S}$ requires C, 52.57; H, 7.07; N, 12.27%).

p-(NN-Diethylamino)benzenesulphonhydrazide. (315)

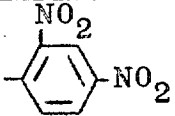


p-(NN-Diethylamino)benzenesulphonyl chloride was added to a stirred solution of hydrazine hydrate (25 ml.), in acetone (40 ml.), cooled in ice. The mixture was left for 12 hours and then was poured into excess water; the oily precipitate crystallized on scratching and cooling. It was collected, dried and recrystallized from chloroform-petroleum (b.p. 60-80°) to yield the sulphonhydrazide (7 g., 66%), m.p. 130°. (Found: C, 49.79; H, 6.97; N, 17.53; S, 12.89. $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$ requires C, 49.37; H, 7.05; N, 17.28; S, 13.15%).

ω -N'-Isopropylidene-p-(NN-diethylaminobenzenesulphonhydrazide.



p-(NN-Diethylamino)benzenesulphonhydrazide was dissolved in acetone (20 ml.) and left to stand for 7 weeks. The acetone was distilled off to leave an oily solid which was chromatographed on alumina, using chloroform as eluent, to give the hydrazone, (which was recrystallized from chloroform) m.p. 182°. (Found: C, 54.89; H, 7.56. $\text{C}_{13}\text{H}_{21}\text{N}_3\text{O}_2\text{S}$ requires C, 55.08; H, 7.47%).

I.C. No.	COMPOUND	M.Pt.	Recrystal- ization Solvent	FOUND				MOLECULAR FORMULAE	% yield	THEORY				Page No.
				C	H	Cl	N			C	H	Cl	N	
160	RN:CHC ₆ H ₅	79°	B-Pet (b.p.40- 60°) (1:1)			22.4	8.86	C ₁₇ H ₁₈ Cl ₂ N ₂	53			22.17	8.72	197, 232.
163	RN:CH-  -COOCH ₃	129°	C-B			18.44	7.23	C ₁₉ H ₂₀ Cl ₂ N ₂ O ₂	74			18.69	7.39	233
164	RN:CH-  -NO ₂	189°	B-Pet (b.p.40- 60°)			17.16		C ₁₇ H ₁₆ Cl ₂ N ₄ O ₄	63			17.25		234
176	RN:CH-  -COOH	197°	B	58.89	5.27		7.51	C ₁₈ H ₁₈ Cl ₂ N ₂ O ₂	64	59.13	4.96		7.66	357
184	RN:CH-  -NMe ₂	126°	C-E (1:1)			19.48	11.37	C ₁₉ H ₂₃ Cl ₂ N ₃	80			19.46	11.53	375

SOME SCHIFF'S BASES

p-Aldehydobenzoic acid. (266)

Methyl p-aldehydobenzoate (0.5 g.) was boiled under reflux with 2N hydrochloric acid (25 ml.) and glacial acetic acid (1 ml.), for 2 hours. On cooling, white crystals were deposited; they were collected, washed and dissolved in saturated sodium bicarbonate solution and reprecipitated with dilute hydrochloric acid. The white solid was filtered off, washed and dried, to give the free acid (0.34 g., 76%), m.p. 254°, Lit, 256°.

The following method is one example of the general procedure adopted for this series of compounds. For a list of the compounds prepared, their physical constants and analyses, see the Table on p.181.

N'-p-Carboxybenzylidene-p-(NN-di-2-chloroethyl)-p-phenylenediamine. (357) RN:CH.C₆H₄COOH(p)

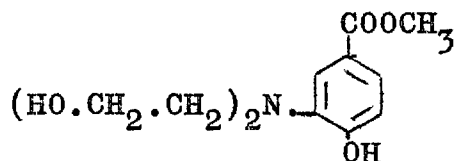
Nitrogen mustard A(I) (0.6 g.) in chloroform (10 ml.) was added to a suspension of p-aldehydobenzoic acid (0.34 g.) in chloroform (25 ml.). The mixture was shaken for 6 weeks and the unreacted material was filtered off. The filtrate was concentrated and chromatographed (alumina, benzene), to yield the Schiff's base (0.52 g., 64%) which was

recrystallized from benzene; it had m.p. 197° . Analysis: see Table.

A DIFUNCTIONAL AROMATIC NITROGEN MUSTARD

Methyl p-hydroxy-m-(NN-di-2-hydroxyethylamino)benzoate.

(397, 401)



Methyl p-hydroxy-m-aminobenzoate (3.34 g.) was suspended in a mixture of water (15 ml.) and glacial acetic acid (50 ml.). To this suspension was added ethylene oxide (16 ml.), and the mixture shaken for 24 hours. The acetic acid was distilled off and the residue made alkaline with saturated sodium bicarbonate solution, and extracted into ethyl acetate (3 x 30 ml.). The ethyl acetate extracts were combined and dried (MgSO_4) and concentrated to give a gum which crystallized from benzene to yield the hydroxyalkylated amine (3.06 g., 60%), m.p. 79° , ν_{max} 1700 cm^{-1} ($-\text{COOCH}_3$ aromatic). (Found: C, 56.57; H, 6.80; N, 5.46. $\text{C}_{12}\text{H}_{17}\text{NO}_5$ requires C, 56.46; H, 6.71; N, 5.49%).

Methyl p-hydroxy-m-(NN-di-2-chloroethylamino)benzoate.

(437, 439)

Methyl p-hydroxy-m-(NN-di-2-hydroxyethylamino)benzoate (3.4 g.) was dissolved in dry benzene (25 ml.), to which

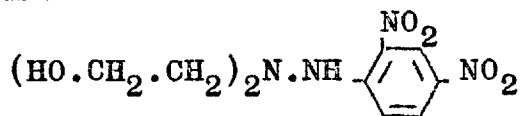
was added redistilled thionyl chloride (12 ml.). The mixture was boiled under reflux for 20 minutes, then the precipitated solid was filtered off and added to ammonia (d. 0.880) (100 ml.), and extracted into chloroform (50 ml.). The extract was washed with water (3 x 50 ml.), dried (MgSO_4) and concentrated to an oil. This was dissolved in acetone and decolourized (charcoal), then concentrated to an oil which was chromatographed (alumina, chloroform) to give the difunctional nitrogen mustard, (which crystallized from acetone petroleum), m.p. 76° , ν_{max} 1700 cm^{-1} ($-\text{COOCH}_3$ aromatic). (Found: C, 49.46; H, 5.36; N, 4.34. $\text{C}_{12}\text{H}_{15}\text{Cl}_2\text{NO}_3$ requires C, 49.32; H, 5.18; N, 4.78%). It was insoluble in saturated sodium bicarbonate solution.

Some precursors of difunctional nitrogen mustards

NN-Diethanolhydrazine⁶¹ (281)

This was prepared by the method according to Plisov, from ethylene oxide and hydrazine hydrate. It had b.p. $138^\circ/8.6 \times 10^{-3} \text{ mm.}$

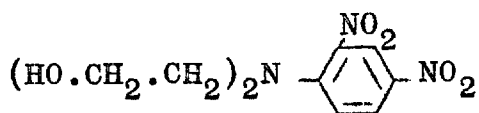
2,4-Dinitro-p-(NN-di-2-hydroxyethylhydrazino)benzene. (283)



NN-Diethanolhydrazine (6.01 g.), 2,4-dinitrochlorobenzene (10.13 g.) in methanol (70 ml.), were boiled under

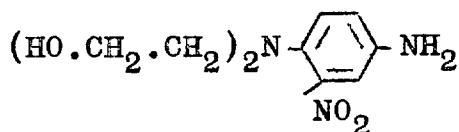
reflux for 2 hours. 1.4N Alcoholic potash (5 ml.) was then added to the mixture which was boiled under reflux for a further 2 hours. The ethanol was distilled off to give an oily solid which was recrystallized from water to yield the trisubstituted hydrazide, m.p. 145°. (Found: C, 41.73; H, 4.67. $C_{10}H_{14}N_4O_6$ requires C 41.94; H, 4.92%.)

2,4-Dinitro-p-(NN-di-2-hydroxyethylamino)benzene⁵³
(235, 286)



This was prepared by the method of Ross from ethanolamine and 2,4-dinitrochlorobenzene, to give a yellow crystalline compound. It had m.p. 98°; γ , 1.50, 1.82, 2.50 p.p.m.

2-Nitro-4-amino-p-(NN-di-2-hydroxyethylamino)benzene.
(280)



2,4-Dinitro-p-(NN-di-2-hydroxyethylamino)benzene (5.66 g.) was dissolved in vigorously stirred boiling water (60 ml.) to which was added sodium polysulphide (5.57 g.). After 45 minutes the mixture was cooled and the precipitate filtered off. The precipitate was boiled for 15 minutes in 10% hydrochloric acid (40 ml.) and the sulphur filtered off. Excess ammonium hydroxide solution precipitated the required compound, which was recrystallized from water to

yield the p-amino compound, m.p. 115° ; τ , 2.45, 2.52, 2.84 p.p.m. (Found: C, 49.98; H, 6.16. $C_{10}H_{15}N_3O_4$ requires C, 49.78; H, 6.24%). For proof of structure see page 167.

QUANTITATIVE HYDROLYSES

The standard conditions used by Ross were employed to determine the extent of hydrolysis of the nitrogen mustards in the following table. The mustard (0.5 m.mole) was dissolved in acetone (25 ml.), water (25 ml.) was added and the solution was boiled (66°) under reflux for 30 minutes, then cooled quickly in ice and the liberated hydrogen ion determined by the use of standard sodium hydroxide, employing methyl red (M) or phenolphthalein (P) as indicator.

In some cases these conditions were modified, due to lack of material, or insolubility of the nitrogen mustard derivative. Where the proportion of acetone used was higher than 1:1, then the hydrolysis figures will be lower than those conducted under standard conditions.

COMPOUND	I.C. No.	Wt. taken (mg.)	Vol. Me ₂ CO (ml.)	Vol. H ₂ O (ml.)	H ⁺ % (indic.)
R'SO ₂ NH-  -OH	146	239.1	25	25	5 (M)
R'SO ₂ NH-  -SO ₂ NH ₂	147	270.7	25	25	4 (M)
R'SO ₂ NH-  -COOH	149	65.0	25	25	2 [⌘] (M)
R'SO ₂ O-  -NHCOCH ₃	153	260	25	25	2 (M)
R'SO ₂ NHNH-  -COOH	154	95.5	11	9.2	16 [⌘] (P)
RNHSO ₂ -  -NHCOCH ₃	155	200.2	25	25	13 (M)

[⌘]This is a corrected figure, arrived at after allowing for the sodium hydroxide required to neutralize the carboxylic acid.

BIOLOGICAL RESULTS

BIOLOGICAL RESULTS

The biological examination of some of the compounds produced was conducted under the direction of Professor J. F. Danielli, at King's College, London, by his co-workers.

Special and grateful acknowledgment is made to Dr. R. I. Hawkins for the full and concise information which she has made available, and which is summarized below.

TEST CONDITIONS.

Animals. All the animals used in the experimental work were Wistar albino rats.

Tumour. The tumour used was the Walker Carcinoma 256 which occurs in two forms, solid and ascites (suspension of tumour cells). The ascites tumour was not used for experimental work, but was used to form the solid tumour after 5 days' growth in the peritoneal cavity.

Administration of Drugs. This depended primarily on their solubility. The drug was suspended in Arachis oil or dissolved in saturated bicarbonate solution and the pH of the solution adjusted to 7. The intramuscular route was used when the volume to be injected was less than 0.5 ml. The intra-peritoneal injections were given when the volume of

material was from 0.5 ml. to 1.0 ml. The oral route was used when even larger volumes of material had to be administered.

Toxicity. This was determined on non-tumour-bearing rats, and although the more popular toxicity is the LD₅₀, which is the dose at which 50% of the animals die, here the median lethal dose (M.L.D.) was determined. This is the mean between the lowest dosage which would kill the rat and the highest dosage from which it would recover.

COMPOUND given as I.C. No. See p.171	MLD mg./100 g. Rat	Route	No. of Rats	% of tumour bearing Rats showing GI
146	150+	im	7	Nil
147	100+	im and ip	7	40
148	100+	im and ip	11	20
149	50	im and ip	8	45
150	60+	im and ip	7	15
153	100+	im and ip	10	30
154	100+	im and ip	10	30
170	100+	im and ip	6	16

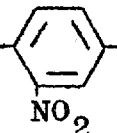
I.C. No.	COMPOUND	HALOGEN	ACUTE LD ₅₀ (mice) mg./Kg.	CHRONIC LD ₅₀ (mice) mg./Kg.	CHRONIC LD ₅₀ (rats) mg./Kg.
20 ⁺	RSO ₂ NH ₂	Cl	600	450	>400 [ⓧ]
56 ⁺⁺	R'SO ₂ NH ₂	Br	>2000	225	80 [ⓧ]
188	R''SO ₂ NH ₂	I	>2000	>600 [ⓧ]	>200 [ⓧ]

[ⓧ]Preliminary Result only.

⁺Prepared by Benn.²⁴

⁺⁺Prepared by Creighton.²¹⁰

In the foregoing biological results the route chosen for administration of the drugs is given, where im = intramuscular; ip = intraperitoneal and sc = subcutaneous.

COMPOUND	LD ₅₀ mice mg./Kg.	Dose mg./Kg./ day.	Route	No. of rats	Effect on Tumour	Effect on Rat % wt. loss	Anti- tumour activity
RN:CHC ₆ H ₅	60	40 x 5 24 x 5 12 x 5	im im im	2 1 2	PR GI GI	30 5 5	++
RN:CH-  -NO ₂	>1000	400 x 3	sc	2	Nil	5	-
RN:CH-  -NMe ₂	< 500	100 x 5	im	2	PR	15-30	++

CR = Complete Regression; PR = Partial Regression; GI = Growth Inhibition.

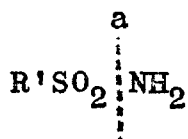
DISCUSSION

The success of the sulphonamide drugs $R'SO_2NH_2$ (CVII) and $R'SO_2NH.NH_2$ (CVIII) prepared by Creighton²¹⁰ initiated a programme of preparation of substituted sulphonamides which was continued by White²⁵ and subsequently extended by the present writer.

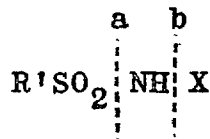
When (CVII) and (CVIII) were administered in amounts required to give 50% and 68% complete regressions of the tumour a novel type of toxicity action was exhibited, since many deaths occurred 12 to 102 days after the commencement of treatment. To explain this action it was suggested that a folic acid deficiency might be caused by these two drugs, by showing similar activity to anti-bacterial sulphonamides. However, administration of folic or folinic acids failed to produce any effect. A microbiological assay of the liver and intestinal epithelium of rats with these apparent folic acid deficiency symptoms showed that there was no lack of either folic or folinic acids. Substitution of groups on the sulphonamido or sulphonhydrazido moieties resulted in a loss of anti-tumoural activity. Thus it can be inferred that a similar action to anti-bacterial sulphonamides is unlikely.²¹³

The loss of anti-tumoural activity in the substituted sulphonamides and sulphonhydrazides is very interesting even though it is not explainable. If comparison is made between

(CVII) and (CV) it is obvious that no enzyme is present or is produced which would cause fission (b), otherwise the free



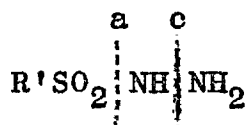
(CVII)



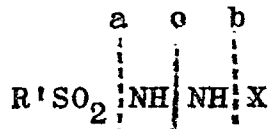
(CV)

sulphonamide would be available in vivo. Again the sulphonamido linkage cannot be split by method (a), since both drugs considered would produce the RSO_2^- ion which would produce in vivo $\text{R}'\text{SO}_2\text{OH}$. If this occurred then all the sulphonamide drugs would have comparable activity, which is not the case. Thus it would appear that it is imperative to have the free - SO_2NH_2 group present.

Comparison of (CVIII) and (CXLII) leads one to similar



(CVIII)



(CXLII)

conclusions. Fission at (b) would release (CVIII) in vivo, but obviously this does not occur, otherwise a good anti-tumour effect should be observed. Fission of (CXLII) along (c) would give (CVII) but for the same reason one can conclude that it does not occur. Similarly fission of (CXLII) at (a) would give all the sulphonhydrazides a comparable activity. Thus it would appear that it is imperative to

have the free - $\text{SO}_2\text{NH.NH}_2$ group. However, it is realised that (CVIII) could split at (c) to give (CVII), the fact that (CXLII) does not split at (c) does not exclude the possibility of (CVIII) doing so.

Chemically the ω -amino group of both these compounds, i.e. (CVII) and (CVIII) are very different, one (CVII) having an acidic nature compared with the highly basic character of (CVIII). It has been found that whatever group is substituted on these ω -amino positions there is a loss in biological activity. Thus one is led to postulate that the substituted sulphonamides and sulphonhydrazides are sterically incapable of attacking alkylatable centres, whereas the unsubstituted ones can. However, this does not explain the apparent folic acid deficiency symptoms shown by (CVII) and (CVIII). For if these compounds were alkylating pteridine components and so competing with p-aminobenzoic acid for active enzyme sites then there would in fact be a deficiency of folic acid. This, however, is not the case.

The effect on the toxicity of these drugs when the β -halogen atoms are changed is shown in the table, It will be seen that the iodo compound, see p.193, is less toxic than the bromo derivative.

The biological tests on the Schiff bases were conducted in the University of Buffalo by Professor Danielli and his co-workers, using Swiss mice to determine the LD_{50} figures.

Two of the compounds showed reasonable anti-tumour activity, i.e. (CXXXII) and (CXXXIII). The toxicity figures are interesting; (CXXXVI) was the least toxic, and by the hypothesis quoted (p.162) this could be expected by the fact that the 2,4 dinitro groups are removing electrons from the system. Similarly the higher toxicity of (CXXXIII) can be explained by the presumption that electrons are being fed into the system. It would have been thought that the toxicity of the compound (CXXXII) would have occupied an intermediate position between those of (CXXXIII) and (CXXXVI); unfortunately, only an upper limit has so far been recorded for the toxicity of the dimethylamino-compound, and whether it is in fact more toxic than the parent compound (CXXII) has yet to be established.

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