A thesis entitled

"SYNTHETICAL APPROACHES TO POTENTIALLY CYTOTOXIC AROMATIC POLYFUNCTIONAL NITROGEN MUSTARDS"

submitted by

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ABSTRACT

The first part of this work reviews some of the more important aspects of the chemotherapy of cancer with special emphasis on the modes of action of some widely-used chemotherapeutic agents, and on their use in combination therapy in efforts to overcome drug resistance. The design of such agents, particularly of the nitrogen mustard type, is also discussed.

There follows a review of the methods of preparation and chemistry of aryl nitrogen mustards with particular emphasis on the preparation of the intermediate bishydroxyethylamino derivatives via hydroxyethylation of anilines or via the nucleophilic substitution of halogenonitrobenzenes.

The novel use of zinc chloride-catalysed thionyl chloride chlorinations and the use of mesyl chloride in pyridine led to the greatly improved syntheses of methyl 4-hydroxy-3-(bis-2-chloroethylamino)benzoate and of methyl 4-(bis-2-chloroethylamino)salicylate. The N-phenylcarbamate of the former has also been synthesized. Syntheses of methyl 5-(bis-2-chloroethylamino)salicylate, methyl 5-(bis-2-chloroethylamino)-2-nitrobenzoate, and of methyl 4-(NN-di-2-chloroethyl)-2,4-diaminobenzoate and its 2-chlorophenylcarbamate are also described. Part of the work involved studies on the hydroxyethylation of several ring-substituted anilines and considerable variations in reactivity have been revealed. Finally, the successful nucleophilic substitution of dichloronitrobenzenes with diethanolamine led to the syntheses of various aminophenyl chloro nitrogen mustards and to their respective 2-chlorophenylcarbamates.

The research has thus led to the synthesis of new types of polysubstituted aromatic nitrogen mustards, which are of potential interest, particularly in the form of carbamate derivatives, as antitumour agents.

I dedicate this to
Mary Anne
my parents
and
my late grandmother Grazzia Abela.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Professor L. N. Owen for his constant advice and encouragement throughout the course of this work.

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CONTENTS

Page

Introduction..... 8

CHAPTER 1 : CHEMOTHERAPY OF CANCER 11

The Cell Cycle 12

DNA and RNA synthesis 12

Major Types of damage to DNA 16

Chemotherapeutic Agents:

(a) Biological Alkylating Agents 18

(b) Antimetabolites 27

(c) Antibiotics 32

(d) Alkaloids 35

(e) Enzymes 39

(f) Hormones 40

Drug Resistance..... 41

Combination Chemotherapy 44

Drug Design 47

CHAPTER 2 : AROMATIC DIFUNCTIONAL NITROGEN MUSTARDS 50

Preparation of Aryl Dialkanolamines..... 50

Preparation of Aryl Difunctional Nitrogen Mustards 54

Alkylating reactions of Aromatic Nitrogen Mustards 55

Aromatic Nucleophilic Substitution Reactions of Dichloronitrobenzenes..... 61

CHAPTER 3 : DISCUSSION AND RESULTS

Introduction 68

Phenolic Methoxycarbonyl Nitrogen Mustards:

methyl 4-hydroxy-3-(bis-2-chloroethylamino)-benzoate and derivatives 74

methyl 4-(bis-2-chloroethylamino)salicylate and derivatives 76

methyl 5-(bis-2-chloroethylamino)salicylate and derivatives 83

Aminophenyl Methoxycarbonyl Nitrogen Mustards:

methyl 5-(bis-2-chloroethylamino)-2-nitrobenzoate and derivatives 86

methyl 4-(bis-2-chloroethylamino)-2-aminobenzoate and derivatives 92

Aminophenyl Methoxy Nitrogen Mustards:

derivatives of 2-methoxy-4-nitroaniline 95

derivatives of 4-methoxy-2-nitroaniline 96

derivatives of 4-chloro-3-nitroanisole 98

Aminophenyl Chloro Nitrogen Mustards:

derivatives of 4-chloro-2-nitroaniline 101

derivatives of 2-chloro-4-nitroaniline 101

derivatives of 4-chloro-3-nitroaniline 102

from 2,3-dichloronitrobenzene 106

from 2,4-dichloronitrobenzene 106

from 2,5-dichloronitrobenzene 106

from 3,4-dichloronitrobenzene 108

Conclusion 112

CHAPTER 4 : EXPERIMENTAL

Phenolic Methoxycarbonyl Nitrogen Mustards 115

Aminophenyl Methoxycarbonyl Nitrogen Mustards 129

Aminophenyl Methoxy Nitrogen Mustards 143

Aminophenyl Chloro Nitrogen Mustards 151

REFERENCES 167

"..... that whatever has been proposed for the curing of cancers, are merely palliative medicines; and that no real specific has been hitherto discovered for that fatal disorder, although the physicians of all nations, from the time of Hippocrates to the present, have, by numberless researches and experiments, made trial of every thing in nature, from the most innocent drug, to the most virulent poison, both in the mineral and vegetable kingdoms; yet the disease still baffles the power of physic ....."

Burrows
(A New Practical Essay on Cancers)
London, 1767.

INTRODUCTION

Cancer is known to occur in all species of animals and in plants. Geographically, it is found in every corner of the Earth, ethnologically affecting all racial groups and sub-groups.

The "disease" has been known throughout history. Copisarow[1] quoted three references from the Old Testament possibly referring to Cancer; e.g. 11 Chronicles 21: 18-19 may have referred to an "incurable" cancer of the bowel. Archigenes of Apamea, a contemporary of the famous Cornelius Celsus (25B.C. - A.D.50) performed hysterectomies, limb amputations and excisions on mammary carcinomas.

There is some evidence to show that it was once less prevalent than present. Several factors may be attributed to explain the higher incidence of cancer at present. In the first instance, there has been a great reduction in death from infectious diseases and major diseases. This naturally leads to a relative increase in deaths from other causes, as cancer. Secondly, the average human lifespan has increased steadily from around 30 in the first century to around 70 years at present. As cancer shows a strong dependence on age, its incidence would be expected to increase with increased lifespan. Furthermore, diagnosis of diseases has been greatly perfected and deaths from formerly unknown causes may now be ascribed correctly to cancer. Finally, the modern environment may be subjecting living matter to a wide range of carcinogens such as cigarette smoke, questionable food additives, air, water and smoke pollutants, radiations, industrial wastes etc. Whether as a result of the factors specified above or as a result of other unknown causes, cancer has become the second leading cause of death in developed countries.

To define cancer, one must consider a disease that may be induced by a host of chemical, physical and biological agents giving rise to an excessive proliferation of abnormal cells unrestricted to any particular tissue or organ. Cancer is in fact a generic term embodying a whole group of pathologic states with one common factor - disordered growth that is not subject to normal control systems or to pre-existing cell architecture but proliferates continuously, losing all resemblance to its tissue of origin.

It is therefore not surprising that cancer is nowadays one of the major areas of medical, chemical and biological research endeavour giving rise to a co-ordinated approach involving surgery, pathology, clinical medicine, immunotherapy, radiotherapy, genetics, biochemistry

and chemotherapy among others.

The fundamental modes of cancer therapy at present are surgical treatment, radiotherapy, chemotherapy and immunotherapy. In recent years, as a result of using a combination of modalities of two or more of the above, cure rates have been greatly improved.

Surgical treatment has been, up to the 1930's, practically the only mode of treating cancer patients. With the advent of post-operative radiotherapy, cures for cancer improved greatly but only were significant when surgery and radiotherapy were followed by chemotherapy. More recently, the crucial importance of immunotherapy has been established. The existence of tumour-specific antigens and of an immune response to them through the formation of specific antibodies has been now demonstrated in both experimental and human malignant disease[2]. Immunologic factors are therefore important in the prevention of cancer with their defence mechanisms searching and destroying any appearance of malignant cells[3].

Furthermore, the initial in vivo immunological resistance to cancer is important - as indirectly evidenced by the high recurrence of secondary tumour formation in patients receiving immuno-suppressive drugs during organ transplant operations[4]. Cortisone, an immuno-depressant, has also been found to enhance tumour growth as a result of a reduction in the host immunity[5] while many carcinogens produce immuno-suppression resulting in carcinogenesis[6]. Patients who cannot develop certain immune responses, e.g. delayed cutaneous hypersensitivity to 2,4-dinitrochlorobenzene, a skin allergen, have been found to experience an early recurrence of cancer after surgical treatment but patients with average immunologic activity received a good prognosis[7,8].

One of the most important factors governing successful treatment of a cancer patient is the realization that there exists a critical antigenic mass beyond which immunologic defence mechanisms in vivo become ineffective[9]. Thus, in patients with widely disseminated neoplastic tumours, the size of the tumour is usually in the region of 10^{12} cells (about 1Kg. of tumour cells). This would invariably necessitate the removal of the large primary tumour by surgical treatment in an attempt to reduce the amount of tumour cells at any given time to a level that would activate the immunologic defence mechanisms to overcome the microscopic foci of neoplastic tumours.

Various immunotherapeutic techniques have been developed. These include the enhancement of immunogenicity of the tumour cells by tumour vaccines[10], alterations of tumour cell surfaces using neuramin-

idase enzyme[11,12], effective transfer of the tumour immunity using sensitized lymphoid cells[13] and the use of bacillus Calmette-Guerin (BCG), an immunologic adjuvant[14,15]. All these techniques are, however, valuable only if the tumour size is in the region of 10^6 cells or less. A chemotherapeutic success in the treatment of cancer is dependent on the synergistic influences of the host immunity[16] so that prolonged exposure to strong dosages of anticancer agents, usually immunosuppressive, may lead to an enhancement of the tumour due to severe inhibition of the immunologic defence systems[17]. On the other hand, the combined modality of chemotherapy and immunotherapy against acute lymphoid leukemia[18] has been found more effective than if either modality was used alone. Surgical removal of the bulk of the antigenic tumour mass not only reverses the immunologic defence inertia but also enhances the effectiveness of chemotherapy[19]. In order to effect a cure, the immunologic defence systems must not be destroyed or greatly impaired by chemotherapy. Furthermore, it has to be realized that surgical treatment, radiotherapy or chemotherapy alone or combined, cannot achieve a hundred per cent destruction of malignant cells, essential for a cure. One can therefore employ excisional surgical treatment followed by radiotherapy to reduce the tumour size to a level that can be effectively coped with by chemotherapy which, in turn, would reduce the size of the remaining tumour cells to a small enough level to be completely destroyed by the host immunologic defences.

However, a given dosage or course of chemotherapy only destroys the same percentage of tumour cells, not the identical absolute number[20]. In using chemotherapeutic agents to treat leukemias, drug approach to the malignant cells is relatively facile and chemotherapy as well as immunotherapy have been fairly successful. Considerable difficulty has, however, been experienced in treating solid tumours chemotherapeutically[21]; more consecutive treatments of anti-cancer drugs need be administered which greatly increase the chances of ensuing prohibitive toxicity, especially as most chemotherapeutic agents have relatively low therapeutic indices.

It is therefore essential that surgery and / or radiotherapy reduce the total number of tumour cells to the lowest possible number in order that chemotherapy be most efficient; the total number of tumour cells hopefully being reduced to the level where host immunity would destroy each and every remaining neoplastic cell, essentially the pre-requisite to a pronounced cure.

CHAPTER 1.

CHEMOTHERAPY OF CANCER

The Ebers Papyrus (1500 B.C.)records the oldest known approach to the treatment of cancer. It involved the application of garlic to skin cancers - an extraordinary achievement considering that garlic has now been found to contain a weak anti-tumour agent. Over the centuries, several chemicals have been reported used in the treatment of cancer, in particular, mercury, belladonna and various inorganic compounds of arsenic. In 1851 William Marsden was using local application of arsenious acid to treat certain types of cancer.

The principles of chemotherapy were established by Ehrlich at the turn of the century. Chemotherapy is the study of selective inhibition by chemical means of a parasite species with minimum damage to the host species; and the ratio of the median lethal dose (LD_{50}) to the minimum dose necessary to cause 90% complete regression of the tumour (ED_{90}) is termed the Chemotherapeutic Index (CI) indicating the efficiency of a drug.

Nevertheless, forty years ago cancer chemotherapy was not considered practical despite the successes achieved in the chemotherapeutic treatment of bacterial and parasite infections. This stemmed partially from Warburg's work[22] in which he showed that neoplastic cells did not have specific morphological features to distinguish them from normal cells. To design chemotherapeutic agents, it was realized that one had to consider a number of small differences which when taken together could give rise to specificity[23,24]. The limited success obtained in the treatment of Hodgkin's disease by di-(2-chloroethyl)methylamine (HN2) proved an important starting point for cancer chemotherapy since it established that systemic administration of a chemical could induce regression of a tumour at doses tolerated by the host[25].

In the early 1950's, the pessimism of the pre 1940's had changed to optimism that random screening and synthesis of analogues of known cytotoxic agents would soon lead to selective agents capable of curing cancer[26]. However, by 1970 more than a quarter million compounds had been screened and it is now accepted that rapid "break throughs" are unlikely and that single cytotoxic agents are not the answer to maximum palliation or possible cures. This is further complicated by the fact that there is no known screening system that can conclusively predict

whether agents active against animal tumours would be clinically useful against human malignancies. Nonetheless, the single agent chemotherapy of the 1950's and 1960's has now been replaced by combination therapy involving known cytotoxic agents that selectively block sequential steps in the cell cycle.

The Cell Cycle.

The cell cycle is characterized by various phases which follow each other in cycle[27,28] (Fig.1). The G_1 phase is a period of protoplasmic growth during which RNA, proteins and various other products required for normal cell functioning are synthesized. The cells in this phase are characterized by having diploid amount of DNA. This is followed by a period of DNA synthesis, the S phase, consisting almost entirely of DNA replication - the various products for which having been prepared in "late G_1". DNA replication produces cells with tetraploid amount of DNA which then move into the G_2 phase in preparation for the mitotic phase, M. The M phase consists of four sub-phases in the order prophase, metaphase, anaphase and telophase. For cells proliferating in exponential growth with no cell loss, the cell number following mitosis is doubled since twice as many cells leave M as enter it. However, certain cells along the cell cycle may move out of the cycle and, while retaining their proliferating capacity, do not actively prepare for division unless stimulated (e.g. most liver tissue). This interphase is described as G_0 and is characterized by resting cells. Cells in the S phase and G_2 phase can now be identified by tritiated thymidine and microspectroscopy respectively, but there is, as yet, no morphological marker for cells in G_1 or G_0 phases[29].

DNA and RNA Synthesis.

It is universally accepted that genetic information is encoded in deoxyribonucleic acid (DNA) as nucleotide sequences. Since DNA is contained in the nucleus of the cell, intermediate molecules, ribonucleic acid molecules (RNA), are needed to transfer the genetic information from DNA to the cytoplasm - the main site of protein synthesis. These RNA are synthesized in the nucleus by transcription of DNA and subsequently migrate to the cytoplasm where they become associated with ribosomes (ribonucleoproteins; particles composed of three specific RNA molecules) as a result of which, the genetic

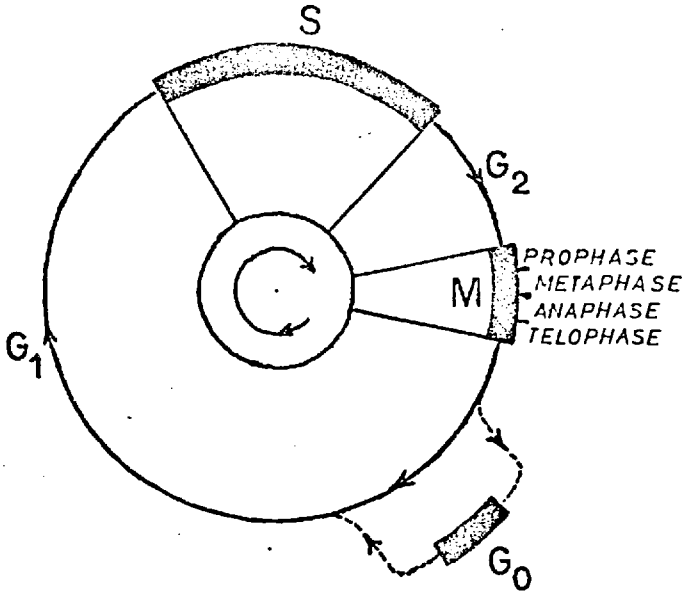

S
G2
M
PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
G1
G0

Fig.1

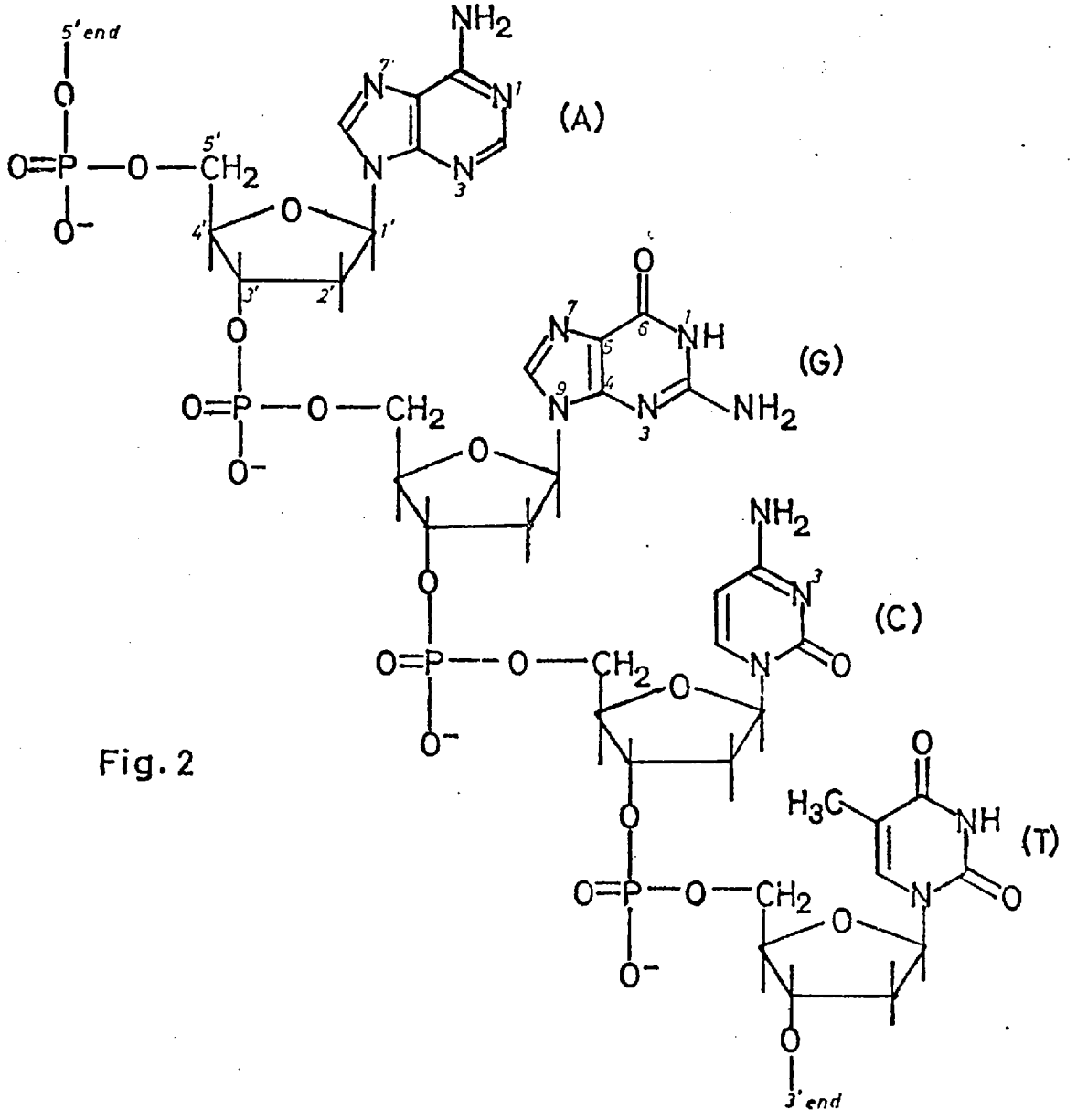

5'end
NH2
(A)
(G)
(C)
(T)
H3C
3'end

Fig. 2

information they transport is translated into the specific polymerisation of the twenty different natural amino acids into proteins.

The building blocks of DNA are nucleotides covalently assembled together by 3',5'-phosphodiester bonds between adjacent pentose moieties. In DNA the nucleotide bases are adenine (A), guanine (G), cytosine (C) and thymine (T) and the sugar moiety is β-2-deoxy-D-ribose (Fig. 2). In RNA thymine is replaced by uracil (U) and the deoxyribose by β-D-ribose. No particular periodicity characterizes the succession of the resulting nucleotides but in 1953 Watson and Crick[30] observed that the A/T and G/C ratios are approximately unity and that the spacial configuration of DNA is uniform. This led them to propose a double helix structure for DNA consisting of two polynucleotide strands twisted around each other in opposite directions to form a regular double helix held together by highly specific hydrogen bonding between complementary nucleotides. Such an organized structure suggested that each strand could serve as a template for the synthesis of its complementary strand, such a template being able, by way of weak secondary bonds, to specifically attract the right building block into the proper position.

The four nucleotides composing DNA are synthesized from simple precursors as carbon dioxide, sugars, amino acids and ammonia. The prototype purine nucleotide, inosinic acid, is synthesized from ribose-5-phosphate and then converted to adenylic acid (AMP) or guanylic acid (GMP), both purine ribonucleotides. The prototype pyrimidine nucleotide, orotidylic acid, is synthesized from aspartic acid and carbamoyl phosphate and converted to uridylic acid (UMP) and cytidylic acid (CMP) - both pyrimidine ribonucleotides. These ribonucleotides are enzymatically reduced to form deoxyribonucleotides which are then phosphorylated. Deoxyuridylic acid is also methylated to form thymidylic acid (TMP) which is subsequently phosphorylated further. The resulting deoxyribonucleotide triphosphates[31] are enzymatically polymerized by DNA polymerase on DNA templates making use of the base-pairing properties of the purine and pyrimidine moieties on the DNA templates, thus achieving polydeoxyribonucleotide chains which are complementary to the DNA templates.

The sequential pathways leading to the synthesis of DNA, RNA and proteins occur in both normal and neoplastic cells. Each pathway in theory, could be inhibited by administration of specific chemotherapeutic agents, but the site most sensitive to the majority of

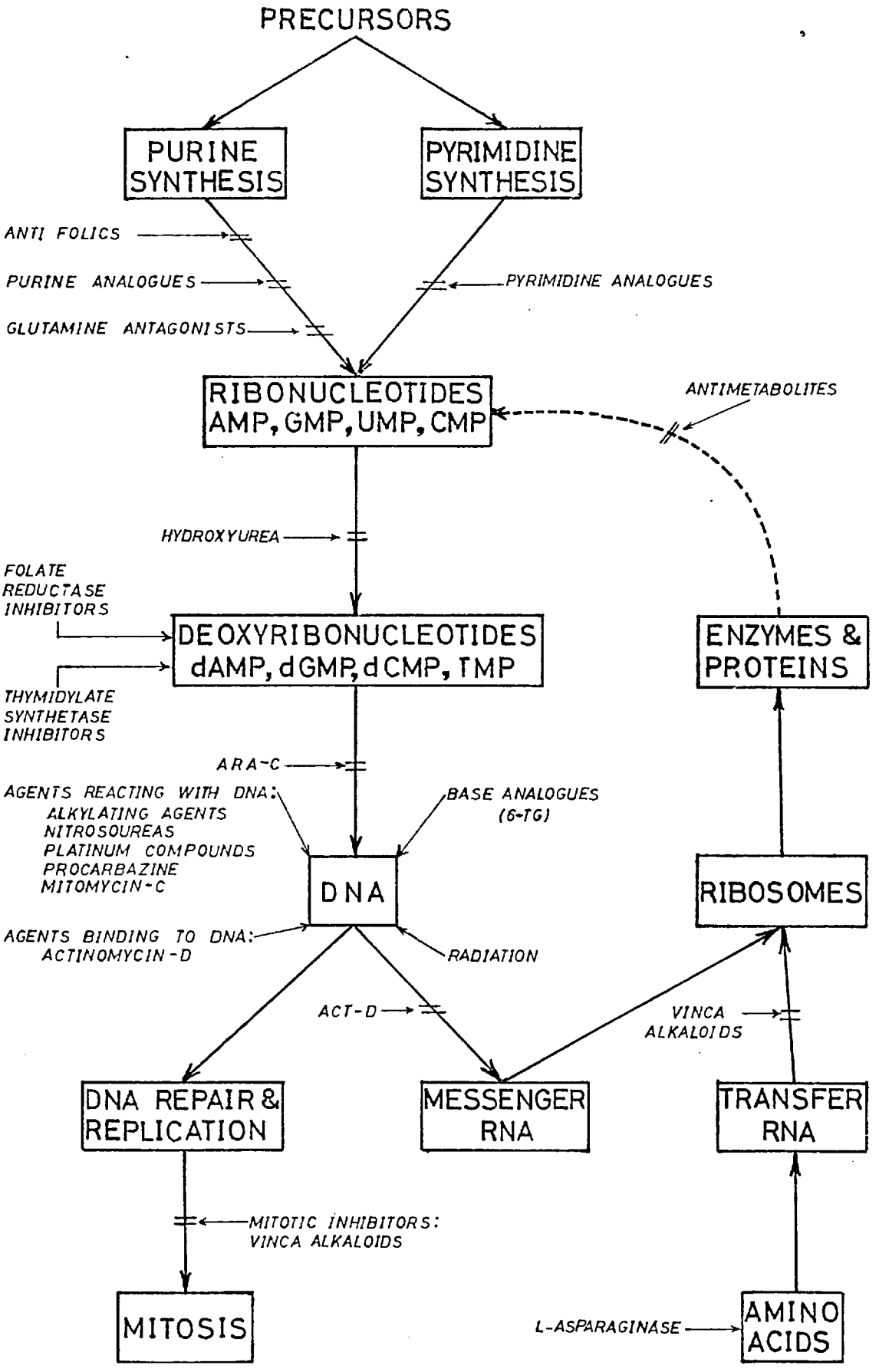


Fig. 3. Anabolic pathway to DNA, RNA and proteins and the effects of chemotherapeutic agents at each stage.

anti-cancer agents, especially to alkylating agents, is DNA[32,33](Fig. 3).

Major Types of Damage to DNA.

Various types of major damage to DNA are recognised. One is the result of single substitution reactions arising from base substitution or phosphate esterification[34] as for example attack by monofunctional alkylating agents. Extensive studies by Brookes and Lawley[35-37] identified the base positions of guanine, adenine and cytosine as the primary sites of attack, substitution at the N-7 position of guanine accounting for about 90% of the total alkylation. N-3 alkylated adenine[36], O-6 alkylated guanine[38], N-3 alkylated guanine[39] and N-3 alkylated cytosine[40,41] have also been identified and their biological significance established[42]. Thus monofunctional alkylating agents alkylate DNA at two sites - bases and phosphates. The toxic action of monofunctional alkylating agents has been correlated with adenine alkylation effecting single-strand DNA breaks[43,44]. In general, monofunctional alkylating agents are very toxic but not active or selective against tumours. Certain exceptions are known but even where monofunctional alkylating agents are active, they do not compare in their spectrum of activity with their respective difunctional derivatives[45,46].

Another type of damage to DNA is the result of difunctional substitution reactions arising from interstrand or intrastrand crosslinks. This is exemplified by the damage caused by difunctional alkylating agents. Interstrand crosslinks in double-stranded DNA or intrastrand crosslinks in single-strand DNA arise from alkylation of guanine moieties on adjacent strands (Fig. 4) or on the same strand of DNA respectively[36].

Alkylated bases involved in single substitution or in crosslinks may be released through the weakening of the glycoside bond. This depurination leaves a gap in the sequence of DNA bases which gives rise to instability and mispairing and possibly chain scission, through weakening of the sugar phosphate backbone, of one strand of a double-helix DNA. If only one strand is involved, single-strand breaks are obtained but if two single-strand breaks occur independently opposite each other in the DNA helix then an irreversible double-strand break, or main chain scission, results[47] (Fig. 5).

Alkylation in itself is a relatively non-cytotoxic process but

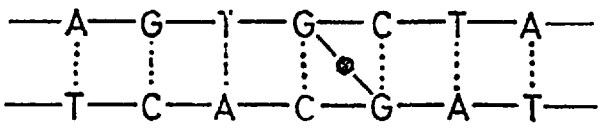


Fig. 4

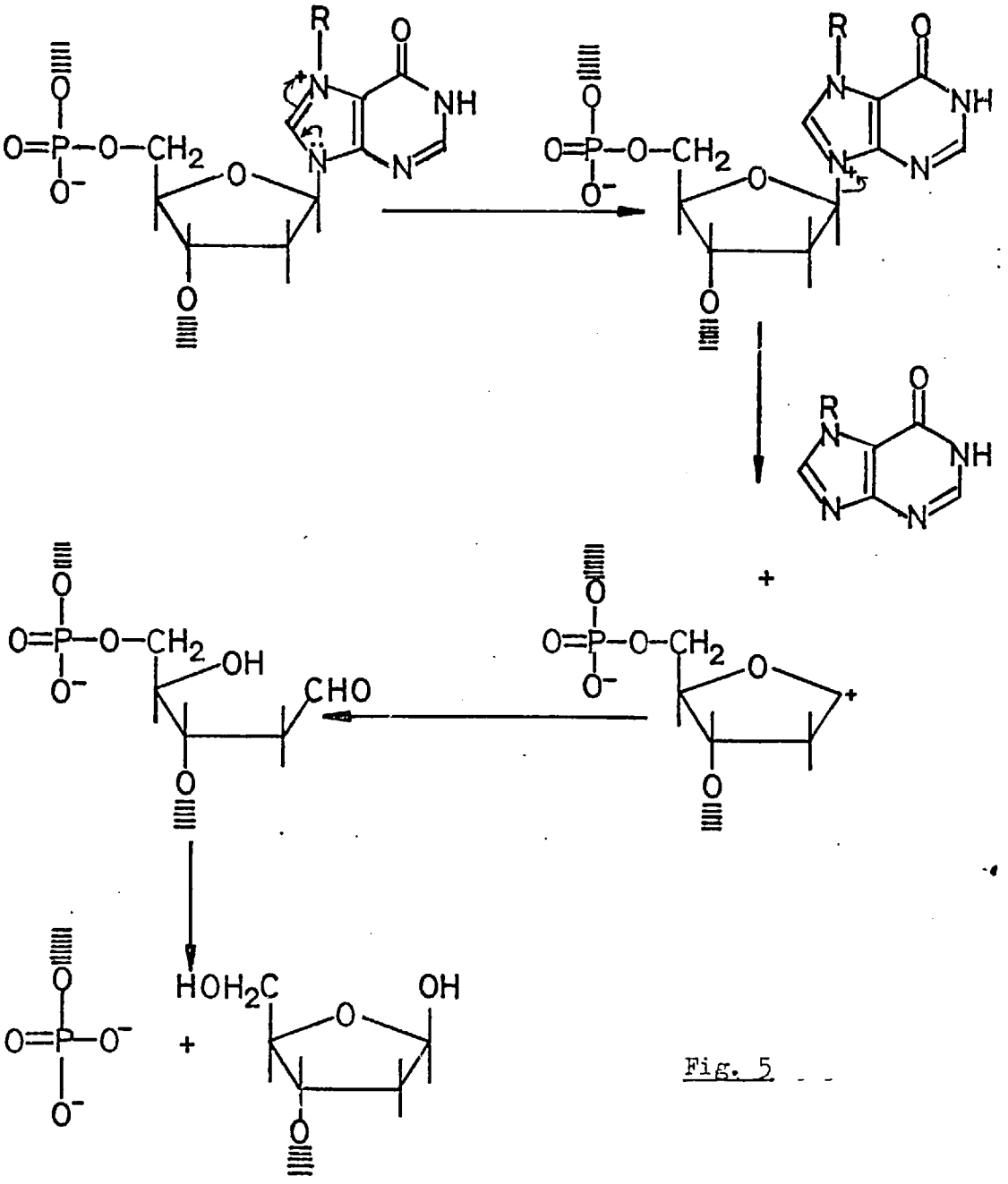


Fig. 5

if the cells are forced to divide before repair of alkylation can be effected, cytotoxicity results[48]. All known alkylating agents kill cells in this way and as such attack rapidly dividing cells. Using spleen colony techniques, it has now been established that alkylating agents are proliferation-dependent but not phase specific[49,50]. It is thus evident that biological activity depends substantially on the bifunctionality or the polyfunctionality of the alkylating agent since difunctionality gives rise to cross-linking which in turn causes depurination and chain scission.

Chemotherapeutic Agents

Most cancer chemotherapeutic agents presently used in clinical trials for the treatment or palliation of cancer can be grouped into six classes, namely: (a) biological alkylating agents, (b) antimetabolites, (c) antibiotics, (d) alkaloids, (e) enzymes and (f) hormones.

It is outside the scope of this work to describe in detail these various classes of agents but an attempt is made to discuss briefly the mode of action of the agents in major clinical use.

(a) Biological Alkylating Agents.

Sulphur Mustard ($\beta\beta'$-dichlorodiethylsulphide) (1) was first prepared by Guthrie[51] and Niemann[52] in 1860. Their observations of its highly vesicant properties led to its production and limited use as a chemical warfare agent in World War I; in consequence, severely gassed people developed erosions and ulcers in the stomach and intestines[53,54]. This prompted further research on sulphur mustards which led to the realization that it affected different tissues at sites distant from the area of application, particularly the hematopoietic system[55,56]. Adair and Bagg[57] reported induced regression following application of sulphur mustard to superficial tumours; Auerbach[58] established that sulphur mustard induced mutation and inhibited mitosis in the vaginal epithelium in mice - a property previously attributed only to radiations. The potential uses of mustard gases for chemical warfare led to the development of NN-di-2-chloroethylamine [59] (mechlorethamine, HN2) (2) and NNN-tri-2-chloroethylamine[60] (HN3) (3) which were found to be non-vesicant but cytotoxic and leucopenic. This observation, that HN2 though highly toxic, affected circulating blood cells and specifically lymphoid tissue and rapidly dividing cells, led Gilman and Philips[25] to suggest its use in the

treatment of leukemia[61,62] and precipitated the search for other chemotherapeutic agents with equal or better anti-tumour action but with reduced levels of toxicity.

HN2 is still in limited clinical use in the treatment of bronchogenic carcinoma either by itself or in combination[63,64]. Its chemical properties[65] and biological effects[66,67] have been extensively studied and reviewed[68]. A great number of aliphatic nitrogen mustards were developed but very few gave any improved selective cytotoxicity towards tumour cells. One such agent is nitromin (4)[69,70] at present extensively used by the Japanese. It was found to have lower host toxicity towards Yoshida carcinoma than HN2 and is presumably reduced in vivo to HN2 by a reductase having a high level of activity in the carcinoma.

In attempts to increase the ease of administration of alkylating agents, various agents were developed in which the chloroethyl groups were attached to a host of organic carriers including amino acids, sugars, vitamins, proteins etc. The synthesis of such aromatic nitrogen mustards have been reported by Ross and co-workers[71] and Owen and his group[72] and excellently reviewed by Ross[73].

4-[Bis-(2-chloroethyl)amino]benzenebutanoic acid (Chlorambucil, Leukeran, CB 1348) (5)[74] is commonly regarded as the safest of alkylating agents so far developed. It effects predominantly the lymphoid system and is the drug of choice in the treatment of malignant lymphoma[75-77]. It is also effective against Hodgkin's disease, chronic lymphocytic leukemia[78,79], polycythemia vera[80], metastatic ovarian carcinoma[81-83] and, in combination with the adrenal corticoid, prednisone, against disseminated breast cancer[84]. Like all other alkylating agents it is however immunosuppressive, binding actively with human γ-globulin[85].

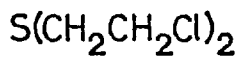
Melphalan, the L-isomer of phenylalanine nitrogen mustard (6; L-isomer) was developed by Bergel and Stock[86,87] with the aim of enhancing the active transport of the drug to the tumour site through the use of a natural amino acid. Clinically, it induces complete inhibition of the Walker rat carcinoma-256, remission in plasma cell myeloma[88,89] especially in combination with the synergistic prednisone[90], and very good remission in malignant melanoma when administered through regional perfusion[91,92]. Its DL-form, Sarcolysin[93] is extensively used by the Russians in the palliation of testicular and liver carcinoma.

The use of nucleic acid bases as carriers of alkylating agents is exemplified by 5-[bis-(2-chloroethyl)amino]uracil[94] (Uracil mustard) (7), effective against chronic lymphocytic and myelogenous leukemia.

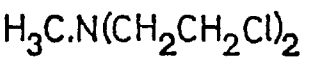
NN-Bis-(2-chloroethyl)tetrahydro-2-oxido-2H-1,3,2-oxazasphorin-2-amine (cyclophosphamide, endoxan, CTX) (8) was designed by Arnold and co-workers[95,96] to be activated by tumour cells possessing high levels of phosphoramidase and has been found extremely effective against Hodgkin's disease, lymphocytic leukemia[97], myeloma[88], ovarian carcinoma[98] and malignant reticulosis[99]. It was soon realized that endoxan was not itself cytotoxic but that it was activated by phosphatase and phosphamidase enzymes in liver microsomes[100]. The active metabolite has not, as yet, been identified due to its relative instability[101] but Colvin and co-workers[102] isolated NN-bis(chloroethyl)phosphorodiamidic acid (9) from incubations of endoxan with murine hepatic microsomes and found this to be a potent alkylating agent. Its metabolism has been extensively studied[103,104] and is believed to act in part through its degradation to nor-HN2[105]. Furthermore, its chemical action and pharmacology in man have been excellently reviewed by Bertino[106].

Recognition of cross-linkage of DNA as the main mode of action of alkylating agents led to an intensive search of known cross-linkage agents used in industry. The reactivity of 2,4,6-triethylenimino-1,3,5-triazine (triethylene melamine, TEM) (10) with cellulose[107] and its similarity to imine formation from nitrogen mustards suggested its use in cancer chemotherapy[108-111], but its toxicity and lack of specificity rendered it useless for clinical administration. Triethylene phosphoramide (TEPA) (11) has also been evaluated clinically[112] in the treatment of malignant melanoma but has since been replaced by the more stable 1,1',1"-phosphino thioylidynetrisaziridine[112,113] (triethylene thiophosphoramide, THIO-TEPA) (12) which is converted <u>in vivo</u> to TEPA and metabolized as such[114]. It is a very active inhibitor of metastasis in rat mammary adenocarcinoma and is clinically used in the treatment of various forms of carcinoma as well as in Hodgkin's disease, neuroblastoma, disseminated melanoma and breast and ovary carcinoma[115].

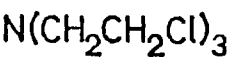
Various aziridinyl derivatives have since been developed and clinically established including 2,3,5-tris-(1-aziridinyl)p-benzoquinone (trenimon, triaziquone) (13)[116] effective against soft tissue



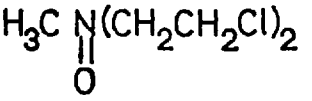
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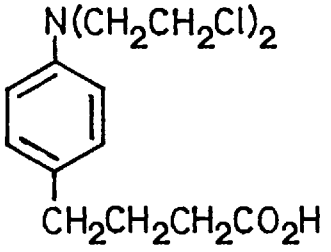
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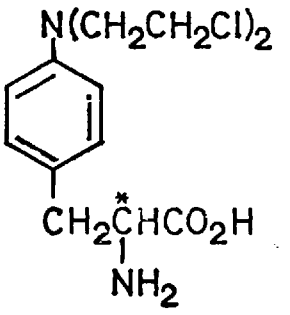
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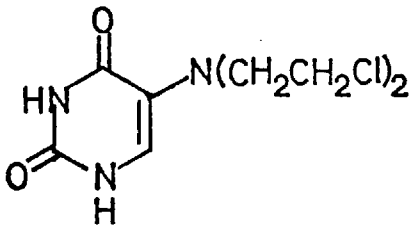
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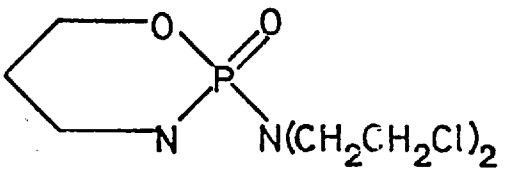
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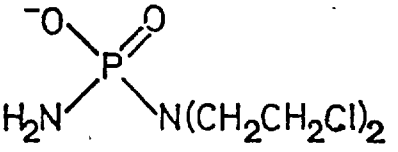
(6)



(7)



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(9)

sarcomas.

In 1950, Timmis[117,118] investigated the use of disulphonic esters as biological alkylating agents and found that they inhibited growth of many transplanted animal tumours as a result of which 1,4-dimethanesulphonyloxybutane (Myleran; Busulphan) (14) was synthesized[119]. Myleran is the therapy of choice against chronic myeloid leukemia but is ineffective against acute lymphocytic leukemia[120-122]. It is effective against polycythemia vera[123]. However, its toxic depression of bone marrow cells limits its use.

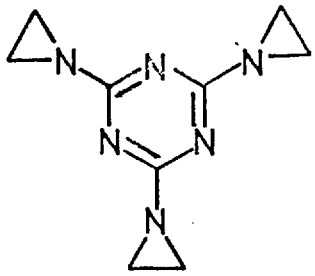
Bisepoxides were also found to induce cross-linking of cellulose and were in use in the textile industry[124]. Various epoxides were investigated[125] but results proved clinically disappointing. The most useful agent proved to be butadiene dioxide[126] (15) but the margin between the lethal dose and that required for inhibition was very small. In fact, the only antineoplastic bisepoxide still in limited clinical use is 1,2,15,16-diepoxy-4,7,10,13-tetraoxahexadecane (ethoglucid, epodyl)[127] (16).

A number of alkylating agents linked to carbohydrates have been synthesized and found to be clinically promising. Attachment to a sugar molecule conferred water solubility and active transport properties <u>in vivo</u>. Clinically established agents of this type include 1,6-di-(2-chloroethylamino)-1,6-dideoxy-D-mannitol (Degranol; Mannomustine)[128] (17; X = $-NHCH_2CH_2Cl$), and 1,6-di-O-methanesulphonyl-D-mannitol (Mannitol myleran; mannogranol)[129] (17; X = $-OSO_2Me$).

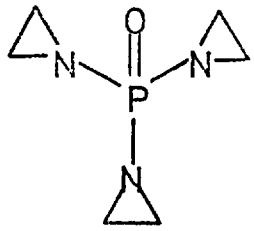
A group of halogenated sugars have also been found to have similar properties to the difunctional alkylating agents. 1,6-Dibromo-1,6-dideoxy-D-mannitol (Mitobronitol; myclobromol)[130] (17; X = Br) has been proved of major clinical use in the treatment of chronic myeloid leukemia and polycythaemia vera[131]. Jarman and Ross[132] have established that degranol, mannogranol and mitobronitol are converted under physiological conditions to 1,2,5,6-dianhydro-D-mannitol (18) which is over fifty times as active as the parent compounds.

Another halogenated sugar, 1,6-dibromo-1,6-dideoxygalactitol (Dibromodulcitol; mitolactol)[133,134] (19) has been found to give reasonable response against carcinomas of squamous origin and has a pronounced inhibitory effect on granulopoiesis.

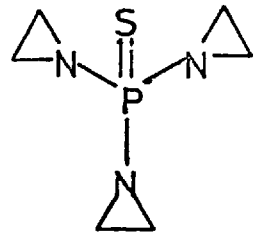
1,2,5,6-Tetramethanesulphonyl-D-mannitol (Zitostop)[135] (20) has been found to have a wider spectrum of action on solid tumours as mammary, pulmonary and ovarian carcinomas than degranol and mannogranol,



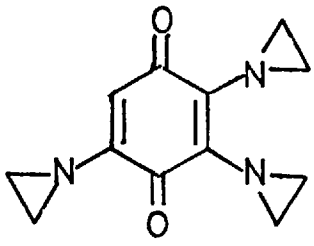
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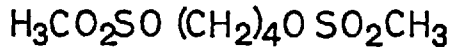
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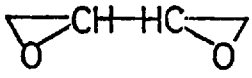
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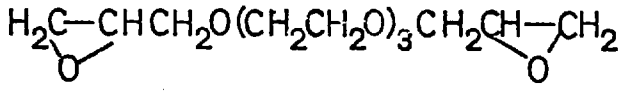
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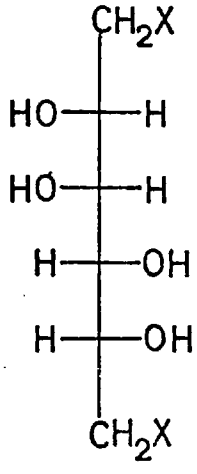
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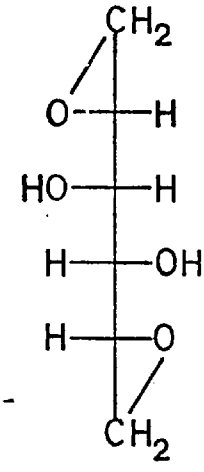
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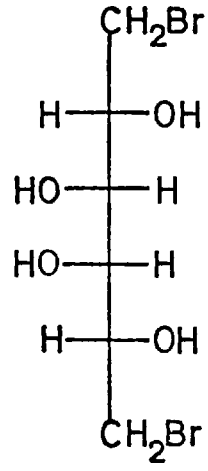
(16)



(17)



(18)



(19)

but has as yet not been fully evaluated clinically.

Several anti-cancer agents have been developed that cannot strictly be referred to as alkylating agents but which show a close resemblance to such through the formation of eletrophilic reactions in vivo.

The simplest of these is hydroxyurea (hydre; hydroxy-carbamide)[136-138] (21). It is currently one of the most widely used indirect inhibitors of DNA synthesis[139] since it can be administered for long periods of time without seriously affecting survival at levels producing 90% inhibition of DNA synthesis[140,141] and without concomitant effects on RNA and protein synthesis. It is therefore used at high dose levels in the management of chronic myeloid and granulocytic leukemia. It is specific to the S phase of the cell cycle and its primary mode of action is believed to be the inhibition of ribonucleoside diphosphate reductase[142,143].

Most alkylating agents mentioned above are not very soluble in lipids. As such they could not be used to treat various brain tumours. However, several nitrosoureas have been found to be effective inhibitors of DNA and RNA synthesis as well as being highly lipid soluble. As such they are able to ford the blood-brain barrier to gain access to the cerebral spinal fluid and the blood stream simultaneously. 1,3-Bis-(2-chloroethyl)-1-nitrosourea[144] (BCNU) (22) is clinically effective against brain tumours, particularly gliomas[145,146]. It is more toxic to proliferating cells than to G_0 cells but is not phase specific[147]. 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (23; X=H) and its trans-4-methyl derivative (MeCCNU) (23; X=Me) have also been found to be highly active and are now clinically established, MeCCNU being particularly active against cells that are not proliferating and hence against a number of solid human carcinomas. The mechanism of action of the nitrosoureas is exemplified by that of BCNU (Fig. 6).

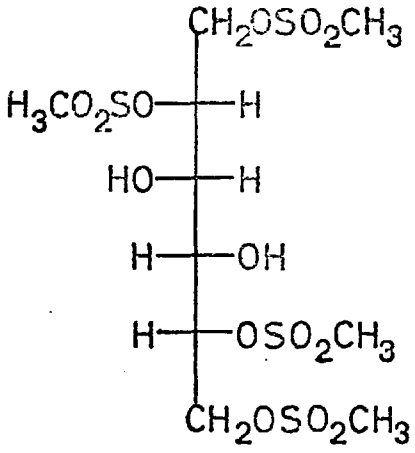
Nitrosoureas are highly selective due to their unique breakdown into highly active products which act on different metabolic pathways. The highly reactive carbonium ions formed alkylate nucleic acids and inhibit DNA and RNA synthesis, while the carbamoylation of proteins with the isocyanate formed, inhibits protein synthesis thus inactivating enzymes necessary for the repair of the damaged DNA[148].

Some triazine derivatives have also been found to be active against tumours in particular against TLX-5 lymphoma which is

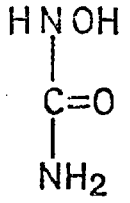
unresponsive to all difunctional alkylating agents. 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide[149] (DIC) (24) is probably the most effective agent in the treatment of melanoma. Its cross-resistance to BCNU indicates a similar metabolic pathway as nitrosoureas. It is believed to be enzymatically degraded to methyl carbonium ions which methylate essential macromolecules[150].

N-Isopropyl-α-(2-methylhydrazino)-p-toluamide[151,152] (Natulan, Procarbazine) (25) was originally developed as a potential monoamine oxidase inhibitor and subsequently found to be a very active tumour inhibitor with no cross-resistance to other agents[152]. It has been clinically tried[153] and found useful in the treatment of advanced Hodgkin's disease[154,155], malignant lymphomas[156], and advanced brain tumours[157]. Natulan and its metabolites are easily transported across the blood-brain barrier[158] and as such may give rise to unwanted side effects on the central nervous system[159]. Its mechanism of action[160] is believed to be the selective transmethylation of the N-methyl group onto the N_7 guanine moiety of transfer RNA. This inhibition of the functional transfer RNA leads to inhibition of protein synthesis and concomitant inhibition of DNA and RNA synthesis.

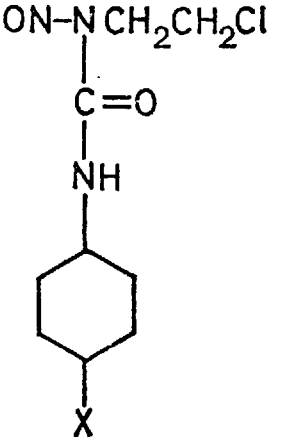
A chance observation by Rosenberg and co-workers[161] led to the discovery of tumour-inhibiting platinum compounds. Two active platinum complexes were formed following the application of an alternating current, across platinum electrodes, to a liquid culture of Escherichia Coli in the presence of ammonium and chloride ions, resulting in unbalanced growth due to inhibition of mitosis. Cis-dichlorodiammine platinum (II) (26) and cis-tetrachlorodiammine platinum (IV) (27) were found to be powerful inhibitors of Sarcoma-180[162] and (26) is currently being used in the treatment of ovarian carcinoma with acquired resistance to nitrogen mustards. Trans isomers were found to be toxic but not active[163]. The mode of action of these platinum complexes is believed to be the selective inhibition of DNA synthesis arising from intrastrand cross-linking of complementary strands in DNA with primary attack on the N-7 and 6-NH_2 positions of adenine[164,165]. Reticulocytopenia, lymphocytopenia and kidney damage are the limiting factors in their management but alicyclic diammine analogues[166] have greatly reduced toxicity and have higher selective activity against tumours, achieving chemotherapeutic indices of over 200 as compared to dichlorodiammine platinum, which has a C.I. of 8. A major problem with these platinum complexes is their poor



(20)



(21)



(23)

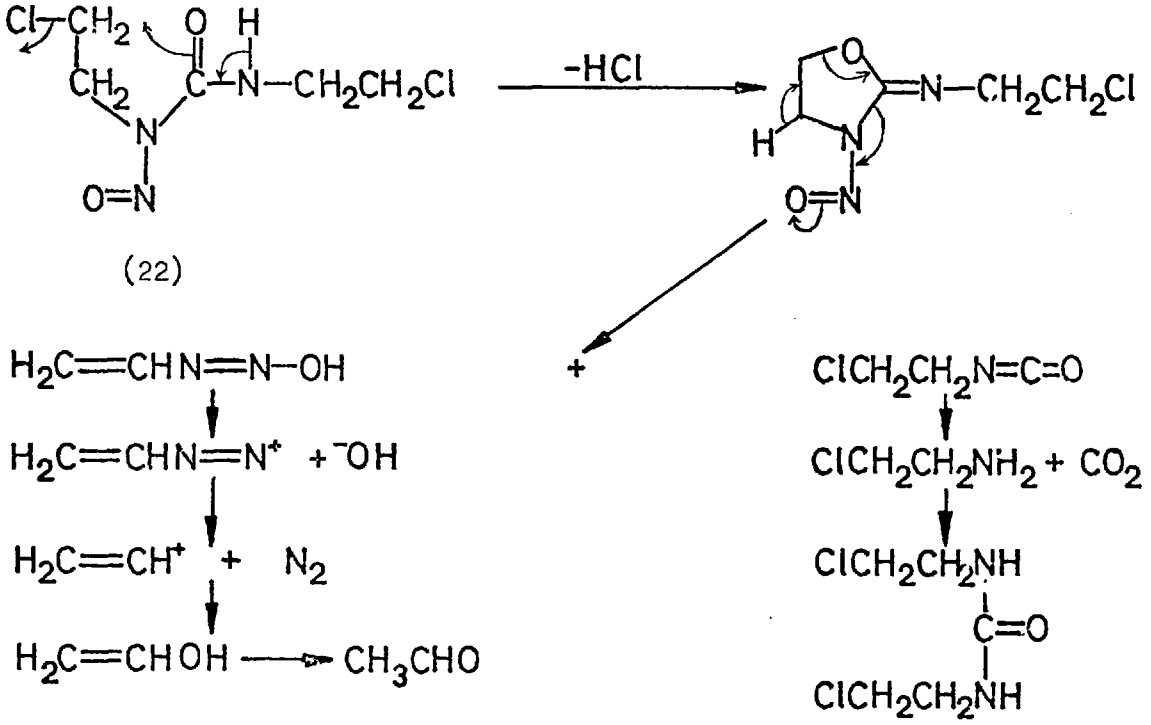
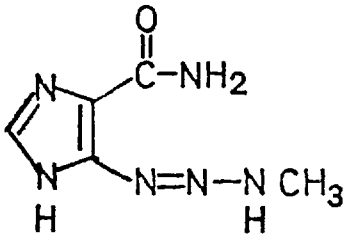
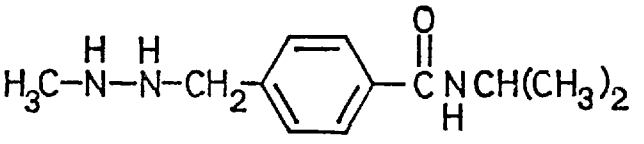


Fig. 6.



(24)



(25)

solubility; but highly soluble platinum diamminechloride complexes with pyrimidines have recently been synthesized and might eventually be more clinically useful.

The reader is referred to excellent reviews on biological alkylating agents by Ross[73,167], Connors[168], Haddow[169] and Ludlum[170].

(b) Antimetabolites

Antimetabolites are competitive substrates that act on enzymic reactions along the metabolic pathway of normal metabolites and inhibit the enzymes that catalyse subsequent reaction pathways. They may also follow the entire sequence of the normal metabolites including incorporation into DNA, RNA and proteins thereby causing inhibition of polymer chain growth, genetic mutations and erroneous transcription and translation of the genetic code resulting in faulty enzymes.

There are three main classes of antimetabolites, namely: folic acid, purine and pyrimidine antagonists.

Precursors of pteroylglutamic acid (folic acid) (28; R_1=OH, R_2=H, n=2) were known to be essential for the synthesis of red blood cells. Folic acid is known to be converted via tetrahydrofolic acid into the active co-enzyme, folinic acid. This conversion is achieved through the use of folic reductase and dihydrofolic reductase enzymes. But administration of folic acid was found to accelerate anaemia in leukemic children. Moreover, pteroyltriglutamic acid induced tumour regression in single spontaneous breast cancers in mice[172] and in disseminated cancer in man[173,174]. These results led Farber[171] to investigate the use of folic acid analogues in the management of acute leukemia in children. A folic acid antagonist, aminopterin[175,176] (28; R_1=NH_2, R_2=H, n=2) induced temporary remissions in acute leukemia. The isosteric replacement of an -OH group at position 4 with an -NH_2 group extensively affects the ionization and hydrogen bonding of the substituted pyrimidine ring and the π-electron system of the pyrazine ring, making it less susceptible to reduction by enzymes, and results in a three-thousandfold increase in the binding strength to dihydrofolate reductase[177]. Thereby the complexing of the normal metabolite to the enzymes is profoundly reduced and this results in the inhibition of tetrahydrofolate synthesis and consequently the inhibition of folinic and inosinic acid synthesis and thus of DNA and RNA synthesis[178].

Aminopterin has since been replaced by the more active but less

toxic N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl] L-glutamic acid (Methotrexate, MTX, amethopterin)[179,180] (28; R_1=NH_2, R_2=CH_3, n=2). Its mechanism of action is similar to that of aminopterin[181]. Methotrexate also inhibits thymidylate synthetase[182] and lower-affinity receptor sites necessary for the maintenance of deoxyuridine metabolism[183]. It blocks mitosis at the metaphase-anaphase transition and is phase specific affecting cells in early G_1, at the G_1-S transition and in the mid-S stage, mid-G_1 cells being apparently resistant[184]. Thus, during the S phase, thymidylate synthetase produces thymine nucleotides and an equivalent amount of dihydrofolate. Since dihydrofolate reductase is partially inhibited, dihydrofolate will accumulate and nucleic acid synthesis ceases resulting in "thymine-less" death. Its limiting bone-marrow toxicity can, however, be reversed by leucovorin (5-formyltetrahydrofolate), a synthetic preparation of citrovorum factor identical to folinic acid. Leucovorin blocks methotrexate uptake by competing with it for the same mechanism[185,186]. Leucovorin is usually administered within four hours of methotrexate administration and in this way a much improved chemotherapeutic index is achieved[186]. Certain neoplastic cells are affected much faster by methotrexate than normal bone-marrow cells. So the delayed administration of leucovorin rescues normal but not neoplastic cells. Methotrexate is particularly effective in the treatment of acute leukemia in children, of choriocarcinoma and of head and neck cancer[196], and is currently used in combination with purine and pyrimidine analogues[187]. Its chemical action and pharmacology in man have been extensively reviewed by Bertino[106]. Various analogues of folic acid have been synthesized and clinically tested but only one, dichloromethotrexate (chlorine atoms at 3' and 5' positions), has been found superior to methotrexate and is currently undergoing clinical investigations.

The first anti-purine to be clinically studied was 2,6-diaminopurine[189-191] (29) but, following the development of other more active and less toxic purine analogues, its clinical use was discontinued due to its severe gastro-intestinal side effects[192]. Mitosis depends on the synthesis of DNA which requires purine and pyrimidine precursors (Fig. 3, page 15). Purine antagonists therefore inhibit DNA synthesis indirectly and consequently mitosis. Two of the most important anti-purines in present clinical use are 1,7-dehydro-6H-purine-6-thione (6-mercaptopurine, 6-MP, Puri-Nethol)[193-195] (30) and 6-thioguanine (Lanvis, 6-TG)[189] (31). 6-Mercaptopurine (30) is a thiol derivative

of hypoxanthine, the precursor of inosinic acid. It is an anti-metabolite of hypoxanthine and adenine and is activated via the anabolic conversion to its nucleotide, thioinosinic acid, which subsequently inhibits the de novo purine synthesis and interconversions, thereby affecting DNA and RNA synthesis[197]. It may also be anabolized to 6-thioguanine nucleotides and subsequently incorporated into DNA[198]. Its limiting toxicity is bone marrow depression.

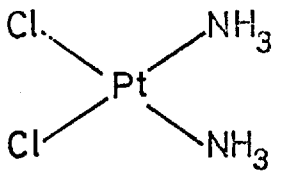
6-Mercaptopurine is particularly effective against acute myeloid leukemia in children and has also caused substantial remissions of acute leukemia in adults. Its current use is mainly in combination with prednisone, methotrexate and vincristine (VAMP regimen)[199,200]. 6-Thioguanine[31] is mainly incorporated into DNA and RNA and its anti-tumour activity is related to its level of incorporation into DNA, maximum incorporation being achieved in sensitive tumours. It is used mainly in combination therapy and produces effective remission in early chronic myelocytic leukemia. 8-Azaguanine[201] (32) has also been found active against tumours and its mechanism of action is believed to be its anabolism to azoguanylic nucleotides and subsequent incorporation into messenger RNA, thereby displacing a large portion of guanine residues.

The finding by Rutman and co-workers[202] that uracil was incorporated more in the synthesis of nucleic acid in a liver tumour than in normal liver tissue led Heidelberger and co-workers to the synthesis, mechanistic elucidation and clinical testing of a host of pyrimidine antagonists - the most useful of which is undoubtedly 5-fluorouracil (5-FU)[203,204] (33). Its metabolism and mechanism of action were elucidated[205]. Fluorouracil is anabolized to fluorouridine (FUR) which is subsequently converted to fluorouridine monophosphate (FUMP) and enzymatically reduced to 5-fluoro-2'-deoxyuridine monophosphate (FdUMP). This nucleotide at first competitively and then irreversibly inhibits thymidylic acid synthetase and thus blocks the methylation of deoxyuridylic acid to thymidylic acid resulting in inhibition of DNA synthesis[205]. Fluorouridine monophosphate is furthermore converted into fluorouridine triphosphate (FUTP) which is incorporated into RNA in place of the uridylate moiety and thus affects protein synthesis. Fluorouracil is one of the most effective cancer chemotherapeutic agents known. It is used as an adjuvant agent in operable and inoperable cancer of the bowel[206], and produces objective responses in the treatment of gastro-intestinal cancer[207], breast cancer[208] and cancer of

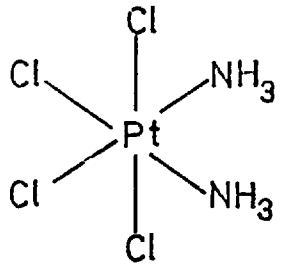
the head and neck[209]. In combination with other drugs it gave a 90% response rate to breast cancer[210] and a fair control of metastases of the lungs[211]. Fluorouracil in cream form (Efudix) is highly effective in the topical management of superficial pre-malignant and malignant lesions, keratoses and extensive multiple superficial epidermal carcinomas, achieving over 80% success rate[212,213].

Pyrimidine antagonists have also been prepared by forming ribosides where the structural alteration is in the sugar moiety. Minor changes in naturally occuring pyrimidine nucleosides can result in extensive cytotoxicity. Thus the epimerization of the 2'-hydroxy group of the ribose moiety of cytidine affords 4-amino-1-(β-D-arabinofuranosyl)pyrimidine-2-one (cytosine arabinoside, 1-β-D-arabinofuranosyl cytosine, ara-C)[214] (34). Ara-C was shown to be a strong tumour inhibitor[215] and the most effective cytotoxic agent in the treatment of acute lymphoblastic monocytic and myeloblastic leukemia[216,217] especially in combination with 6-thioguanine[218]. It has however little therapeutic effect on solid tumours in man, the most responsive of these being breast adenocarcinoma (15%), gastro-intestinal (11%) and soft-tissue and bone sarcomas (11%)[219] the responses of which are of short duration and so rarely of clinical benefit. Ara-C is intracellularly converted to arabinosyl cytosine-5'-triphosphate (ARA-CTP) which in turn inhibits DNA polymerase[220,221] during the S-phase of the cell cycle. Ara-C is therefore scheduled to take advantage of the S-phase specific indirect inhibition of DNA in the treatment of leukemia[222,223]. It probably also inhibits cytidine diphosphate reductase[224]. It is furthermore incorporated directly into DNA and RNA[225,226]. Ara-C incorporation into DNA can be reduced by pre-treatment with methotrexate but incorporation into low molecular weight RNA prevents uridine incorporation and results in acute cell death[226]. Ara-C is also catabolysed to arabinosyluridine (Ara-U) by deaminase enzymes and this provides a natural resistance to the drug. 1-β-D-Ribofuranosyl-4-hydroxy-3,4,5,6-tetrahydropyrimidine-2-(1H)-one (tetrahydrouridine), however, inhibits deaminase enzymes in the liver and administration of this with ara-C prolongs its activity[227]. The most common resistance to ara-C is, however, acquired through the reduction in the level of deoxycytidine kinase activity which converts ara-C to its active metabolite ara-CTP. Administration of deoxycytidine-5'-triphosphate, however, reverses these effects[228].

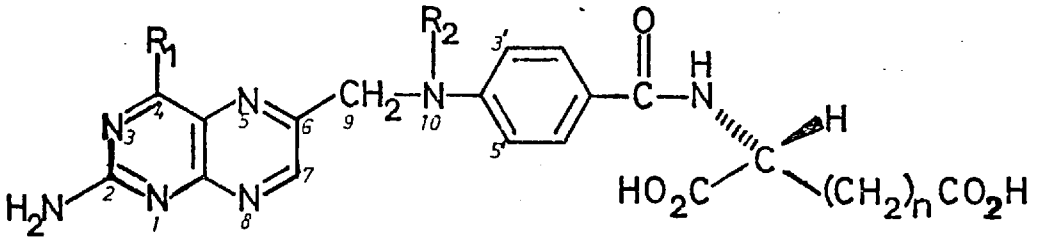
Various analogues of ara-C containing alkylated sugar hydroxyls



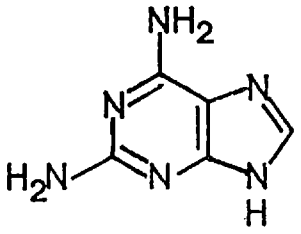
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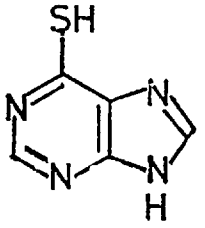
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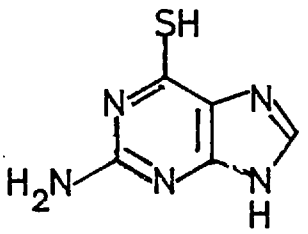
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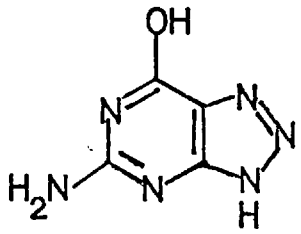
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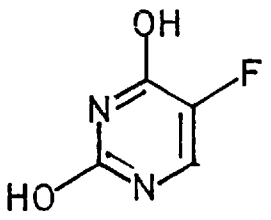
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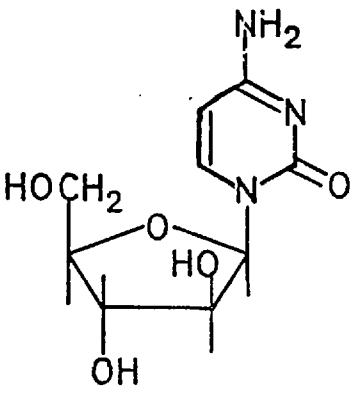
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(34)

have now been developed and found to have improved chemotherapeutic activity coupled with higher resistance to deactivating enzymatic deamination. One such analogue is 5'-O-methyl ara-C.

Various amino acid antagonists were synthesized by Connors and Ross[229] but were found to be poor tumour inhibitors. Various cyclic derivatives of methionine, cysteine etc. were prepared[230,231] but although improved activities were recorded, the results obtained did not warrant clinical investigations.

Todd[232] suggested that ethyl urethane (urethane) (35) was an antimetabolite of an aliphatic amine in purine biosynthesis. Furthermore Lefevre[233] had observed that seedlings exposed to various carbamates showed retarded growth. This led to the clinical trial of urethane with chronic myeloid leukemia[234], a fair response being obtained. It has also been used in the treatment of multiple myeloma[235]. Its mechanism of action is probably linked with pyrimidine metabolism and probably interferes with the methylation of the uracil moiety[236]. Furthermore its effects may be reversed by prior administration of various pyrimidines.

The reader is directed to various reviews on antimetabolites by Timmis[237], Delmont and Jukes[238], Bertino and Johns[239], Hitchings and Elion[240], Mead[241], Montgomery[242], Townsend and Cheng[243], Bardos[244,245] and Grindey and co-workers[246].

(c) Antibiotics.

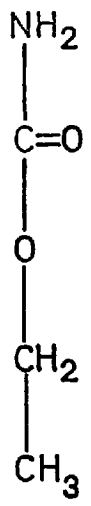
O-Diazoacetyl-L-serine (Azaserine)[247,248] (36) and 6-diazo-5-Oxo-L-norleucine (DON)[249,250] (37) are two naturally occuring and very active glutamine (38) antagonists isolated from broth filtrates of Streptomyces. L-Glutamine (38) is required at three stages of purine biosynthesis. Glutamine antagonists block the conversion of formylglycinamide ribotide to the corresponding formyl glycinamidine ribotide - a process requiring glutamine and 5'-phosphoribosyl formylglycinamidine synthetase[251]. DON binds some forty times more strongly to this enzyme than azaserine, and as such is much more active. Both glutamine analogues temporarily inhibit the enzyme reversibly but quickly inactivate it irreversibly through alkylation (by the $COCHN_2$ moiety) of the sulphydryl group (-SH) of a cysteine residue in the active site of the enzyme[252]. These antitumour agents therefore block protein synthesis and thus indirectly inhibit DNA synthesis. They have been used in the management of acute leukemia in children but are now more commonly used in combination therapy.

Mitomycin-C[253,254] (39, page 36), though an antibiotic, is probably converted *in vivo* to an active alkylating agent[255]. Its quinone and urethane moieties also probably contribute to its effective activity. Loss of the methoxy group at position 9' forms a difunctional alkylating agent composed of the active aziridine ring and the active methylene group at position 10. It therefore inhibits DNA synthesis and mitosis[256,257] and has been reported to cause effective remission of lymphomas, chronic leukemias, breast cancer and some other forms of solid tumours[258,259].

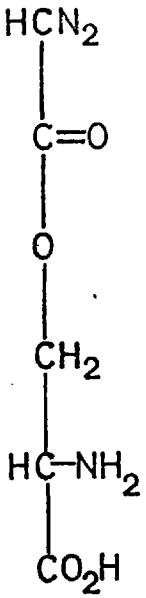
The most important cytotoxic alkaloid to date is probably actinomycin-D (Act-D, Dactinomycin, Actinomycin C_1)[260-262] (40). Actinomycin-D binds to double-stranded DNA[263] thus changing the DNA template and inhibiting the DNA-dependent RNA polymerase responsible for almost all cellular RNA synthesis[264,265]. It slows the overall protein synthesis and inhibits the degradation of tyrosine amino transferase and its mRNA[266]. As such it maintains specific enzyme synthesis through stabilization of mRNA[266]. It is cell cycle specific to mitosis and to the G_1/S transition state but its effects can be reversed by addition of excess of native DNA[267]. The specific binding of actinomycin to deoxyguanosine[268,269] as explained by Hamilton's theory[270] has however been disputed by Wells[271] who postulated that the presence of deoxyguanylic acid was neither necessary nor sufficient for the binding reaction, contrary to the predictions of Goldberg and Reich[264]. Hamilton had postulated that actinomycin is hydrogen bonded to the outside of the DNA helix through hydrogen bonding between its quinone oxygen and the 2-amino group of guanine as well as between its amino group and N_3 of guanine, and between the peptide lactones and phosphodiester oxygens. Wells[271] on the other hand supported Müller and Crothers' theory[272] of intercalation of actinomycin into the DNA chain and maintained that the apparent specificity of binding to guanine was only a secondary effect. Wells[271] also maintained that a marked nucleotide sequence preference existed for the binding reaction and Krugh[273] established such a sequence on the basis of the intercalation model.

Actinomycin has brought about remarkable remissions, possibly cures, in Wilms tumour in children[274] particularly when used in combined modality therapy[275]. Remission has also been obtained in uterine choriocarcinomas[276,277]. It is currently of major importance in combination therapy.

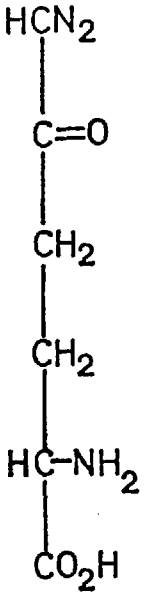
Other antibiotic agents have been used in the treatment of



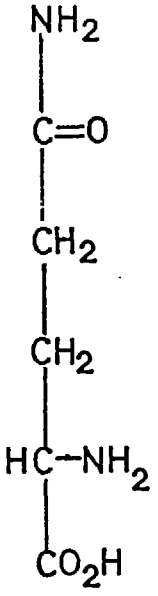
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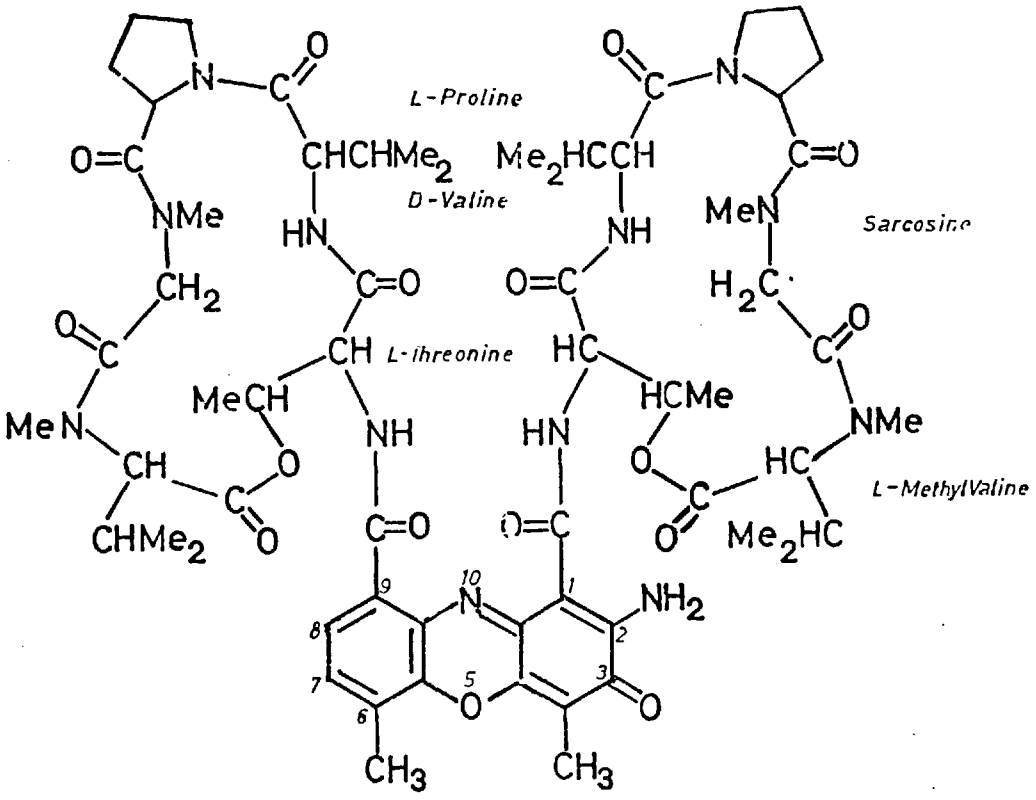
(36)



(37)



(38)



(40)

cancer. Such agents include mithramycin[278] (41) in the treatment of actinomycin-D resistant testicular tumours[279], daunorubicin (daunomycin)[280,281] (42; R=CH_3) in the treatment of acute leukemia[282] and of neuroblastomata in children, and adriamycin (14-hydroxydaunorubicin; doxorubicin) (42; R=CH_2OH) in the treatment of neuroblastoma, soft tissue sarcoma, acute and chronic leukemia and breast and lung cancer[283,284]. The mechanism of action of daunomycin and adriamycin is probably similar to that of actinomycin-D but possibly block different grooves in the DNA by the non-intercalcated part of the antibiotics[269].

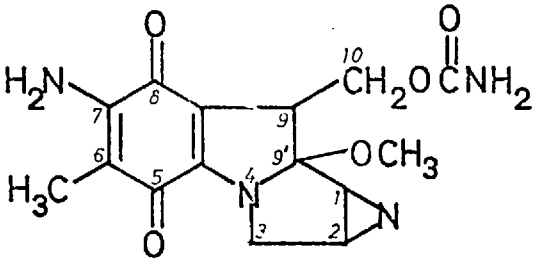
Bleomycin, a combination of a dozen or more compounds with the molecular weight of the main constituents in the region of 1300 to 1800, was obtained from the aqueous phase of Strain-843 S. Verticillus cultures on extraction with organic solvents[286]. It has proved to be extremely effective due to its virtual absence of bone marrow depression[287]. Bleomycin inhibits DNA synthesis but not RNA or protein synthesis. It also inhibits mitosis by drug exposure in late G_2 phase[288]. It has proved particularly effective against Hodgkin's disease when the tumour was resistant to conventional agents[289].

For a review on the use of antibiotics in cancer chemotherapy the reader is directed to the work of Burchenal and Kreis[290].

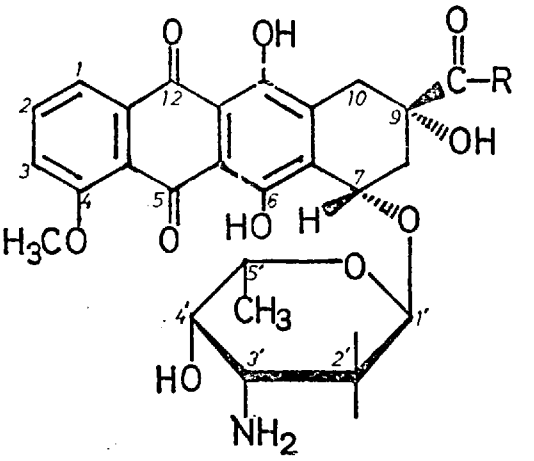
(d) Alkaloids

Colchicine (43; X=$COCH_3$) was originally administered to gouty cancer patients[291] and then found to have anti-tumour activity against chronic myeloid leukemia. However, it was also extremely toxic. Demecolin (43; X=CH_3) was active and had only one fourth the toxicity of colchicine[292] but was still considered very toxic.

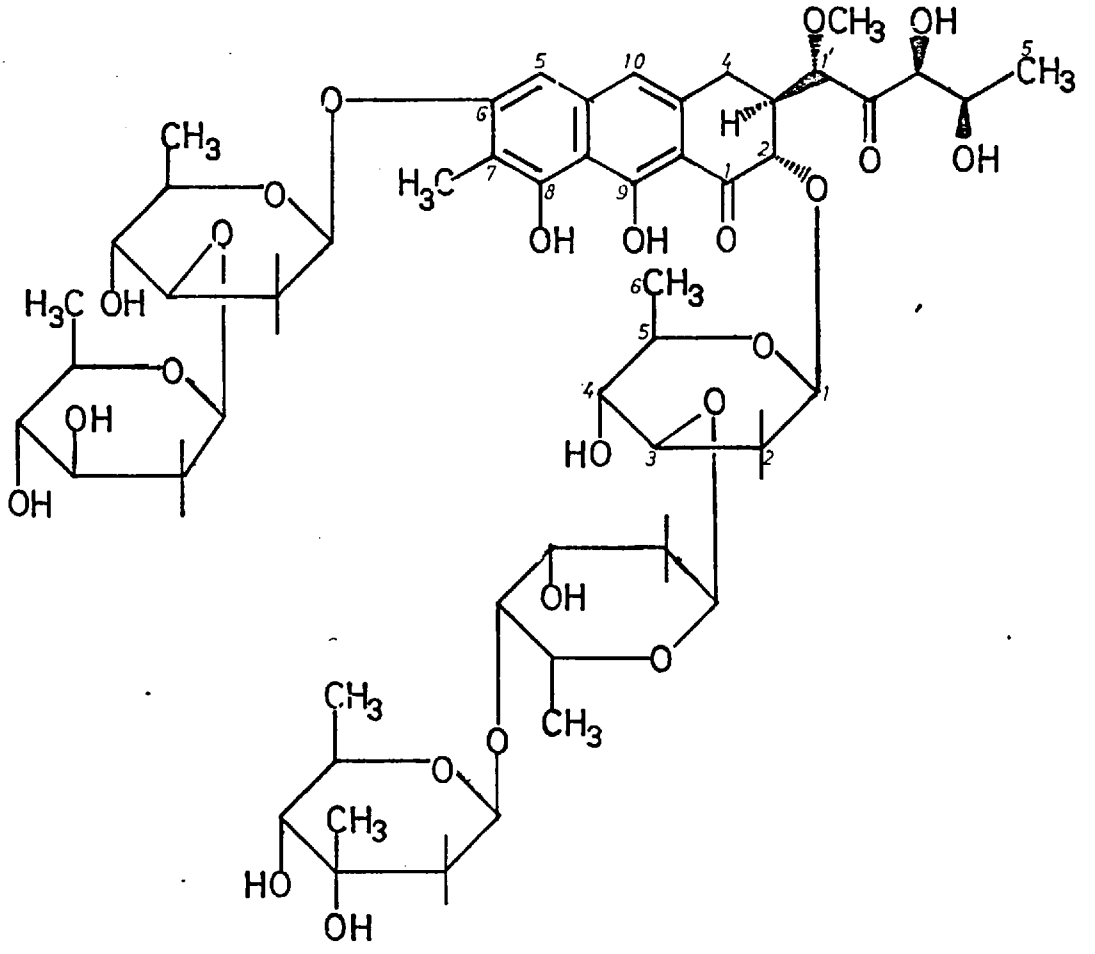
While investigating historical hearsay that a tea made from the leaves of white and pink flowered varieties of Vinca Rosea was supposedly useful in the control of Diabetes Mellitus, Beer and co-workers[293] found that single injections of extracts of this plant to mice led to their death, autopsies on which showed multiple abscesses, granulocytopenia and bone marrow depression. This chance observation led to the isolation[294,295], mainly as the sulphates, and the identification of the seven or so active constituents in the extracts; the two major alkaloids, Vincaleukoblastine (Velbe, Vinblastine) (44; R=Me) and Vincristine (Oncovin, Leurocristine) (44; R=CHO), have since been extensively evaluated and have provided a major contribution towards the treatment of cancer. Their structure was determined by Neuss and co-workers[296-298]. These alkaloids prevent the incorporation



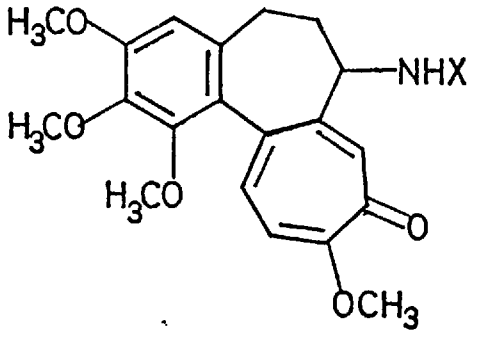
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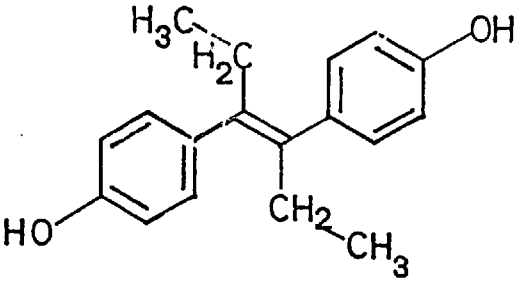
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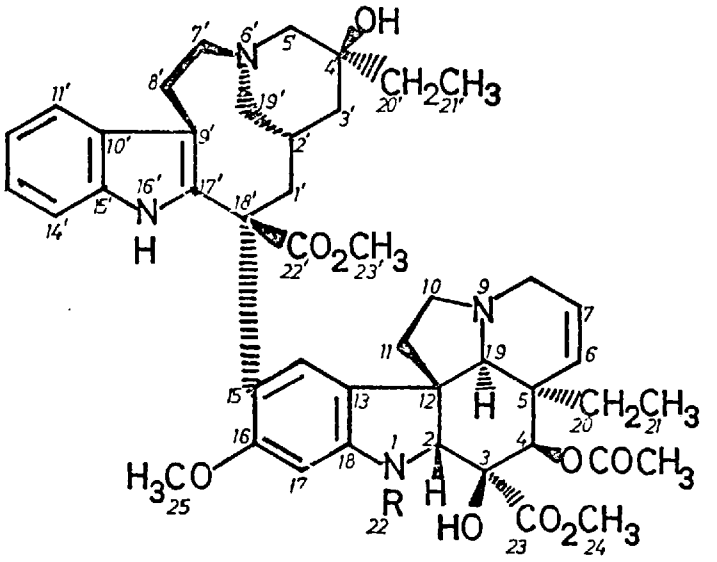
(41)


H3CO
H3CO
H3CO
NHX
O
OCH3

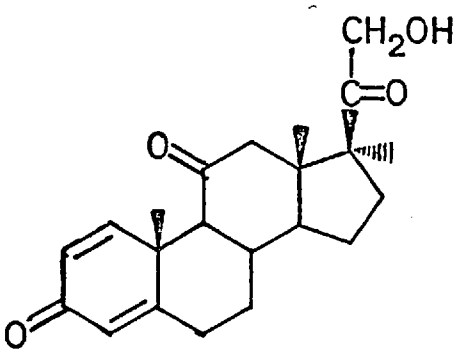
(43)


H3C
H2C
OH
HO
CH2
CH3

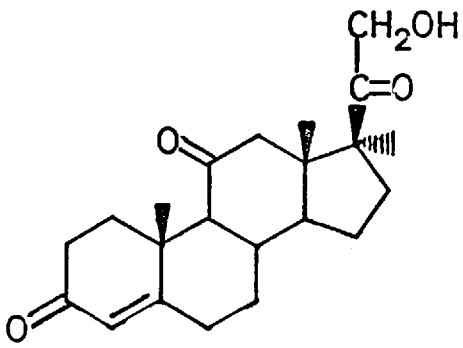
(47)


OH
CH2CH3
CO2CH3
H
N
H3CO
OCOCH3
HO
CO2CH3

(44)


CH2OH
C=O
O
O

(45)


CH2OH
C=O
O
O

(46)

of amino acids as glutamic acid and arginine into transfer RNA. They therefore inhibit protein synthesis at levels that do not effect incorporation of uridine into RNA. A number of amino acids, as glutamic and aspartic acid, can completely reverse the antimitotic properties of vinblastine indicating a similar transport mechanism even though their structures are so different[299,300]. Cells are uniquely sensitive to the Vinca alkaloids during the prophase to metaphase transition of mitosis when the cell membrane becomes temporarily permeable to them. Yet although Vinblastine and Vincristine differ practically by only two hydrogen atoms, their mode of action is substantially different. While both inhibit RNA synthesis, vincristine has no effect on the S phase while vinblastine reduces thymidine incorporation by 25%. Furthermore vinblastine is particularly cytotoxic to cells in mitosis while vincristine is not. This difference in their mode of action is exploited in the management of cancer especially as there is no appreciable cross-resistance between the two agents. Thus vincristine decreases RNA and protein synthesis in human neoplastic leukocytes but not normal leukocytes[301]. Vinblastine exerts severe bone-marrow depression while vincristine exerts prominent neurotoxic effects on the peripheral nervous system (especially in the legs) and to a lesser extent on the autonomous nervous system[302,303]. Their lack of cross-resistance enables vinblastine to be used on patients with intracranial gliomas that had become resistant to vincristine.

Vincristine has been used with good results in the treatment of lymphosarcoma and of reticulum cell sarcoma that had previously showed poor response with cyclophosphamide[304]. It has also been used in the management of breast cancer[305] and in the treatment of acute leukemia in children, especially as children under ten years of age have been found resistant to neurotoxicity[306]. In combination, vincristine has been used with cyclophophamide in the management of neuroblastoma, achieving complete remission in seven out of nine patients[307]. Vinblastine achieved clinical remissions in advanced Hodgkin's disease that was no longer responsive to alkylating agents[308], in chorio-carcinoma resistant to other therapy, and in 23% of breast cancer patients[305]. It has also been used in combination with fluorouracil in the treatment of breast cancer.

The use of alkaloids in cancer chemotherapy has been reviewed by Creasey and co-workers[309], Connors[310], Wilms[311], Svobcda[312] and Bhakuni[313].

(e) Enzymes.

The chance observation that mice and rats bearing transplanted Gardner lymphosarcoma when injected with normal guinea-pig serum had striking regressions of the tumour[314] led to the identification of the active constituent in the serum which was identified by Broome[315,316] to be L-asparaginase, which converts asparagine to aspartic acid. A further important contribution was the finding that L-asparaginase could be obtained on a large scale from Escherichia Coli B[317], thereby providing the impetus for detailed research. Studies on its mechanism of action revealed the very important concept that, contrary to the statements of Warburg[22] and Danielli[23], there may be a gross difference between animal and possibly human tumours, and normal tissues, in their ability to synthesize certain vital amino acids. The action of L-asparaginase is based on the metabolic defect of some neoplastic cells which synthesize endogenous asparagine very slowly, unlike normal tissues, and therefore rely on an exogenous supply. L-Asparaginase destroys the asparagine supply of both normal and neoplastic cells but whereas normal cells can adapt themselves efficiently to asparagine deficiency by synthesizing their own endogenous supply, neoplastic cells cannot cope. L-Asparaginase inhibits protein synthesis but is relatively non-toxic. It lowers serum asparagine to almost zero and renders lymphocytes unreactive to mitogenic stimulation in humans[318]. Its C.I. in animals bearing advanced Gardner lymphosarcoma can be as high as 7000 and against acute lymphocytic leukemia in humans the chemotherapeutic index is over 500. Clinically, however, its cytotoxic effects have been less impressive[319] and although it induces up to 60% remission in acute lymphoblastic leukemia these remissions are short lived due to the development of resistance[319-321]. The anti-tumour effect of L-asparaginase is augmented by a significant, though small, contribution from the host's immune system, failure of which results in incomplete cures and relapse to asparaginase resistant cells, where the tumour cells acquire the ability to produce their own endogenous supply of asparagine by producing asparagine synthetase. L-Asparaginase is, therefore, mostly used in combination with other agents which potentiate its effects.

Cysteine has also been found to be essential for certain tumours in experimental systems while serine is required for chronic myelogenous leukemia cells in man[318] but no enzymes have yet been found that can selectively inhibit these amino acids.

L-Asparaginase has been extensively reviewed and the reader is referred to reviews by Wriston and Yellin[322], Burchenal and Kreis[290], Meshkov and Levitov[323], Patterson[324] and Cottgen[325].

(f) Hormones.

Endocrine therapy owes its origin to the pioneering research of C. Huggins who established that some cancers in man are hormone dependent[326-328]. This led to the wide acceptance of hormone therapy in advanced prostatic and breast cancer.

Androgens, oestrogens, corticosteroids and progestational agents have been found very effective, especially in combination with cytotoxic agents, in the management of prostatic cancer.

Thus prednisone (45), a corticosteroid, can be administered, especially in combination with cytotoxic agents with which it is synergistic, to advanced metastatic breast cancer patients achieving dramatic subjective benefit without fear of stimulating the activity of the disease[329]. Its limiting toxicity acts primarily on protein balance. Cortisone (46) has also been used in the treatment of acute leukemia, being a strong depressant of phagocytic activity[330]. Diethylstilboestrol (stilboestrol) (47), an oestrogen, acts by suppression of pituitary gonadotropin or by direct action on the tumour cells, and as such, has proved a very effective agent in the treatment of prostatic cancer especially when administered as its water-soluble diphosphate (Honvan). Some of the most clinically useful hormones, apart from the ones mentioned above, are: 17-α-hydroxy-6-α-methyl-pregn-4-ene-3,20-dione (Provera, medroxyprogesterone acetate)[331] (48) in the treatment of advanced endometrial adenocarcinoma[332,333], metastatic hypernephroma[334] and metastatic renal cancer[335]; 17-α-hydroxyprogesterone caproate (Depostat, delalutin)[336] (49) in the treatment of prostatic cancer[337] and metastatic endometrial carcinoma[338,339]; 17-α-ethynyl-19-nor-testosterone (Norethisterone acetate, norlutin, primolut, SH 420)[340] (50) in the treatment of selected cases of breast malignancy; and chloro-tri-(p-methoxyphenyl)ethylene (chlorotrianisene, TACE)[341] (51) used in the treatment of prostatic cancer[343].

It is outside the scope of this work to go into further details on endocrine therapy but the reader is directed to an excellent book, edited by Stoll[344], for a comprehensive review on the subject.

Drug Resistance.

Drug resistance was first recognized by Ehrlich[345] at the turn of the century and has moved in step with chemotherapy ever since. Following the remarkable regressions of prostatic cancer in man achieved with diethylstilboestrol[328], acquired resistance in cancer chemotherapy was first noticed when a transplantable mouse Lymphosarcoma sensitive to cortisone developed resistance[346]. Nearly all known anti-cancer agents have since been shown to develop drug resistance in experimental neoplasms[347], and it seems apparent that neoplastic cells have a greater degree of biochemical adaptation than normal cells, especially in their ability to repair damaged cells. Thus, "as long as cancer cells are able to divide, Darwinian evolution is inevitable"[348].

Drug resistance has been associated with chromosomal change[349,350]. L1210 leukemia cells resistant to methotrexate have been found to have the subtelocentric chromosome missing and this has been correlated with an increase in dihydrofolate reductase activity[351-353]. Various factors may give rise to drug resistance such as:-

(a) altered cell membrane permeability - resistance to HN2 involved a decrease in cell permeability and hence a decrease in the uptake of the drug by active transport systems[354,355]. Resistance to other alkylating agents results from a reduction in the level of alkylation[356] due to a decrease in cell permeability[357]. Similarly a decreased capacity of the cell membrane to be permeable towards such agents as actinomycin-D, mithramycin, vinblastine, vincristine, mitomycin-C and daunomycin, leads to resistance to these drugs[358]. Moreover a decreased transport of methotraxate into leukemic cells led to resistance being developed in patients with acute myelocytic leukemia[352, 359, 360];

(b) increased degradation of the drug - the natural resistance to ara-C in being catabolized by deaminase enzymes has already been mentioned (page 30). Similarly, resistance to 6-thioguanine develops as a result of increased guanine deaminase activity[361];

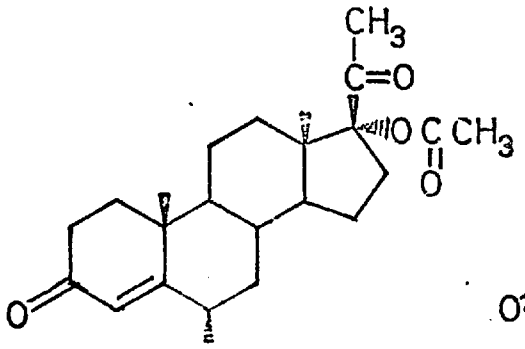
(c) decreased "lethal synthesis" - a reduction in the enzyme level decreases the synthesis of the active metabolite from the less active chemotherapeutic agent. L 1210 leukemia resistant to 6-mercaptopurine and 6-thioguanine was found to have much lower guanine-hypoxanthine phosphoribosyltransferase activity and thus these agents were converted to their active mononucleotides to a much lesser degree[362].

Similarly, leukemias resistant to ara-C have a significantly lower ability to phosphorylate ara-C to its active nucleotide as a result of reduced levels of deoxycytidine kinase[363,364]. Resistance to 5-fluorouracil also arises as a result of considerably lower levels of uridine phosphorylase and uridine kinase, its anabolic enzymes;

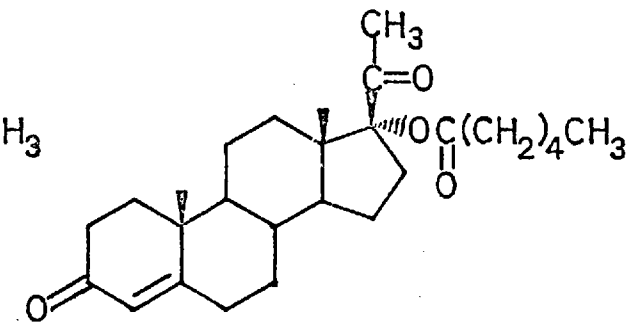
(d) increased detoxifying enzyme levels - resistance to L-asparaginase has already been briefly mentioned (page 39). It is the result of increased synthesis of L-asparagine through induction of asparagine synthetase. Gallo and co-workers[366] suggested that in sensitive cells this enzyme is repressed but that in resistant cells it is re-induced thus forming endogenous asparagine. Similarly administration of alkylating agents is followed by increased levels of enzymes involved in repair of DNA[367];

(e) altered enzymes - the entire dihydrofolate reductase prior to methotrexate administration to rat liver consisted of one enzyme of molecular weight of 20,000. Following its administration however, a new type of dihydrofolate reductase with molecular weight of 24800 was induced which competitively inhibited the original enzyme and thus induced resistance to methotrexate[368,369]. Similarly, resistance to 5-fluorouracil also arises as a result of alteration of thymidylic acid synthetase, the altered enzyme being uninhibited by the active metabolites of 5-fluorouracil[370].

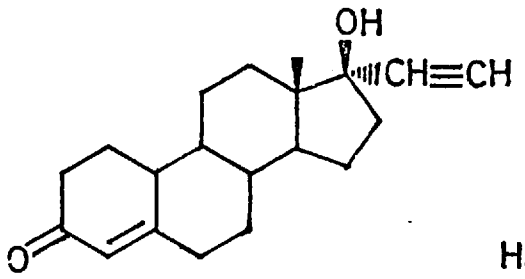
Furthermore, the initial damage to DNA by alkylating agents may be modified by intracellular enzymes which recognize and remove the alkylated base by a series of repair processes[371,372]. Cleaver[373] showed that skin cells of the human genetic disease Xeroderma Pigmentosum were deficient in the excision repair of UV lesions or alkylation caused by alkylating agents. Cleaver[374] also showed that repair replication was independent of the biochemical defect and that this repair replication was inactivated by high doses of alkylating agents. Such excision-repair processes probably follow the pathway shown in Fig. 7. $\overline{PyPy}$ indicates intrastrand crosslinking of the pyrimidine dimer by an alkylating agent. Endonuclease enzymes recognize the abnormal region and excision of this and other neighbouring deoxyribonucleoside-5'-phosphates is followed by repair of the break, making use of the complementary DNA strand as template for the deoxyribonucleoside-5'-triphosphate substrates. Ligase enzymes complete the bonding of the repaired nucleotides to reform the original non-



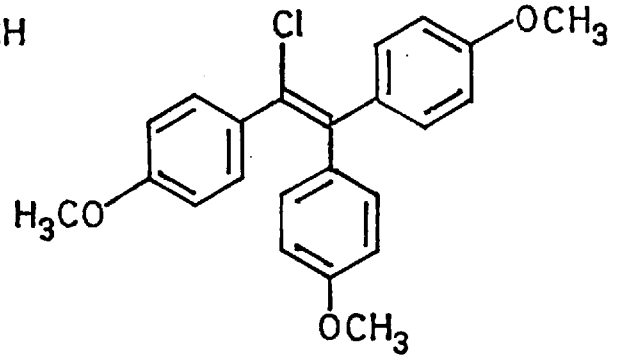
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(49)



(50)



(51)

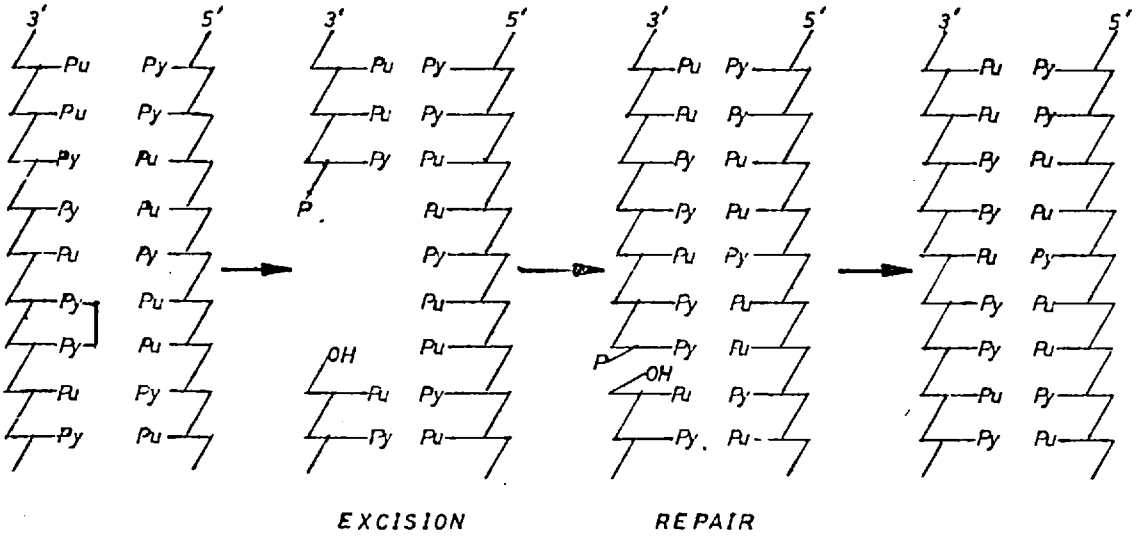


Fig. 7.

alkylated DNA molecule, thus achieving resistance to alkylation.

Overall, drug resistance exposes a gloomy outlook to the use of single chemotherapeutic agents in the treatment of cancer but the advent of combination therapy and better predictability models for drug resistance[375] leading to better understanding of the mechanisms involved will eventually lead to the use of synergistic regimes that will substantially overcome drug resistance.

Combination Chemotherapy

Single cytotoxic agents may only be administered to patients at doses tolerated by them. More often than not, such doses are below those required to achieve the destruction of the tumour. However, the combination of two or more drugs with different tissue-specific toxicities exhibited no additive increase in dose limiting toxicity and decreased to a great extent the development of resistance[376]. Thus, if the spontaneous mutation rate leading to resistance to each drug is 10^{-6} then the simultaneous resistance to two drugs is 10^{-12}. So the actual resistance to the regimen is enormously reduced.

As early as 1951, Potter[26] had hypothesized that agents which blocked sequential steps in a metabolic pathway by acting on different enzymes in the pathway could be used effectively in combination. Soon after, methotrexate and 6-mercaptopurine were found to be synergistic against L1210 leukemia[378,379]. Similarly, cytosine arabinoside in combination with 6-mercaptopurine were more effective when applied together than when they were used singly against L1210 mouse leukemia[380] and later 6-thioguanine and ara-C were found even more effective in the treatment of adult acute leukemia[381].

A Combination Toxicity Index (CTI)[382] was developed to indicate the effectiveness of the regimens. It is the ratio of the LD_{10} doses in mice for individual agents to the sum of the decimal fractions of individual LD_{10} doses for combination. If CTI is less than unity, the toxicity is greater than additive and the drugs in the regimen are antagonistic. If CTI is unity, additive toxicity is apparent. But if CTI is greater than one, then, less-than-additive toxicity is obtained and the drugs are synergistic. Thus, CTI for the ara-C - thioguanine regimen is 2.3.

Two types of synergism are recognized. Therapeutic synergism is achieved when a greater therapeutic effect is noticed on using the

regimen. Metabolic synergism results when the dose of each agent can be reduced in combination while still obtaining the same degree of inhibition.

There are various factors which influence the choice of agents in a particular regimen as for example complementary inhibition, induced enzyme activity, collateral sensitivity, adaptive selection and transport interactions.

Drugs that cause direct damage to DNA or RNA as mitomycin C or uracil mustard can be combined with drugs that inhibit the synthesis or utilization of their precursors, as 5-fluorouracil, hydroxyurea or cytosine arabinoside. Complementary inhibition is thus achieved. Danielli[382] showed that certain tumours develop resistance to urethanes through the induction of enzymes that hydrolyse them to inactive products. He then utilized these enzymes by administering inactivated aromatic nitrogen mustard carbamates following pre-treatment by urethane. This hydrolyses the carbamate to the active phenol or aniline derivative thus causing selective cytotoxicity towards the tumour. The use of 6-mercaptopurine in combination with methotrexate has been mentioned above. Alterations in dihydrofolate transferase following mercaptopurine administration induces resistance to the drug. It, however, also induces collateral sensitivity towards methotrexate[384]. This synergism is further improved by adaptive selection where the two agents are used in a cycle - inducing sensitivity to one drug while resistance develops towards the other. Concurrent addition of hypoxanthine with methotrexate induces hypoxanthine-phosphoribosyltransferase activity. When resistance to methotrexate develops, concurrent addition of 6-mercaptopurine and folic acid is adopted. Due to the higher enzyme activity, 6-mercaptopurine is activated to a greater extent, while at the same time folic acid decreases the level of dihydrofolate reductase thus restoring sensitivity to methotrexate[385]. Glucose pre-treatment to various alkylating agents, particularly epoxides, has been adopted to enhance the characteristic lower pH of neoplastic cells relative to normal cells arising from the increased levels of lactic acid generated by glycolysis in the tumour[386]. To increase the permeability of the plasma-cell membrane to actinomycin-D, Tween 80, a detergent, has been used in combination[387]. Furthermore, the vinca alkaloids are unique in that they increase active transport to methotrexate. Thus vinblastine-resistant cells have been shown to induce collateral sensitivity to methotrexate[388,389].

Present day use of combination therapy involves the use of four or more drugs in each regimen. The combinations are selected on the basis of some of the factors discussed above, their scheduling and the dosages required being carefully computed from various complicated mathematical calculations[390].

One such regimen, VAMP (vincristine, amethopterin, mercaptopurine, prednisone)[199,200], achieved a 90% response to acute lymphocytic leukemia. Another regimen, MOPP (mustine, oncovin, prednisone, procarbazine) has replaced single agent therapy in advanced Hodgkin's disease achieving 70% complete remissions of five years or more[391-393]. These agents are usually administered in short intensive bursts with rest periods allowing host recovery but not tumour regrowth. As the treatment of cancer becomes more motivated towards achieving a cure rather than palliation, the schedules become more and more complex. Thus, pre-arranged schedules of the induction regimen COAP (cyclophosphamide, oncovin, ara-C, prednisone) followed by maintenance regimens of POMP (prednisone, oncovin,methotrexate,mercaptopurine), CART (cytosine arabinoside, asparaginase, rubidomycin, thioguanine) and COAP are in clinical use in the treatment of acute lymphoblastic leukemia.

Finally, some measure of success has now been achieved in the synchronization of some tumour cell populations. This involves the partial arrest of cell populations at a particular drug-sensitive phase in the cell cycle, usually the S-phase, which can then be treated more effectively with phase-specific agents. In vivo partial synchronization with hydroxyurea has been obtained[394,395]. Furthermore vinblastine synchronized L1210 leukemia in vivo by mitotic arrest and therapeutic synergism was achieved when cytosine arabinoside was administered 15 to 24 hours later[396]. Thus proper timing of a phase specific agent to a synchronized population results in greatly improved cytotoxicity but only a short exposure to the drug. Unfortunately each indiviual patient responds differently to synchronization and maximum synchronization is currently not possible.

All these factors indicate the importance of understanding more fully the mode of action of chemotherapeutic agents in order to be able to design better drugs.

Drug Design.

The selectivity of action of a drug is enhanced by a number of variables involving water-lipid solubility, permeability through cell membranes, chemical reactivity and potentiation by enzymes[23,383]. Several of these variables have already been discussed briefly.

Water and lipid solubility are very important for drug design and have led among others to the synthesis of chlorambucil, cyclophosphamide and the nitrosoureas. Alkylating agents need to penetrate cellular and nuclear membranes to reach their site of action. To achieve this, active difunctional groups have been attached to carrying structures related to natural substances involved in cell growth. Such carrying structures may be amino acids (as in the case of melphalan), carbohydrates (e.g. mannitol derivatives), nucleic acid bases (e.g. uracil mustard) or steroid derivatives (e.g. phenestrin (52))[397]. Various drugs have been designed in this laboratory to be activated by transport mechanisms but results have been disappointing[398,399]. Various metabolites require permease enzymes to pass through cell membranes. Antimetabolites utilize such mechanisms less efficiently[400] and have to compete with exogenous supplies of the natural metabolite. Antimetabolites are therefore designed to be less polar thereby by-passing such active transport mechanisms and penetrate the cell membrane by passive diffusion.

Differences in chemical reactivity can be exploited to achieve specific properties in a particular drug. Thus, TEPA, which acts via an S_N2 mechanism, has no effect against mouse leukemia L1210. But its C-dimethyl analogue acts via an S_N1 mechanism through the formation of a stable tertiary carbonium ion and as such has been found effective against the neoplasm[401]. Various aziridines and epoxides are more active at the lower pH prevalent in neoplastic cells and so pretreatment with glucose accenuates the pH differential. The C.I. of TEM increases from 9 to 28 following glucose pretreatment of Walker rat carcinoma-256[402]. Chemical reactivity is also influenced by the redox potential of various tissues. This is lower in neoplastic cells than most normal cells[403]. So quinone derivatives have been designed to be more reactive at lower redox potentials and are activated by *in vivo* reduction[404].

Potentiation by enzymes gives rise to latent activity. These enzymes can be oxidative, reductive or hydrolytic. As tumour cells

have lower redox potentials than normal cells, the design of agents that can be activated by oxidative enzymes is difficult. However, the activity of (NN-di-2-chloroethyl)aniline is consistent with its primary oxidation to the highly active 4-(NN-di-2-chloroethylamino)-phenol (53) *in vivo*. Cyclophosphamide is also believed to be potentiated by oxidative enzymes, one active metabolite isolated from urine after its administration being 4-ketocyclophosphamide[405]. Mitomycin C (39) is likewise activated by reductases which reduce the quinone ring to the hydroquinone structure prior to loss of the methoxy group at 9'.

The most important reductases exploitable by drug design are azoreductases abounding in hepatic tissues. Most of the work carried out in this field has been done by Ross and his co-workers[71] following the discovery that 4-(NN-di-2-chloroethylamino)azobenzene (54: R_1=R_2=H, R_3=H, X=Cl)[406] was active by being reduced *in vivo* to the very active 4-(NN-di-2-chloroethylamino)phenylenediamine. It was further found that the insertion of electron releasing substituents as methyl and methoxy groups and carboxylic acid groups (ionized under physiological conditions)[74] ortho to the azo linkage enhanced cytotoxic activity and facilitated the reduction of the azo linkage[407,408]. This led to the synthesis of 2'-carboxy-2-methyl-4-(NN-di-2-chloroethylamino)azobenzene (54: R_1=H, R_2=CH_3, R_3=CO_2H, X=Cl) (CB 1414), one of a few drugs that have exhibited significant activity towards Lymphoma-8. Although in theory these active azobenzene derivatives were designed to treat liver tumours, in practice these did not respond well to alkylation even though they are situated so that they receive high doses. These liver tumours are less sensitive than bone marrow cells and therefore bone marrow toxicity limits their treatment. Ross and co-workers[409] have developed an azomustard that can be confined to the liver and is hydrolysed to harmless products before reaching the bone marrow. The highly inactivated 2'-carboxy-2-methyl-4-(NN-di-2-bromopropylamino)-azobenzene (54: R_1=R_2=CH_3, R_3=CO_2H, X=Br) is reduced *in vivo* to the corresponding para amino derivative which is extremely active and has a hydrolysis half-life of only 41 seconds compared to the free amine from CB 1414 with a hydrolysis half-life of 8½ minutes and a circulating time of 30 seconds in man. It is the only drug known that can cause complete regression of liver tumours at doses tolerated by the host.

The development of drugs that can be activated *in vivo* by hydrolytic enzymes has been mainly pursued in this laboratory[72].

Various carbamates of the very active nitrogen mustards 4-(NN-di-2-chloroethylamino)phenol (53) (C.I.=2.3) and 4-(NN-di-2-chloroethylamino)phenylenediamine (55) (C.I.<1) have been synthesized. Remarkably high C.I. values were achieved[401], so that, although these carbamates were designed for enzymatic activation by hydrolysis of the carbamate linkage, further activation was achieved by the selective uptake of these agents by specific receptors and site-directed catalysis[410]. Among the carbamates derived from the phenol mustard (53) the most active was found to be 4-(NN-di-2-chloroethylamino)-phenyl N-(4-carboxyphenyl)carbamate (56) (IC 140)[399] having a C.I. of 17. Compared to chlorambucil with a C.I. of 12, this result was very promising. Its reverse carbamate (IC 213) (Table 1) was soon found to be three times as active; and other carbamates (57) of the aniline mustard (55) were found to be three times or more as active as IC 213 (Table 1)[72,411-412] (page 53)

The present work being described here was designed to investigate the effects on the cytotoxicity of carbamate derivatives of types (56) and (57) where the substituent group was introduced in the aromatic ring containing the active nitrogen mustard group.

A very common problem in drug design is to find the optimum substitution on a benzene ring for maximum drug cytotoxicity. So far, the choice of substituent has been based mainly on experience and intuition, but now, a more rational choice may be made based on the developement of the Hansch method[413-415] for the structure-activity correlations involving the partition coefficient of compounds between water and octan-1-ol (log P) (for lipophilicity), Hammett's sigma constant (σ) and Taft's polar substituent constant (σ^*) and steric parameter (E_s)[416,417].

The design and developement of highly active and selective drugs is therefore essential in the drive to achieve cure rates rather than mere palliation in cancer patients and it is hoped that the following work will contribute towards this goal.

CHAPTER 2.

AROMATIC DIFUNCTIONAL NITROGEN MUSTARDS

A survey of the literature revealed that the first aromatic nitrogen mustards to be synthesized were 2-(N-2-chloroethylamino)phenol and (N-2-chloroethyl-N-2-hydroxyethyl)aniline, prepared by Knorr[418] in 1899. However, the first difunctional aromatic nitrogen mustard was only prepared in 1934 by Robinson and Watt[419] when they synthesized (NN-di-2-chloroethyl)aniline. The preparation of an aryl nitrogen mustard usually involves the intermediate preparation of the aryl alkanolamine which is subsequently halogenated to give the nitrogen mustard. In the following short discussion on the preparation of aryl nitrogen mustards attention will only be focused on the preparation of aryl chloroethylamino derivatives.

Preparation of Aryl Dialkanolamines

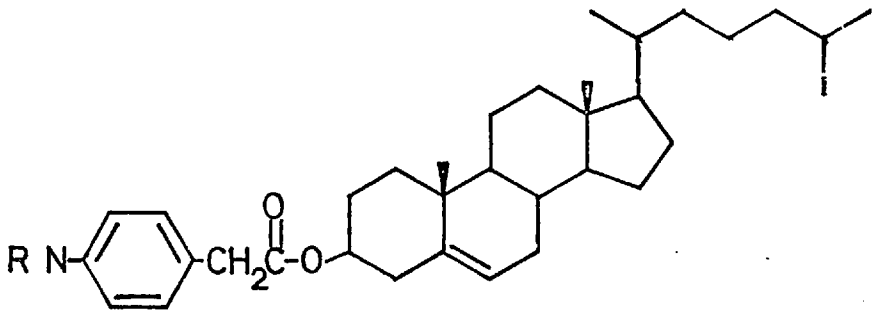
The most commonly used method of preparing di-2-hydroxyethylamino derivatives (henceforth abbreviated 'diol' derivatives) is the reaction of aniline derivatives with epoxides, usually ethylene oxide (58). Various ways of accomplishing such a reaction have been used by various people, ranging from stirring the two reactants at 0° in the presence of a solvent, to the heating of the two reactants with or without solvents in a sealed tube to temperatures as high as 200°C[420]. The various conditions imposed for bishydroxyethylation depend on the basicity and steric environment of the aromatic amine, as well as its solubility. At room or lower temperatures, the solvent normally used is 50% aqueous acetic acid but, depending on solubility and reactivity of the amine, water or glacial acetic acid have been used[418,421]. At these conditions, an excess of liquid ethylene oxide is employed[74] - the acetic acid probably acting as a catalyst as well as a solvent. The more basic the amine, the readier hydroxyethylation is achieved. A striking example of the importance of the basicity effect is the reaction of the three nitroaniline isomers with ethylene oxide respectively. Bieksa and Degutis found that 3-nitroaniline reacts readily with ethylene oxide to give the diol[422], that 4-nitroaniline only gave the diol with great difficulty[423] and that 2-nitroaniline gave only starting material and the monohydroxyethylamino derivative[424]. Results

obtained during the course of this work are in agreement with Bieksa and Degutis' work and are also substantiated by the values of the basic strengths (pK_b) (Table 2) of the respective isomers of nitro, chloro, methoxy, methoxycarbonyl and hydroxyanilines, measured titrimetrically[425-428] at 25° in an aqueous phase or potentiometrically (in terms of the half neutralization voltage $E_{\frac{1}{2}}$)[429] in various solvents. Hall[429] concluded that the strength of the amine, in water, is a reliable index of its base strength in organic solvents, differences in solvation, steric effects and entropy not being important enough to cause major disturbances in the order of respective base strengths. One can therefore correlate the basicity of the aryl amine in aqueous acetic acid or in organic solvents with the pK_b values of the aryl amines in Table 2.

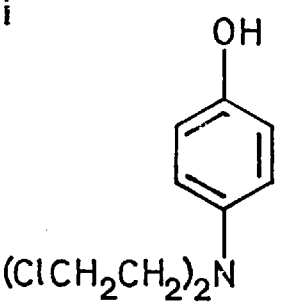
At temperatures of 90° or higher, equivalent quantities of the amine and the epoxide are used, and heating is effected in a sealed container. Under such conditions, much higher pressures than atmospheric are built up. Usually, no solvent is used but occasionally benzene is added to facilitate the addition of the reagents into the sealed container. Ross[74] furthermore emphasized the importance of adding only the theoretical amount of ethylene oxide - polymers of type (59) are otherwise formed. Friefelder and Stone[420] examined in detail the reactions of various anilines with ethylene oxide. Thus, all the methoxyanilines and 3- and 4-chloroaniline react at 90-100° over 5 hours to give the respective diols. However, 2-chloroaniline needed heating at 150° for 15 hours to give comparable results, heating at 90° for 18 hours giving only 45% of the monohydroxyethylamino derivative. At 150° 3- and 4-nitroanilines also gave the respective diols but 2-nitroaniline could not be induced to react[430]

An alternative reagent to ethylene oxide has been reported several times. The reaction of the aryl amine with chlorohydrin is normally achieved in the presence of a base, usually calcium carbonate, at high temperaturee either by refluxing an aqueous solution[342] or by heating without a solvent at higher temperatures than 100°, usually at around 130°C[432]. The base helps to remove the hydrogen halide formed (60).

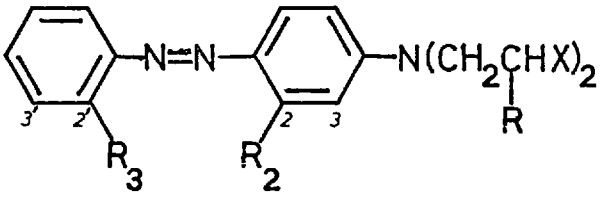
Using these methods, the three isomers of hydroxy-(NN-di-2-hydroxyethyl)aniline[377,431], of chloro-(NN-di-2-hydroxyethyl) aniline[342,433,434], of methoxycarbonyl-(NN-di-2-hydroxyethyl) aniline[74,406,433] and of methoxy-(NN-di-2-hydroxyethyl)aniline[432-434] were prepared.



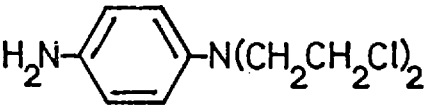
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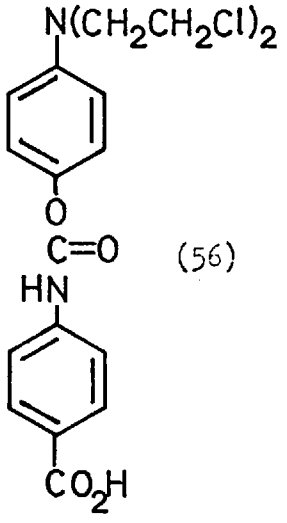
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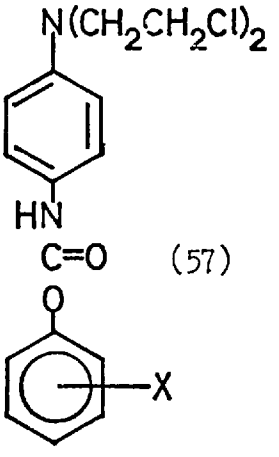
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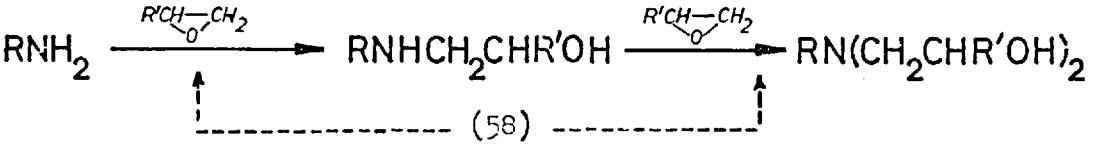
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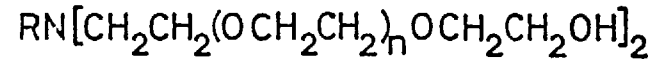
(56)



(57)



(58)



(59)

Table 1. Urethanes of type (57)

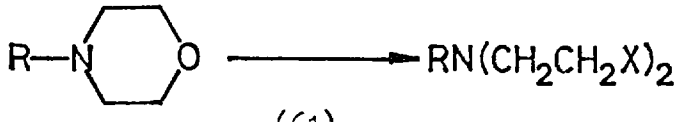
IC No.	X	C.I.
213	p-CO_2H	46
220	p-CO_2Me	137
235	m-CO_2H	85
234	m-CO_2Me	150
290	p-Cl	70
291	p-OMe	17
292	o-OMe	140
296	m-OMe	120
297	p-$CH_2CH_2CO_2H$	140

Table 2. pK_b values for substituted anilines in aqueous solution at 25°C:

X	$-NO_2$	-Cl	$-CO_2Me$	-OMe	-OH	$-CO_2H$	H
ortho	-0.05	2.65	2.23	4.52	4.65	2.2	
meta	2.47	3.67	3.55	4.23	4.25	3.1	4.63
para	0.99	4.15	2.46	5.34	5.55	2.5	

$$RNH_2 + 2XCH_2CH_2OH \xrightarrow{-2HX} RN(CH_2CH_2OH)_2$$

(60)



(61)

The only other general method used to prepare arylalkanolamines is via the aromatic nucleophilic substitution of activated functional groups, mainly halogeno and nitro groups, by alkanolamines, usually monoethanolamine. The first synthesis of an aryl dialkanolamine using this method was reported by Waldkotter who prepared the corresponding 'diol' by refluxing diethanolamine with 2,4-dinitrochlorobenzene in ethanol for 5 hours[436]. The chemistry of and uses of this reaction will be discussed later. A large number of aryl monoethanolamines have been prepared by this method but very few successful preparations of aryl diethanolamines have been achieved, prior to the work being reported here, using this method.

Preparation of Aryl Difunctional Nitrogen Mustards

The dialkanolamines described above have been halogenated by various reagents, the most common of which is undoubtedly phosphorus oxychloride, followed by thionyl chloride and phosphorus pentachloride. Phosphorus oxychloride in chloroform or benzene under reflux conditions has been used as an efficient chlorinating agent[342,431,437]. Phosphorus pentachloride in chloroform with refluxing[342], thionyl chloride in chloroform or in chloroform plus dimethylformamide[412], in benzene under reflux conditions[439] or in pyridine at temperatures below 60°C[440] have also been used. However, thionyl chloride is liable to esterify rather than chlorinate aromatic (NN-di-2-hydroxyethyl)amines, particularly if these aryl 'diols' contain strong electron accepting groups (e.g. nitro groups) in the ortho or para positions to the substituted amine groups[441]. Therefore, aryl alkanolamines containing nitro groups at ortho or para positions are never chlorinated with thionyl chloride, and phosphorus oxychloride is usually used.

Various other chlorinating agents have been used in the synthesis of aryl nitrogen mustards. p-Toluenesulphonyl chloride (tosyl chloride) in dry pyridine at temperatures around 100° is one example - tosylation of the hydroxy groups being achieved first followed by subsequent chlorination at the higher temperatures[442]. White[438] achieved chlorination using milder conditions. He tosylated the 'diol' using tosyl chloride - dry pyridine at 0° for 48 hours. The isolated tosyl derivative was then chlorinated with lithium chloride in dry isobutyl methyl ketone at 100° for 12 hours, obtaining the respective nitrogen mustard. Werner[443] also achieved good yields of the aryl nitrogen

mustards by reacting the tosyl derivative, obtained as in White's method[438], with dry lithium chloride in dry acetone in a sealed tube heated at 80° for 8 hours with stirring. Methanesulphenyl chloride (mesyl chloride) in pyridine has also been used to chlorinate various aryl diethanolamines. The method was first used by Goodman and De Graw[444] by heating the diol with mesyl chloride in pyridine at 80-90° for 20 minutes. Bergel and co-workers[445] refluxed the chlorinating solution for 20 minutes while Ross and co-workers[71] also used this chlorinating agent at 80° for 20 minutes. But Goodman and co-workers[188] found that heating was not always necessary, chlorination being achieved simply by stirring the reactants in pyridine at room temperature for about 18 hours. The need for a reagent that would act rapidly in mild and essentially neutral conditions led Lee and co-workers[446,447] to use triphenylphosphine in excess anhydrous carbon tetrachloride as the chlorinating agent. Refluxing at 80° for one hour was sufficient to achieve chlorination, the only by-products of the reaction being triphenylphosphine oxide and chloroform.

Cerkovnikov and Stern[285] found that the morpholino ring (61; R=Ph) could be cleaved by hot hydrobromic acid to yield the bromo nitrogen mustard (61; R=Ph, X=Br). Bieksa and Degutis[424], however, found that N-(2-nitrophenyl)morpholine (61; R=2-NO_2Ph) was very stable and the morpholino ring could not be cleaved - heating with 48% hydrobromic acid in a sealed tube gave only charred products. This stability was not characteristic only of the nitro group for its corresponding 2-aminophenyl derivative still gave no chlorination product. Vingiello and Duranleau[448], however, succeeded in repeating Cerkovnikov and Stern's method[285]. They managed to obtain the corresponding di-2-chloroethylamino derivatives by heating N-(4-bromophenyl)morpholine, N-(4-tolyl)morpholine or N-(1-naphthyl)morpholine with concentrated hydrochloric acid and zinc chloride in a sealed tube at 180° for about 36 hours, achieving good yields.

Alkylation Reactions of Aromatic Nitrogen Mustards

Alkylation may be defined as the conversion of:

$$A:^- + R\text{-}X \longrightarrow R\text{-}A + X:^- \quad \ldots\ldots\ldots\ldots \text{(i)}$$

where R is an alkyl group and A, in biological terms, is a nucleic acid or a protein. Equation (i) may proceed within or via two extreme mechanisms, namely, S_N1 or S_N2 mechanisms.

An S_N1 mechanism would involve the slow solvent-assisted ionization of the alkylating agent followed by rapid reaction of the resulting carbonium ion with the nucleophilic centres. An S_N1 mechanism implies that the rate of the reaction is independent of the concentration of the nucleophile $A:^-$ ($v_1 = k_1[R\text{-}X]$) :

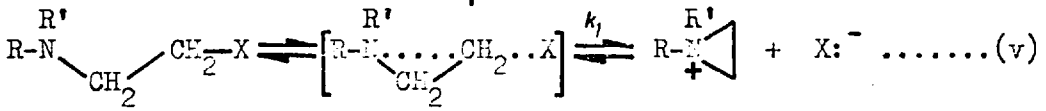
$$R\text{-}X \xrightarrow[r.d.s.]{solvent} R^+_{solv.} + X^-_{solv.} \quad \text{.....(ii)}$$

$$A:^- + R^+_{solv.} \xrightarrow{fast} R\text{-}A \quad \text{.....(iii)}$$

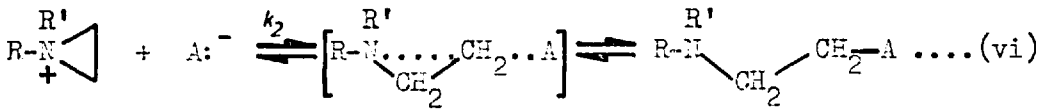
On the other hand, an S_N2 mechanism involves the rate determining formation of an intermediate complex (A--R--X) followed by the fast elimination of the leaving group $X:^-$. As such it is strongly dependent on the concentration of the alkylating agent as well as the concentration of the nucleophile $A:^-$ ($v_2 = k_2[R\text{-}X][A:^-]$) :

$$A:^- + R\text{-}X \xrightarrow[r.d.s.]{} [A\text{--}R\text{--}X] \longrightarrow A\text{-}R + X:^- \quad \text{.....(iv)}$$

In the particular case of nitrogen mustards, the neighbouring group effect of the nitrogen atom on the displacement of the halogen leaving group from the alkyl group assumes particular importance. The nitrogen atom acts as a nucleophile with unshared electrons capable of forming a bond to the β-carbon:



$$\text{.....(v)}$$



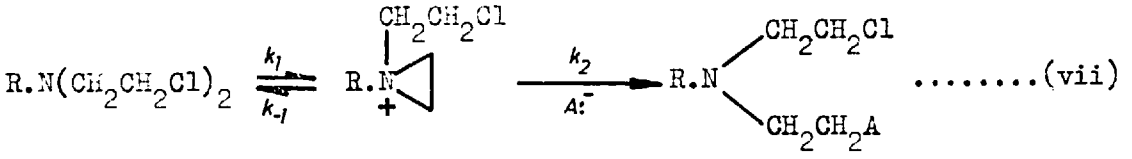
$$\text{....(vi)}$$

The analytical evidence for the formation of the cyclic aziridinium ring during the hydrolysis of HN2 was first presented by Hartley and co-workers[449] and supplemented by Golumbic and co-workers[65]. The overall kinetics are dependent on the relative rates of cyclization and subsequent reaction with nucleophiles. If k_1 is rate determining, then the reaction follows a "superficial S_N1" mechanism. But if k_2 is rate determining, then the reaction follows a "modified S_N2" mechanism. Furthermore, stereochemical and kinetically controlled ring cleavage with ethyleneimines and ring closure and rearrangement with 2-chloroethylamines indicate clearly that each ring cleavage is accompanied by a Walden inversion whether conditions are favourable to S_N1-type or S_N2-type mechanisms. The nitrogen atom, therefore, exercises steric control on the reaction[450].

In aliphatic nitrogen mustards, the cyclisation step is very rapid. In the particular example of HN2, the formation of the

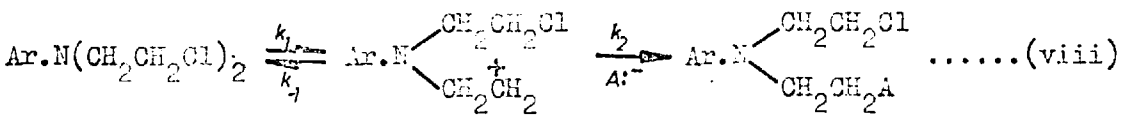
aziridinium ion is complete in two minutes at physiological conditions. Once formed, the aliphatic aziridinium salt is relatively stable, the ring-opening reaction being very slow (the half-life of the hydrolysis reaction of the aziridinium salt of HN2 is more than one week at physiological pH and temperature). The aziridinium salt therefore accumulates and can be detected. Golumbic and co-workers[65] isolated the picryl sulphonate of HN2 while the formation of the aziridinium ring has been established by NMR spectroscopy[451,452]. The formation of the aziridinium salt has been furthermore shown during titrimetric measurements of the hydrolysis of HN2 - a rapid release of one equivalent of chloride ions but a much smaller release up to one equivalent of hydrogen ions results in the formation of the half-hydrolysis product (stabilized as the hydrochloride)[65]. Titration with thiosulphate gives a measure of quaternary nitrogen ions present in solution. In the hydrolysis of aliphatic nitrogen mustards, rapid consumption of thiosulphate indicates rapid formation of the aziridinium salt. Finally hydrolysis of 1-diethylamino-2-chloropropane gave rise to 2-diethylamino-propan-1-ol, confirming that the reaction proceeded via the initial formation of the respective cyclic aziridinium salt[453].

These facts having been established, it is now universally accepted that the reactions of nucleophiles (including water) on aliphatic nitrogen mustards proceeds via the "modified S_N2" mechanism:



.....(vii)

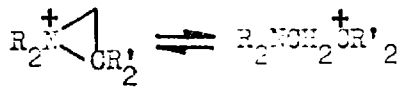
However, two major conflicting theories have been expounded on the mechanism of alkylation of aryl nitrogen mustards. As a result of the initial synthesis of several cytotoxic aryl nitrogen mustards, Ross and co-workers[342] examined the hydrolysis rate of the various aryl di-2-chloroethylamines they had synthesized. They developed a method of measuring the percentage hydrolysis under standard conditions (20 mmoles of the nitrogen mustard per litre dissolved in 50% aqueous acetone and heated at 60° for 30 minutes). From such a study, Ross and co-workers[73,342,406] and Bergel[454] asserted that the mechanism of nucleophilic substitution (hydrolysis or alkylation) for aryl nitrogen mustards was different to that of aliphatic nitrogen mustards, and they proposed that the reaction followed an S_N1 mechanism giving rise to a carbonium ion intermediate rather than an aziridinium salt:



.....(viii)

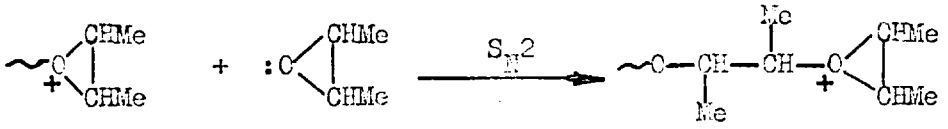
Ross and Bergel based their assumptions on the experimental observations that: (a) the reaction followed first order kinetics, (b) chloride and hydrogen ions were simultaneously liberated in equivalent quantities without an intermediate half-hydrolysis product being obtained, (c) the instantaneous thiosulphate titre was negligible, (d) the rate of alkylation (or hydrolysis) was independent of the nucleophilic centres, (e) electron donating substituents on the aromatic ring facilitated ionization of the carbon-halogen bond resulting in easier formation of carbonium ions, and hence, in an increase in the rate of alkylation or hydrolysis (as expected of an S_N1 mechanism) - electron donating substituents increase the basicity of the nitrogen atom making it more reactive, (f) the presence of added chloride ions reduced the degree of hydrolysis, (g) introducing an electron donating group (e.g. methyl group) at C_2 of the chloroethylamino group, or an ethyl group on the nitrogen atom, resulted in a considerable increase in the reactivity, (h) the relative amounts of ester and hydroxy compounds formed when the halogen of 2-naphthyl nitrogen mustard was chlorine or bromine, and when the ions were added, were the same, indicating a carbonium ion intermediate, (i) (N-ethyl-N-2-chloroisopropyl)aniline and 4-(NN-di-2-chloroisopropylamino)chlorobenzene underwent hydrolysis without rearrangement, thereby, according to Ross[406], excluding the possible formation of the intermediate aziridinium ion.

In the fifties and sixties, most workers in this field accepted the S_N1 mechanism, while Connors[168,457] and others were still favouring a superficial S_N1 mechanism as recently as 1975. But there were a few others who proposed different mechanistic pathways. Thus, Hanby and co-workers[455] postulated a possible tautomerism between the aziridinium ions and the corresponding carbonium ions. Leonard and Crist[456] reasserted that such tautomerism was possible, particularly if a stable tertiary carbonium ion can be formed:

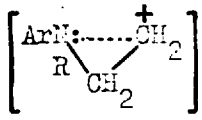


But extensive stereochemical studies of an analogous ring opening reaction, the cationic polymerization of epoxides, showed that

nucleophilic attack on the cyclic oxonium ion proceeded with inversion of configuration at the carbon atom undergoing attack, thus disproving the formation of the carbonium ion from the aziridinium ion:



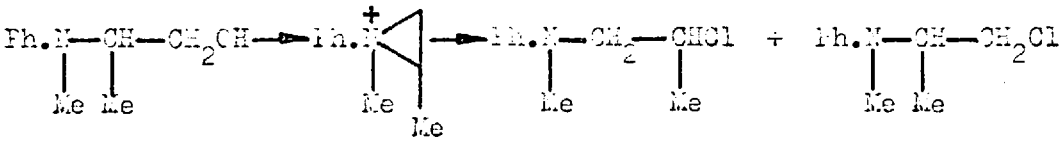
On the other hand, Bardos and co-workers[458] investigated the relative S_N1- and S_N2- reactivities of various aryl nitrogen mustards, as measured by Ross's method of percentage hydrolysis, as well as by their alkylating activity towards 4-(4'-nitrobenzyl)pyridine (NBP). They established that the hydrolysis rate and alkylating activity were dependent on the basicity of the nitrogen atom and so they decided that the transition state for the hydrolysis reaction was a 'solvated carbonium ion - nitrogen dipole', conformationally similar to the aziridinium ion, thus following an S_N1-type reactivity:



Bardos and co-workers also proposed that the alkylation reaction with NBP followed an S_N2 complex mechanism with possible formation of an aziridinium ion-type intermediate.

These mechanisms have been discarded as highly improbable by Owen and co-workers[459], Price[460], Price and co-workers[452] and Williamson and Witten[461]. Instead, they maintained that the involvement of the nitrogen atom in the displacement reactions of aryl mustards can be explained by an identical mechanism to that established for aliphatic nitrogen mustards, namely a 'modified S_N2' mechanism involving conventional aziridinium ions. Scrutinizing the nine points proposed to explain an S_N1 mechanism, listed above, one can see, in respective order of mention, that (a) the observation of first order kinetics does not distinguish between an S_N1 or an S_N2 mechanism particularly as the rate determining step in the solvolysis of aryl mustards can be the actual formation of the aziridine ion giving a rate of reaction normally independent of the concentration of the nucleophile, (b), (c), (d) - these three points can be explained on the basis of the formation of an aziridinium ion provided that k_2 is greater than k_1 (in equation (vii)); moreover, for some aryl mustards, chloride ions were released at a significantly faster rate than the disappearance of activity, indicative of the formation of reactive intermediates[461], (e) the argument that electron donating substituents on the aromatic ring facilitate the ionization of the C-X bond is

untenable since this amounts to the efficient transmission of an inductive effect over more than two carbon atoms. It can however be explained on the basis of an internal S_N2 cyclisation of the C_2 atom onto the nitrogen atom, with formation of an aziridinium ring - electron donating groups increase the basicity of the nitrogen atom and facilitates ring closure and formation of the aziridinium ion, (f) the experimental observation that added chloride ions reduced the degree of hydrolysis can be explained on the basis of the common-ion effect which is observed whenever a discrete reactive intermediate is obtained in a substitution reaction, (g) the increased degree of hydrolysis as a result of a methyl group at C_2 or of an ethyl group on the nitrogen atom can also be explained on the basis of formation of an aziridinium ring - (NN-di-3-chloropropyl)aniline was found to undergo hydrolysis at rates slower than for the corresponding 2-chloroethyl derivative, while (NN-di-6-chlorohexyl)aniline was not appreciably hydrolysed[406], again indicating direct involvement of the nitrogen atom in the fundamental mechanism of hydrolysis or alkylation. Increasing the number of carbon atoms, made entropy requirements for cyclisation too high, making cyclisation less favourable and resulting in a slower reaction, (h) again the observation that the relative amounts of alkylation and hydrolysis products with some chloro mustards and bromo mustards were the same, can also be explained on the basis of an aziridinium ion intermediate; furthermore, Chapman and James[462] found that the rate of release of bromide and iodide ions from corresponding bromo and iodo mustards were much larger than expected of an S_N1 mechanism, (i) lack of rearrangement of (N-ethyl-N-2-chloroisopropyl) anilinecannot be justifiably used to conclude that aziridinium ion intermediates are not formed. It simply indicates that, on thermodynamic grounds, probably as a result of steric inhibition by the ethyl group on the nitrogen, ring-opening of the aziridinium ring is effected by nucleophilic attack on the same carbon atom that originally ring-closed to form the aziridinium ion. In fact, Owen and co-workers[459] have established that [N-methyl-N-(1-methyl-2-hydroxyethyl)]aniline, when chlorinated, gave predominantly [N-methyl-N-(2-methyl-2-chloroethyl)]aniline plus some unrearranged product, and that acetolysis of this mixture gave even more predominantly [N-methyl-N-(2-methyl-2-acetoxyethyl)]aniline:



Work carried out in this laboratory by Baig[463] and Sflomos, on a number of similar compounds, with substituents in the aromatic ring and with methanesulphonyl rather than halogen leaving groups, have further proved the general formation of an intermediate aziridinium ring. Scrambling of the deuterium label was established when three representative aryl mustards, labelled with deuterium at C_2, were hydrolysed or acetolysed[459], again proving the intermediate formation of an aziridinium ion.

Finally, Perges and Peradejordi[464] used the LCBO-MO/LCAO-AO method to calculate the sigma-charge densities and Huckel's LCAO-MO method to calculate the pi-charge densities, of a series of NN-di-2-chloroethylanilines. An independent method using more complicated quantum mechanical procedures showed that both methods gave similar quantitative results from which they concluded that both hydrolysis and alkylation reactions depend largely on the anchimeric power of the nitrogen lone pair, again supporting the formation of aziridinium ions and confirming that ring substituents have a mesomeric influence on the anchimeric power of the nitrogen atom and that the 'modified S_N2' mechanism is equally valid for aromatic as for aliphatic nitrogen mustards. Aryl mustards differ from aliphatic mustards only in their rate of formation of the aziridinium ion, which is slow in aryl mustards mainly as a result of the electron withdrawing properties of the aromatic ring. This effect decreases considerably the overall basicity of the nitrogen atom - the pK_a values of the aniline mustard and HN2 are 6.1 and 2.2 respectively. Such a great difference must obviously affect the rate of cyclisation in such a way that k_1 in equation (vii) will be considerably reduced making k_2 greater than k_1 and therefore giving rise to apparent first order kinetics.

Aromatic Nucleophilic Substitution Reactions of Dichloronitrobenzenes

Holleman and co-workers[465] prepared all six isomeric dichloronitrobenzenes and investigated their nucleophilic substitution reactions with methoxy anions and diethylamine. They found that only one chlorine atom was replaced, that ortho prevailed over para substitution (the 2-chloro atom was replaced much more easily than the 4-chloro atom in nucleophilic reactions with 2,4-dichloronitrobenzene) and that chlorine atoms in a meta position to the nitro group were inactive. Furthermore, with the exception of 2,6-dichloronitrobenzene, the extra

chlorine atom accelerated the reaction of the 2-chloronitrobenzene. Their relative reactivities at 85° are summarized below:

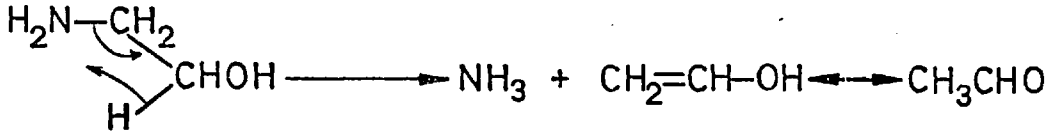
Isomer	2-	2,3-	2,4-	2,5-	2,6-	3,4-	4-
^{-}OMe	0.369	1.74	19.41	3.93	0.135	17.42	1.39
$MeOt_2$ ($x10^{-3}$)	1.4	1.6	27	6.7	0.24	4.4	0.65

They concluded that the introduction of a nitro group in dichlorobenzene greatly enhanced the reactivity (introducing a chloro group in dinitrobenzene had a similar effect), and that increasing the temperature of the reaction from 85° to 110°, increased the rate and ease of reaction eightfold.

The aromatic nucleophilic substitution of active chloronitrobenzenes had been known for a long time. Beilstein and Kurbatow[466] had condensed 2,4-dichloronitrobenzene with ammonia obtaining substitution of the chlorine atom ortho to the nitro group. Since then, several other nucleophiles have been investigated including alkanolamines. Moran[467] was one of the first workers to use monoethanolamine as a nucleophile in the substitution of 2,4-dinitrochlorobenzene. He achieved this by heating the reactants in ethanol, in the presence of a base (sodium hydroxide), at 80°. Karrer and coworkers[468] heated 2-chloronitrobenzene with monoethanolamine, in the presence of anhydrous sodium acetate, for 24 hours at 120°, and later carried out the same reaction by refluxing in pyridine[469] obtaining, in each case, 2-(N-2-hydroxyethylamino)nitrobenzene.

As a result of extensive work at City College, New York, Meltsner and co-workers[470] discovered that the reaction of ethanolamines on nitrohalides brought about, not only condensation, but also reduction to the corresponding azoxy, azo or amino compounds, hydrolysis or combinations of these reactions - the yield of any product depending on the presence of the alkanolamine, any added alkali, and the time of reaction. Kremer[471] repeated Karrer's work[468] on 2-chloronitrobenzene and obtained the monoethanolamine derivative plus a small percentage of 2-chloroaniline after 8 hours heating. 4-Chloronitrobenzene gave corresponding products plus a 20% yield of 4,4'-dichloroazobenzene, while 3-chloronitrobenzene gave only reduction products. Meltsner and co-workers[472] reacted 2-chloronitrobenzene with diethanolamine at 180° for 3 hours. The products of the reaction isolated, even without the presence of added alkali carbonate, were N-(2-aminophenyl)-

morpholine, 2,2'-dichloroazobenzene, 2-chloroaniline and 2-nitrophenol, while a similar reaction with 4-chloronitrobenzene gave only the azo derivative. Bieksa and Degutis[424] confirmed this and stated that reactions of 2-chloronitrobenzene with diethanolamine, under different conditions, yielded complex mixtures, not easily separated by thin layer chromatography, of the azoxy, and azo derivatives. Kremer and Kress[473] investigated the mechanism of such reductions and confirmed that the ethanolamine is decomposed into ammonia and an aldehyde:



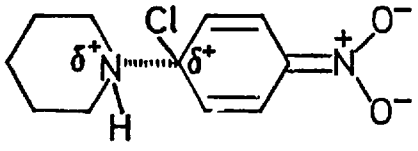
Kremer and Bendich[474] then considered the corresponding reactions of ethanolamine with all six dichloronitrobenzenes. They achieved the respective condensation reactions by refluxing the dichloronitrobenzene with ethanolamine in propyl or butyl alcohol in the presence of magnesium oxide as an acid-binding agent. Their results, as shown below, were in agreement with those obtained by Holleman and co-workers[465] except that 2,5-dichloronitrobenzene was found more reactive than the 3,4-isomer:

Isomer	2,3-	2,4-	2,5-	2,6-	3,4-
% Yield	60	88	70	13	64

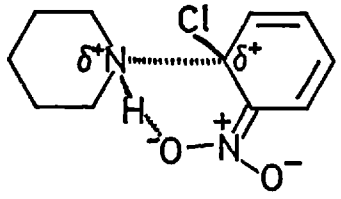
As Holleman[465], Kremer[474] only obtained substitution of the 2-chloro atom in 2,4-dichloronitrobenzene, again indicating the much higher activity of ortho chloro atoms to para chloro atoms in chloronitrobenzenes. However, when Kremer and Bendich[475] progressed to diethanolamine condensations on the isomeric dichloronitrobenzenes by heating with (or without) anhydrous sodium carbonate at about 175° for approximately 4 hours, no condensation products were obtained and only reduction and hydrolysis products and tars were isolated.

An interesting development arose as a result of den Hertog and Jouwersma's observation[476] that the condensation of 2,4-dichloronitrobenzene with aqueous ammonia gave the expected ortho substitution product, 2-amino-4-chloronitrobenzene, and also a small amount of the para substitution product, 4-amino-2-chloronitrobenzene; while condensation with alcoholic ammonia gave only the ortho substitution product. This established that a change of solvent resulted in a change in the ortho-para substitution ratio. Bunnett and Morath[477] developed this

work and established that the o/p ratio for chloronitrobenzenes decreased as the solvent became more polar - decreasing from 80 in xylene to 1.4 in 1% dioxan-99% water. They showed that the rate for the ortho isomer remained relatively unchanged while that for the para isomer increased. Following the observations that para isomers of halonitrobenzenes react faster than the corresponding ortho isomers with methoxide anions[465,478], but that the same para isomers react slower with amines[465,479] led Bunnett[480] to summarize the then known data on such substitutions and propose a mechanism for them. Thus, nitro groups accept electrons by mesomeric interactions and so, in general, activate about equivalently the o/p positions but not the meta position. Predominance of ortho substitution is partly the direct result of additional inductive effects of the ortho nitro group. The observation that piperidine and N-deuteropiperidine react at similar rates with both 2- and 4-chloronitrobenzene[479] indicates that the N-H bond of piperidine is not broken in the transition state, indicating further that the transition state is composed of a metastable zwitterionic tetrahedral intermediate:



(62)



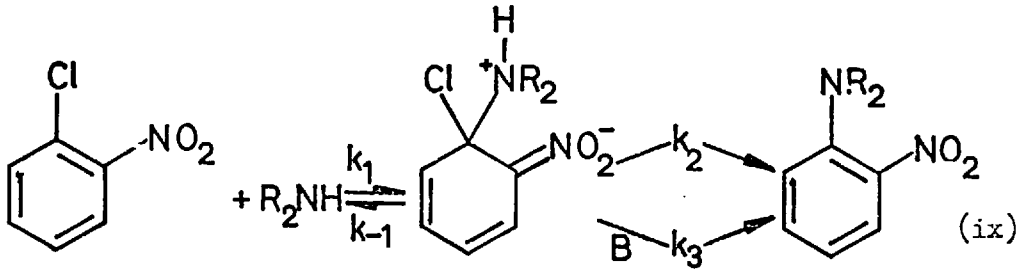
(63)

Such extensively ionic transition states would be strongly solvated and thus the rates of reaction would be solvent dependent, as observed[476,477]. The para transition state would obviously be greatly solvated but the electrostatic interaction between adjacent negative (oxygen atoms on nitrogen) and positive (nitrogen atom of the amine) ends of the dipolar ortho transition state reduces the requirements for solvation giving rise to strong "built-in" solvation which increases the entropy of activation thus favouring ortho substitution.

However, steric interactions between the nitro group and the ortho chlorine atom in the ortho isomer was believed to offset partially such preferential ortho substitutions. To achieve maximum activation, the nitro group must achieve coplanarity with the aromatic ring in the zwitterionic intermediate. An ortho group would partially impair attainment of coplanarity. Yet, attainment of double-bondedness of the nitro group is critical in order to accomodate the negative charge of the pentadienate system, so that steric compression may arise, in the case of a group ortho to the nitro group, which would increase to some degree the free energy of the ortho transition state as compared to the

unhindered para transition state. This effect was believed to be powerful enough to overcome the inductive effect and the "built-in" solvation effect in the reaction of chloronitrobenzenes with alkoxide ions and to induce para substitution. A very minor effect could also influence such preferential para substitutions - electrostatic repulsion in the transition state between the negatively charged oxygen atoms of the nitro group and the partly negative incoming nucleophile would oppose ortho substitution. But in reactions with amines, "built-in" solvation and inductive effects overcome any steric compression effects and establish overall ortho substitution. In any case, in the reaction of 2,4-dichloronitrobenzene with nucleophiles, its nitro group would be bounded by an ortho group and would be potentially sterically hindered irrespectively of whether ortho or para substitution prevails, so that, all things being equal, ortho substitution dominates.

Several mechanisms for such aromatic nucleophilic substitution reactions have been proposed but the multi-step addition-elimination mechanism is now generally accepted[481] particularly as strong evidence of base catalysis has been established:



(ix)

If the steady-state treatment applies the mechanism of equation (ix) gives the rate equation (x) where k is the second order rate coefficient (Rate / [Substrate][R_2NH]):

$$k = \frac{k_1k_2 + k_1k_3[B]}{k_{-1} + k_2 + k_3[B]} \qquad (x)$$

Thus, by adding or omitting bases in the reaction mixture, one can exert considerable control on the relative ratios of product formation or reversion to reactants and therefore on the overall rate-determining steps. The intermediate can proceed to products either spontaneously (k_2) or through general base catalysis (k_3). At low base concentrations $k_3[B] \ll k_{-1} + k_2$ and equation (x) describes a straight line indicating linear dependence of k on [B]. At very high concentrations of base, however, $k_3[B] \gg k_{-1} + k_2$ and equation (x) is simplified to $k = k_1$. In the majority of reactions, however, no base catalysis was observed,

or where base catalysis was observed, k was linearly dependent on [B] over the entire concentration range. Absence of base catalysis would mean that $k_2 \gg k_{-1}$. On the other hand, the linear dependence on base, when base catalysis was observed, indicated that $k_3[B] \ll k_{-1}$ and that $k_2 \ll k_{-1}$, so that:

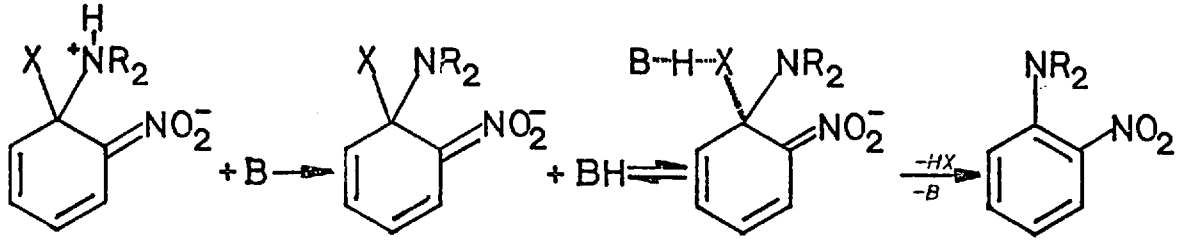
$$k = \frac{k_1 k_2}{k_{-1}} + \frac{k_1 k_3}{k_{-1}}[B]$$

Moreover, as the base is usually the nucleophile itself, first order kinetics is usually observed. Several factors determine whether a reaction is susceptible to base catalysis or not. One such factor is the leaving group. In several aromatic nucleophilic substitutions, the nucleofugicity (capability of leaving group) has been found to increase with decreasing pK of the conjugate acid of the leaving group[484,485]. Chloride ions are therefore among the best leaving groups and base catalysis is usually not observed, implying that $k_2 \gg k_{-1}$. (Alkoxide ions, on the other hand, are very poor leaving groups and make the k_2 term undetectable). Changes in k_2/k_{-1} ratios as a function of the leaving group are mainly due to changes in k_2 since k_2 refers to the bond-breaking process. But k_{-1} is also affected since the leaving group may alter the electrophilic nature, through its inductive and polarisability effects, of the tetrahedral carbon in the intermediate. Another factor is the entering amine. Different amines affect the k_2/k_{-1} ratio mainly through changes in k_{-1} ; k_2/k_{-1} increases with increasing pK of the amine. In reversing the zwitterionic intermediate back to reactants, a charge neutralization would be obtained so that k_{-1} would increase considerably in non-polar solvents reducing the k_2/k_{-1} ratio. This ratio has been observed to be smaller for secondary amines as compared to primary amines possibly due to steric compressions in the intermediate[486]. The steric strain is partly released on reverting to reactants so that, k_{-1} increases and k_2/k_{-1} decreases. But the reaction of 1,3,5-trinitrobenzene with several amines indicated that k_{-1} was similar when methylamine, dimethylamine or piperidine[487] were used indicating that steric strain was not in fact important. Pietra and Del Cima[482], furthermore, investigated the condensation of 2,4-dinitrochlorobenzene with piperidine, 2-methylpiperidine or 2,6-dimethylpiperidine in dimethylsulphoxide and came to the conclusion that steric inhibition was, again, not important.

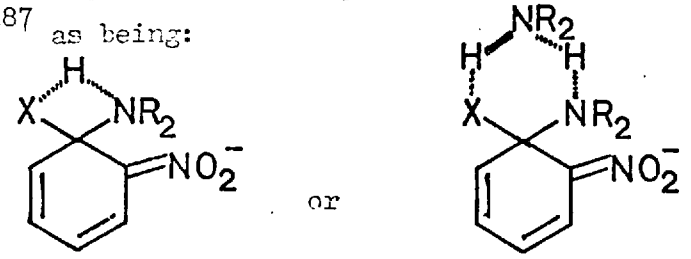
Bernasconi[483] showed that an ortho nitro group gives rise to intramolecular hydrogen bonding in the intermediate complex. For a

2,4,6-trinitrobenzene derivative in aqueous solution, a hydrogen bond strength of around 9.6kJmol^{-1} was suggested. With all the negative charge being concentrated on only one nitro group, as in 2,4-dichloronitrobenzene, the hydrogen bond would be much stronger and full proton transfer may even result[488]. In going from the intermediate (63) to the product, the hydrogen bond must be broken which would therefore require extra activation energy. But there is no hydrogen bonding in intermediate (62) and no extra activation energy would be needed. On the other hand, the drastic increase in the k_2/k_{-1} ratio on moving the nitro group from the para to the ortho position in reactions using the same secondary amine can be explained on the basis that hydrogen bonding decreases k_{-1} much more than k_2 the stronger the hydrogen bond is. Introducing an ortho nitro group, therefore, brings about a change from $k_2 \ll k_{-1}$ to $k_2 \gg k_{-1}$ even though the ortho nitro group could give rise to steric hindrance. In fact, reversion to reactants would also require the breaking of the hydrogen bond and more activation energy would be needed, again indicating that k_{-1} would be decreased considerably.

The mechanism of the base-catalysed step (k_3) involves a fast acid-base equilibrium between the zwitterionic intermediate and its conjugate base followed by rate limiting general acid catalysis by BH resulting in the release of the leaving group:



The mechanism for the uncatalysed step (k_2) must involve simultaneous removal of the ammonium proton and the halogen leaving group to avoid charge creation. A plausible mechanism was proposed by Bernasconi[487] as being:



where R_2NH is the actual nucleophile acting as solvent when used in excess. Support for such mechanisms was shown by an investigation of the proton NMR spectrum of the reaction of methyl picrate with methoxy anions, Meisenheimer complexes being directly observed.

CHAPTER 3

DISCUSSION AND RESULTS

Introduction

The importance of aryl nitrogen mustards in cancer chemotherapy has already been fully emphasized.

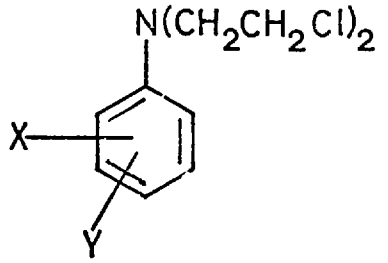
In synthesizing such mustards for biological screening, in the past, most workers have concentrated on disubstituted aryl mustards but comparatively few trisubstituted aromatic mustards have been synthesized and still fewer have been screened.

A comprehensive literature survey of almost all the trisubstituted mustards synthesized up to the end of 1974 revealed that very few aminophenyl and even fewer phenolic trisubstituted mustards have been prepared and that the most common substituents involved were, in order: carboxy groups and their esters, methyl, methoxy, sulphonamido and halogeno groups. Rose and co-workers [407] furthermore have prepared several active azobenzene derivatives particularly with 2-methyl and 2-methoxy groups, while Popp[603,609] prepared several Schiff bases of 2- or 3-substituted 4-(NN-di-2-chloroethylamino)benzaldehyde. A summary of this is given in Table 3.

Most aryl nitrogen mustards, though tumour-active, have very low chemotherapeutic indices making them unsuitable for clinical use. The exceptions, as mentioned on page 49, are mustards containing urethane linkages. Carbamates of the disubstituted mustard phenol (53) (e.g. IC 140) and of the disubstituted mustard aniline (55) (e.g.IC 220, 234, 290, 292 and 296) have been found to have very high chemotherapeutic indices. In such carbamates, the substituents causing highest activity (methoxycarbonyl, methoxy or chloro groups) were always placed in the aromatic ring not containing the mustard substituent.

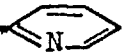
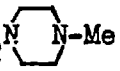
No single carbamate of the trisubstituted aryl mustard type has ever been reported. The main aim of this work was to attempt the synthesis of several trisubstituted mustard phenols or anilines having a substituent of the type mentioned above and subsequently to convert them to the respective phenyl carbamates using isocyanates or chloroformates.

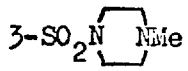
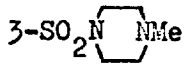
Table 3. Summary of a literature survey on aryl trisubstituted mustards (excluding polycyclic aryl mustards as benzoxazole or naphthyl derivatives)

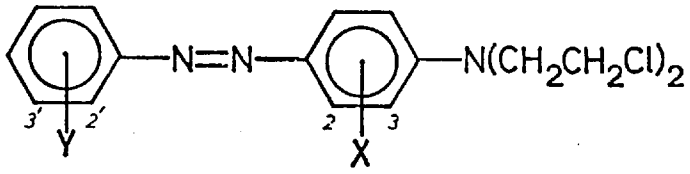


X	Y	Remarks	References
2-OH	5-CO_2Me		439
3-OH	4-CO_2Me		421
3-OH	4-CO_2H		421
4-OH	3-CO_2H		519
3-OH	4-OH		600
5-OH	2-Me	dihydrogen phosphate	601
4-NH_2	3-CO_2Et		523
2-NH_2	5-CO_2Et		530
2-NH_2	5-CO_2H		530,531
4-NH_2	3-CO_2H		523
2-NH_2	5-CH_2CO_2H		531
2-NH_2	4-SO_2NH_2	hydrochloride	441
2-NH_2	4-SO_2NEt_2	hydrochloride	441
2-NH_2	4-SO_2N(CH2CH2)NMe	hydrochloride	602
2-NH_2	4-NH_2	dihydrochloride	603
2-NH_2	4-Me		604
4-NH_2	3-Me	hydrochloride	377,605,409
4-NH_2	3-OMe		377
3-OAc	4-CO_2H		421
2-NHAc	5-CO_2Et		530
4-NHAc	3-CO_2Et		523
2-NHAc	5-CH_2CO_2H		531
2-NHAc	4-NH_2	hydrochloride	603
2-NHAc	4-NHAc	hydrochloride	603
2-NHAc	4-NO_2		603
2-NHAc	5-NO_2		603
2-NHAc	3-Me		377

X	Y	Remarks	References
4-NO	3-Me		377,606
4-NO	3-OMe		377
4-NO	3-Cl		606
4-NO_2	2-Me		604
5-NO_2	2-Me		601,604,610
2-NO_2	4-Me		607
2-NO_2	4-NO_2		342,459,603
2-NO_2	4-SO_2NEt_2		441
2-NO_2	4-SO_2-N(CH$_2$CH$_2$)N-Me		602
3-NO_2	4-CHO		608
2-Me	4-CHO	*	608,609
2-Me	5-CHO		610
3-Me	4-CHO	*	611,612
3-CF_3	4-CHO	*	608
3-F	4-CHO	*	608
3-Cl	4-CHO	*	608,612
2-OMe	4-CHO	*	608
3-OMe	4-CHO		613
2-OEt	4-CHO		608
Me	CO_2Me	all 10 isomers	601,614,615
Me	CO_2H	all 10 isomers	617,618,619
		2-Me-5-CO_2H is an effective anti-tumour agent	616
2-Me	4-CH_2CO_2H		619
2-Me	4-$(CH_2)_2CO_2H$		619
2-Me	4-$(CH_2)_2CO_2H$	toxic but not cytotoxic	619
2-Me	5-COCl		610
3-Me	4-CN		608
2-Me	6-Me		406
2-Me	5-$CONH_2$		409,605,618
2-Me	5-CONH-(2-thiazolyl)		605
2-Me	5-SO_3H		605
2-Me	5-SO_2NH_2		620
2-Me	5-SO_2NH-(2-thiazolyl)		620
2-Me	5-SO_2NH-(2-pyrimidinyl)		620

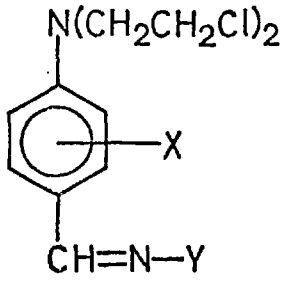
X	Y	Remarks	References
2-Me	5-SO_2NH-		620
3-Me	4-NHCOPh		377
4-Me	3-Cl		433
OMe	OMe	all 6 isomers	433,630
2-OMe	5-CO_2H		601,621,615
2-OMe	5-CO_2Me		615,621
2-OMe	5-SO_2NH-		620
2-OMe	5-SO_2NH-		620
5-Cl	2-CO_2Et		622
4-Cl	3-SO_2NMe_2		623
3-Cl	4-Cl		433
3-Cl	5-Cl		433
2-Et	5-CO_2H		601,615,624,616
2-Et	5-CO_2Me		615
4-NEt_2	3-CO_2H		523,531
2-NEt_2	5-CO_2H		531
4-NEt_2	3-SO_2NEt_2		625
2-NEt_2	5-SO_2NH_2		626
4-NEt_2	3-SO_2-		627
5-CO_2Me	2-Pro		615
5-CO_2Me	2-isoPro		615
5-CO_2Me	2-tertBu		615
5-CO_2H	2-CO_2H		616
5-CO_2H	3-CO_2H		628
5-CO_2H	2-Ph		616
5-CO_2H	2-isoPro		616
5-CO_2H	2-piperidino		531
5-CO_2H	2-morpholino		531
3-CO_2H	4-morpholino		531
3-CO_2H	4-piperidino		531
5-SO_2NH_2	2-piperidino		626
5-SO_2NH_2	2-morpholino		626
3-SO_2NEt_2	4-morpholino		625
3-SO_2NEt_2	4-piperidino		625

X	Y	Remarks	References
3-	4-piperidino		627
3-	4-morpholino		627
3-I	4-$(CH_2)_3CO_2H$		629
3-I	4-$CH_2CH(NH_2)CO_2H$		629
2-OMe	4-$CH{=}C(CN)_2$		608
3-Cl	4-$CH{=}C(CN)_2$		608



2-Me	4'-OMe		407
2-Me	2'-Cl		407
2-Me	2'-NO_2		407
2-Me	2'-CO_2H	very active	407
2-Me	4'-CO_2H		407
2-Me	2',3'-benzo		407
2-Me	4'-Me, 2'-CO_2H		407
2-Me	2'-OH, 5'-NO_2		407
2-OMe	2'-CO_2H	very active	407
3-OMe	3'-CO_2H		407
3-OMe	4'-CO_2H		407
3-OMe	3'-NO_2		407
3-OMe	4'-NO_2		407
2-OMe	2'-OH		407
2-OMe	2'-OMe		407
2-OMe	2'-Me		407
2-CO_2Me	4'-NO_2		407
2-CO_2H	4'-NO_2		407
2-CO_2H	4'-CO_2H		407
2-CO_2H	2'-CN		407

* A fairly large number of Schiff bases were prepared by Popp[608,609] by condensing the aldehyde with various amines. These were of the general structure:



where X was 2-OEt, 2-OMe, 2-Me, 3-Cl, 3-F, 3-CF_3 and particularly 3-Me, and YNH_2 were amines as: p-fluoroaniline, cyclopentylamine, 3-aminopyridine, p-bromoaniline, p-aminophenylmercaptoacetic acid, methyl 3-amino-4-hydroxybenzoate, 4-aminoantipyrine, 1-aminobiphenyl, 1-aminoanthracene, fluorenone hydrazone, N-benzoyl-L-tyrosinehydrazide, 2,4,6-trifluoroaniline, 2-fluoroaniline, 4-fluorobenzylamine, 4-fluoro-3-methylaniline, 4-fluoro-2-methylaniline, 2-amino-4-nitrobiphenyl, 1-aminoindane, 1-aminopyrene and N-amino-1,2,3,4-tetrahydroquinoline.

Of these, three have been found to be very active anti-tumour agents and have undergone clinical trials (X = 3-Me, Y = p-FC_6H_4, o-$C_6H_5C_6H_4$ and cyclopentyl).

Phenolic Methoxycarbonyl Nitrogen Mustards

One such nitrogen mustard was first synthesized by Johnson[439] in this Laboratory though, unfortunately, the yield of the chlorination step was not reported. Johnson[439] had prepared methyl 4-hydroxy-3-(NN-di-2-chloroethylamino)benzoate (67) from orthocaine (methyl 4-hydroxy-3-aminobenzoate) (65) by hydroxyethylation followed by selective chlorination of the resulting triol (66).

It was decided to repeat the preparation of the above-mentioned mustard (67) starting from methyl 4-hydroxy-3-nitrobenzoate (64). This nitrobenzoate was hydrogenated with Raney nickel and hydrogen at room temperature and atmospheric pressure, essentially as described by Cavill[492], to give methyl 3-amino-4-hydroxybenzoate (65). Addition of excess liquid ethylene oxide to a stirred solution of this amine in aqueous acetic acid afforded methyl 4-hydroxy-3-(NN-di-2-hydroxyethylamino)benzoate (66) in fairly good yields. However, large scale preparations of this triol, using a large excess of ethylene oxide, gave an oil which could not be crystallized even by trituration with prolonged cooling and seeding. Partial distillation of this oil under reduced pressure gave a colourless distillate which was identified from n.m.r. as the product of the condensation of acetic acid with ethylene oxide (72) where n was probably 1. The residue crystallized fairly easily to afford the triol which was subsequently recrystallized. Thionyl chloride chlorination of a solution of the triol (66) in refluxing benzene, as in Johnson's preparation[439], gave a precipitate which was worked up to afford the difunctional nitrogen mustard (67) in poor yields (2%). The filtrate was also worked up from which 6-methoxycarbonyl-4-N-2'-chloroethyl-2,3-dihydro-1,4-benzoxazine (68) was isolated and identified from spectral and microanalytical analysis. This benzoxazine has apparently two distinct melting points depending on the solvent used for crystallization. It was also obtained during the reaction of the difunctional mustard (67) with phenyl isocyanate in the presence of catalytic amounts of triethylamine. This internal cyclisation of one of the 2-chloroethyl arms of the nitrogen mustard onto the phenol group resulting in the formation of a benzoxazine derivative is not unprecedented. Knorr[418] had prepared 4-N-methyl-2,3-dihydro-1,4-benzoxazine (74; R = Me) and 2,3-dihydro-1,4-benzoxazine (74; R = H) from the condensation of 2-(N-methyl-N-2-chloroethylamino)phenol (73; R = Me) and 2-(N-2-chloroethylamino)phenol (73; R = H) respectively. A minor product obtained from the filtrate of the above chlor-

ination reaction had an i.r. spectral band at 1760 cm^{-1} and was attributed to the minor reaction of thionyl chloride with the triol (66) resulting in the formation of the cyclic sulphite (75).

The main distinguishing feature between the difunctional mustard (67) and the monofunctional mustard benzoxazine (68) was their n.m.r. spectra. Otherwise, their melting points and R_f values were very similar.

The reason why thionyl chloride favoured selective attack on the aliphatic hydroxyls and why the difunctional nitrogen mustard was precipitated out of solution is still not known.

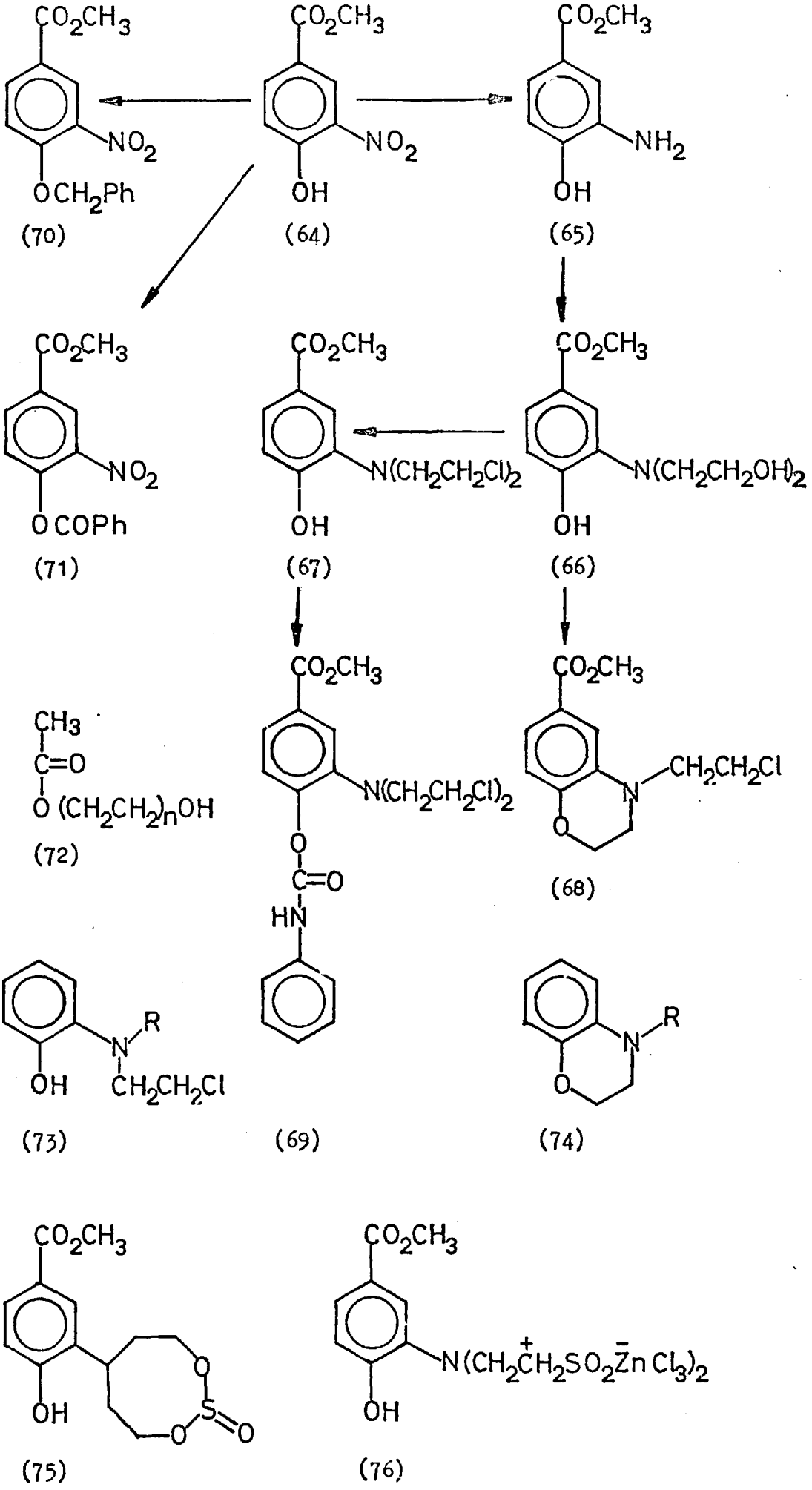
In an effort to improve the yield of methyl 4-hydroxy-3-(NN-di-2-chloroethylamino)benzoate (67), protection of the hydroxyl group at the 3-nitro derivative stage was attempted. The preparation of methyl 3-nitro-4-benzyloxybenzoate (70) had previously been described by Sabalitschka[489] but no mention of the yield obtained was given. Sabalitschka's method, as well as that involving the initial reaction of the 3-nitro derivative with potassium methoxide in methanol followed by addition of benzyl chloride under reflux conditions[496], gave very poor yields of the benzyloxy derivative (70). Benzylation in pyridine, as a homogeneous proton extractor as well as solvent, was also attempted but very poor yields were obtained. Benzoylation with benzoyl chloride in pyridine at room temperature, however, gave high yields of methyl 3-nitro-4-benzoyloxybenzoate[490,493] (71). However, a three-fold improvement in the yield of the di-2-chloroethylamino derivative (67) by a direct method made it unnecessary to proceed further via the benzoyl-protected route.

Schmidt and co-workers[494] recently found that zinc chloride catalysed the thionyl chloride chlorination of aliphatic alcohols, resulting in much improved yields. They proposed that ions are involved in the transformation of aliphatic alcohols to alkyl chlorides and that zinc chloride complexes at one or more stages of the reaction making the reaction species more ionic thereby speeding up the reaction. Their method was therefore extended to the possible selective chlorination of the above triol (66) or its isomers, possibly via the ionic intermediate (76). Using these low acidity, mild temperature conditions the chloro derivative (67) was obtained in good yields (58%) by dropwise addition of thionyl chloride on a solution of the triol in refluxing benzene containing catalytic amounts of zinc chloride. The residue was, once again, worked up to afford the chloro derivative as an oil

which crystallized on trituration with acetone-petroleum.

Various attempts at preparing the N-phenylcarbamate (69) of 4-hydroxy-3-(NN-di-2-chloroethylamino)benzoate (67) gave mainly the starting material and possibly some of the benzoxazine derivative (68). These reactions were carried out in refluxing benzene or toluene containing catalytic amounts of dry triethylamine. But addition of phenyl isocyanate gave no carbamate even after 21 hours refluxing. Yet Pringle[495] succeeded in obtaining the carbamate (78) of 2-(NN-di-2-chloroethylamino)phenol (77) by reacting this phenol under identical conditions with 4-methoxycarbonylphenyl isocyanate. Steric hindrance by the ortho di-2-chloroethylamino group could not, therefore, be the primary reason causing non-reactivity. A possible explanation of the difficulty of reaction of phenyl isocyanate with methyl 4-hydroxy-3-(NN-di-2-chloroethylamino)benzoate (67) lies in the deactivation of the phenol group by the para methoxycarbonyl group. If this were the case, then one would expect that by heating the two reactants in the presence of the catalyst and proton extractor without using a solvent, the concentration of the reactants would be so increased that reaction might be facilitated. When this was attempted a very good yield of 2-(NN-di-2-chloroethylamino)-4-methoxycarbonylphenyl-N'-phenylcarbamate (69) was obtained, the reaction being carried out in a sealed tube to prevent any reaction of the phenyl isocyanate with atmospheric moisture. A small amount of the benzoxazine derivative (68) was also obtained, as explained above. This internal condensation with elimination of hydrogen chloride is, in fact, quite rational especially in the presence of a base. It probably also resulted during the reaction of 2-(NN-di-2-chloroethylamino)phenol with the isocyanate[495] but was undetected.

Baker and co-workers[421] achieved the synthesis of another phenolic methoxycarbonyl nitrogen mustard - methyl 4-(NN-di-2-chloroethylamino)-salicylate (82). They hydroxyethylated methyl 4-aminosalicylate (80) to obtain the corresponding triol (81). However, all their attempts to chlorinate selectively (81) with phosphoryl chloride or thionyl chloride failed, suggesting that the phenolic group had to be protected prior to chlorination. Methyl 4-amino-O-benzoylsalicylate (85) was therefore prepared by them and hydroxyethylated in aqueous acetic acid, but only starting material and monohydroxyethyl derivative (40%) were obtained - the very weakly basic nature of the amine group precluded bishydroxyethylation. Methyl 4-(NN-di-2-hydroxyethylamino)-O-benzoyl-salicylate (83) was however obtained from the amine (85) when glacial



(70) (64) (65)

(71) (67) (66)

(72) (68)

(73) (69) (74)

(75) (76)

acetic acid was used. It was also obtained from the triol (81) by selective benzoylation. Chlorination of (83) gave the blocked nitrogen mustard (84) which was found to be very stable and was only debenzoylated in hot methanolic hydrogen chloride. Attempts to hydrolyse the resulting methoxycarbonyl nitrogen mustard (82) resulted mainly in decarboxylation and they could only achieve a 4% yield of 4-(NN-di-2-chloroethylamino)salicylic acid.

Since 4-aminosalicylic acid and some of its derivatives have been found to possess strong tuberculostatic activity[497,498] and to cause irreversible inhibition of lactic acid dehydrogenase and glutamic acid dehydrogenase[501] it was decided to attempt the synthesis of methyl 4-(NN-di-2-chloroethylamino)salicylate (82) along the above-mentioned pathway and then to react it with various phenyl isocyanates.

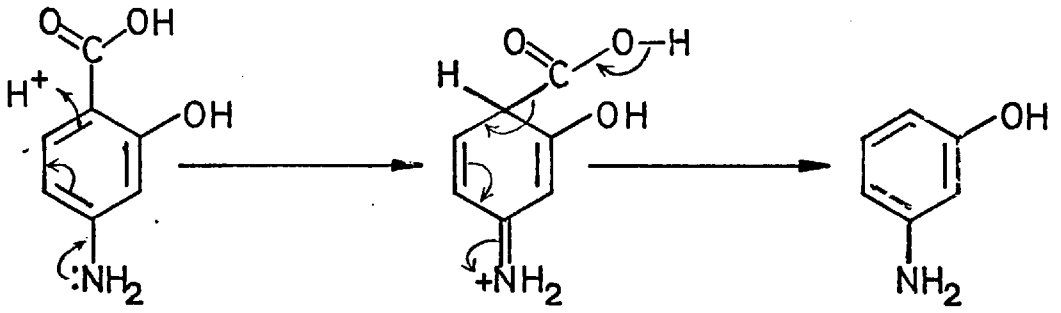
However, when 4-aminosalicylic acid (79) was esterified with methanol saturated with hydrogen chloride, only very small yields of the ester were obtained and the main product isolated was 3-aminophenol (86). As the melting points of the methyl ester (80) and of 3-aminophenol are almost identical, only spectral data can confirm the preparation of the methyl ester. A literature search on the direct esterification of 4-aminosalicylic acid to its methyl ester, showed that although such a reaction has been well documented with over twenty publications since 1920, most of these gave only the melting point as proof of the formation of the methyl ester. Seidel and Bittner[499] first prepared the ester from methyl 4-nitrosalicylate. Drain and co-workers[500] synthesized the ester from various routes including that from the nitrosalicylate, or from 4-aminosalicylic acid by reaction with diazomethane in ether or with methanol in concentrated sulphuric acid. They furthermore characterized the ester by its ultraviolet spectrum. Several other workers [502-504] reported good yields when concentrated sulphuric acid was used as catalyst. Iwatare[505] used 1% hydrogen chloride in methanol but his method is doubtful especially as his reported yield of the ester was greater than theoretical. Schaefer and Doub[506] concluded that direct esterification of 4-aminosalicylic acid (79) by conventional methods under various conditions led to very low yields, the main product in each case being 3-aminophenol. They therefore, adopted Sowa and Nieuwland's method[507] of esterifying salicylic acid and obtained very good yields on using boron trifluoride as catalyst.

Esterification of 4-aminosalicylic acid was achieved in very good

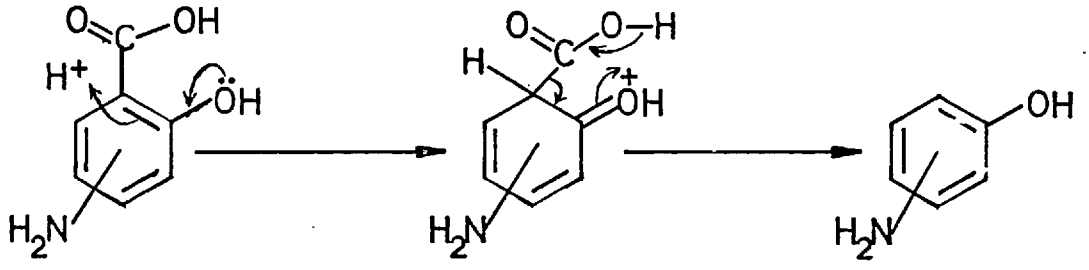
yields using both Schaefer and Doub's method[506] (BF_3 catalyst) and Mc Cubbin and Moir's method[504] (conc H_2SO_4 catalyst). In each preparation the methyl ester obtained was identified by its melting point as well as by its spectral characteristics.

The decarboxylation of 4-aminosalicylic acid has been investigated by several workers[509,510]. Thus above 80°, aqueous solutions of the acid or its hydrochloride decarboxylate to 3-aminophenol[509]. This decarboxylation is dependent on the pH and temperature of the solution - decomposing rapidly at high temperatures and low pH[510]. It is therefore not surprising that 4-aminosalicylic acid is extremely unstable in refluxing methanol saturated with hydrogen chloride.

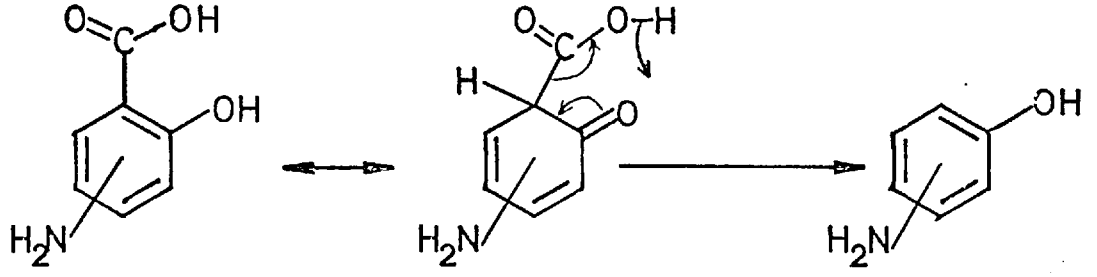
At least three mechanisms for the decarboxylation reaction can be formulated. The first mechanism is dependent on the position of the amino group:



(A 5-amino group would not induce decarboxylation by this mechanism). The second possible mechanism is independent of the position of the amino group:



The third mechanism is less likely as it involves the initial tautomerism of the phenol to its keto form, but the substituents on the ring could stabilize the intermediate to some extent:



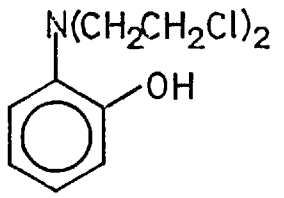
It is uncertain yet which mechanism predominates, but the first two are the most likely. However, as no decarboxylation was noticed in the esterification of 5-aminosalicylic acid (99) (see below), the first mechanism seems to be the most likely.

Methyl 4-aminosalicylate (80) was dissolved in 80% aqueous acetic acid and hydroxyethylated with ethylene oxide to obtain methyl 4-(NN-di-2-hydroxyethylamino)salicylate (81) in fairly good yield. However, as in the hydroxyethylation of methyl 3-amino-4-hydroxybenzoate (65) mentioned above, a large excess of ethylene oxide resulted, in one preparation, in the formation of an intractable oil which made the purification of the product extremely difficult.

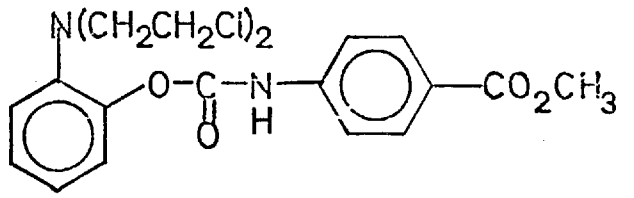
Attempts to selectively benzoylate the phenol group in methyl 4-(NN-di-2-hydroxyethylamino)salicylate by Baker and co-workers' method [421] gave only oils which when chromatographed by dry column chromatography[511] followed by further t.l.c. purification gave crystals of the tribenzoylated derivative (87) plus two viscous oily products, one of which was indicated by its n.m.r. spectrum to be that of the protected phenol derivative (83) but which was still too impure to be crystallizeable.

Baker[421] had attempted the chlorination of methyl O-benzoyl-4-(NN-di-2-hydroxyethylamino)salicylate (83) using thionyl chloride in refluxing dichloromethane but only obtained the corresponding cyclic sulphite. When Baker[421] however, used thionyl chloride in refluxing chloroform some of the desired mustard (84) was obtained in low yields after a reaction time of 20 hours. It was therefore decided to attempt the direct selective chlorination of the triol (81) using the zinc chloride-catalysed thionyl chloride reaction which was so successful in obtaining methyl 4-hydroxy-3-(NN-di-2-chloroethylamino)benzoate (67). The product obtained, however, was an almost colourless oil which was characterized by its spectral data and microanalysis as a 1:1 molecular complex of two isomers , methyl 3-chloro-4-(NN-di-2-chloroethylamino)salicylate (90) and methyl 5-chloro-4-(NN-di-2-chloroethylamino)-salicylate (91), arising from the chlorination of the aromatic ring by the zinc chloride-thionyl chloride Lewis acid ($Cl_3Zn^-SO_2$). An assignment of the n.m.r. spectrum of this 1:1 complex is given with structures (90) and (91) and are in agreement with the structures proposed.

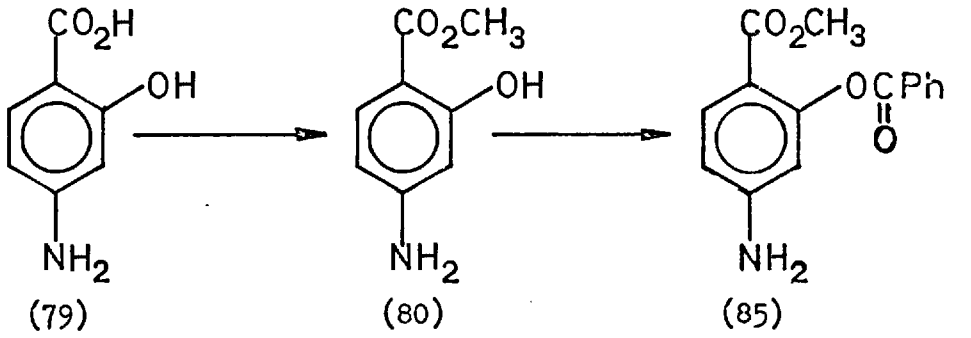
It is very difficult to assign the bands at -1.55 τ and -0.72 τ to the proper phenolic proton. However, 4-chlorophenol is less



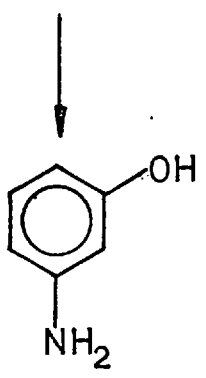
(77)



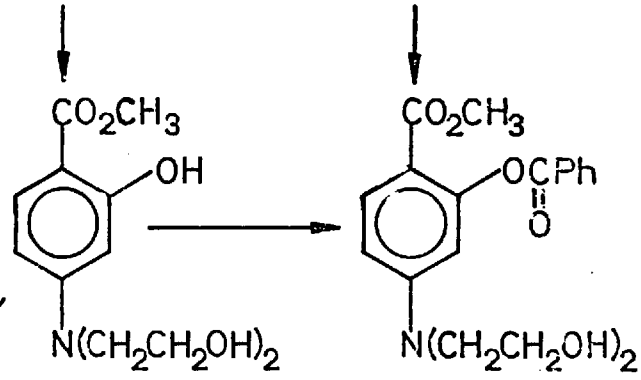
(78)



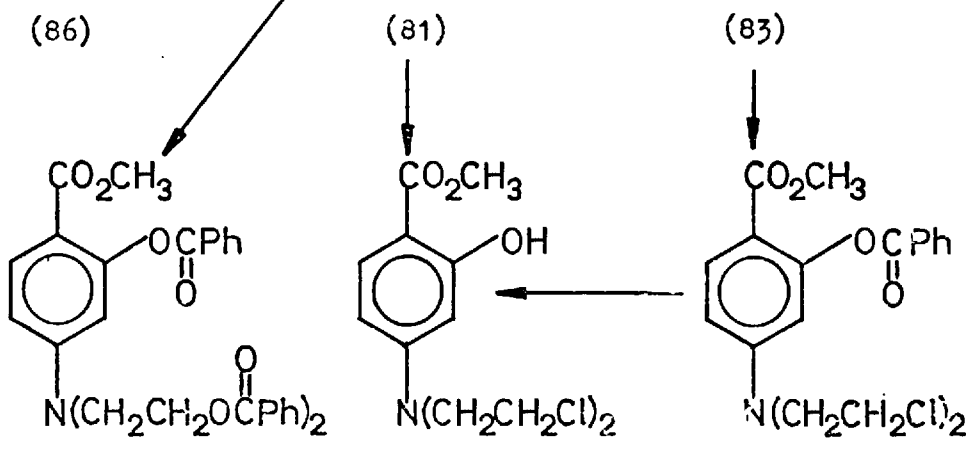
(79) (80) (85)



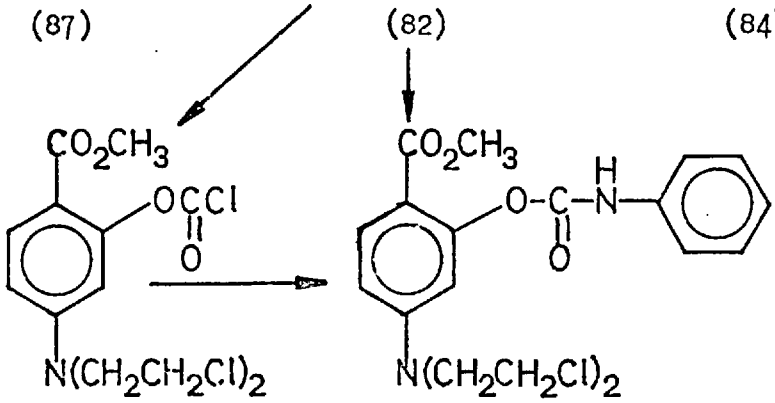
(86)



(81) (83)



(87) (82) (84)



(89) (88)

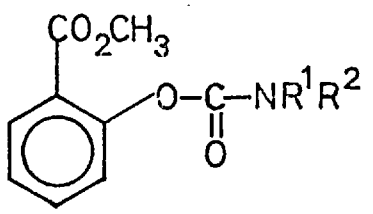
acidic (pK_{25} : 9.20[515], 9.38[516]) than 2-chlorophenol (pK_{25} : 8.1[515], 8.48[516]). The phenolic group in compound (91) is therefore less acidic than that in (90), and consequently (90) is more strongly hydrogen-bonded to the methoxycarbonyl group, and hence more strongly deshielded. The two very low field bands have been assigned on this basis. Furthermore, microanalysis of this oily 1:1 mixture gave a chlorine percentage of 30.74 which although slightly low ($C_{12}H_{14}Cl_3NO_3$ requires 32.56) is much higher than for the trisubstituted aryl mustard (82) ($C_{12}H_{15}Cl_2NO_3$ requires C, 49.30; H, 5.17; N, 4.79; Cl, 24.30%) indicating more than two chlorine atoms in the molecule. The slightly lower chlorine value observed was probably due to the presence of a very small impurity of the trisubstituted mustard (82) due to the difficulty of separating the compounds by t.l.c. (R_f values were very close).

Using a modified version of Szekerke and co-workers' method of chlorination[445], the triol (81) was selectively chlorinated by heating a solution of it in pyridine with methanesulphonyl chloride obtaining methyl 4-(NN-di-2-chloroethylamino)salicylate (82) in good yield (65%). Baker and co-workers' overall yield from methyl 4-amino-salicylate[421] was just under 11% while a yield of over 35% from the same starting material is here being reported.

Several attempts at forming the phenyl carbamate (88) by reacting the mustard phenol (82) in refluxing benzene with phenyl isocyanate gave only starting material and diphenylurea whether pyridine or triethylamine were used as catalysts. Similar results were obtained when the mustard phenol and phenyl isocyanate were combined and heated in sealed tubes in the presence of catalytic amounts of triethylamine at temperatures ranging from 66° (6 days) to 140° (5 days) - at 140° and higher considerable charring occurred.

Attempts to form the chloroformate (89) by reacting the mustard phenol (82) in benzene with phosgene and then condensing the crude chloroformate directly with aniline again failed to give any phenyl carbamate (88), most of the starting material being recovered. This indicates that the mustard phenol (82) is very unreactive, a conclusion that was also indirectly indicated by Baker[421].

Very few examples of carbamate formation with methyl salicylate itself have been recorded. The NN-diethylcarbamate (92: $R_1 = R_2 = Et$) and the N-p-ethoxycarbonylphenylcarbamate (92: $R_1 = H$, $R_2 = PhCO_2Et(p)$) were in fact prepared from methyl salicylate by prior formation of the chloroformate and subsequent reaction with diethylamine or ethyl



(92)

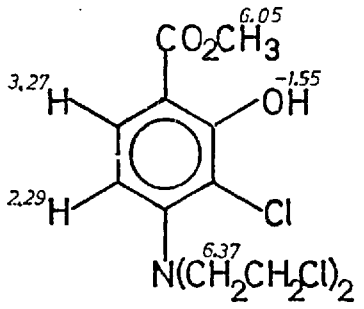
4-aminobenzoate, respectively, in ether[512]. The phenylcarbamate (92: R_1 = H, R_2 = Ph) was obtained by reaction with phenyl isocyanate in the presence of triethylamine at 65° [513] or in the presence of diazomethane as catalyst[514] but Leffler and Matson[513] reported that temperatures higher than 65° promoted the formation of diphenylurea. Bergel and Wade[517] prepared the NN-di-2-chloropropylcarbamate (92: $R_1 = R_2 = CH_2CH(Me)Cl$), again via the initial formation of the chloroformate while Suciu[518] prepared the NN-di-2-chloroethylcarbamate via the initial formation of bis-2-chloroethylcarbamoyl chloride and subsequent reaction with the salicylate.

Artico and Ross[519] obtained 5-(NN-di-2-chloroethylamino)salicylic acid (97) from ethyl O-acetyl-5-nitrosalicylate (94) by reduction, hydroxyethylation in glacial acetic acid, chlorination and hydrolysis. The preparation of 5-(NN-di-2-hydroxyethylamino)salicylic acid (98) from 5-aminosalicylic acid (99) using ethylene chlorohydrin has also been reported[520]. 5-(NN-Di-2-chloroethylamino)salicylic acid has been found to be active against the Walker-256 carcinoma only at near toxic levels.

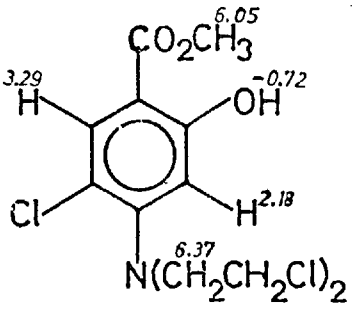
It has been shown that the hydrolysis of a methyl ester is much easier than that of an ethyl ester[521] and that the chemotherapeutic index of a methyl ester is normally much higher than that of the free acid[410] due to the latter's dissociation into anions. In view of this, it was decided to attempt the synthesis of methyl 5-(NN-di-2-chloroethylamino)salicylate (102).

5-Aminosalicylic acid (99) was esterified with methanol in the presence of dry hydrogen chloride and the resulting methyl ester[522] was dissolved in 77% aqueous acetic acid and hydroxyethylated to give methyl 5-(NN-di-2-hydroxyethylamino)salicylate (101) which was identified by its spectral data and by microanalysis. Organic solutions of (101) were found to fluoresce with a blue-green colour particularly at the air-surface interphase.

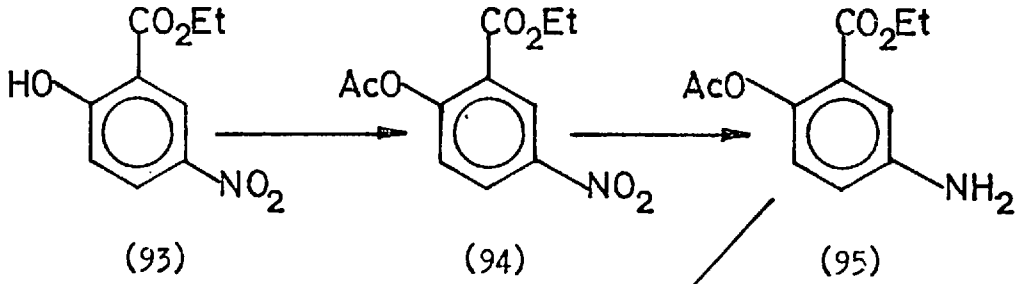
Attempts to block the phenol group of this triol (101) by selective benzoylation[421] or benzylation gave low yields of the respective



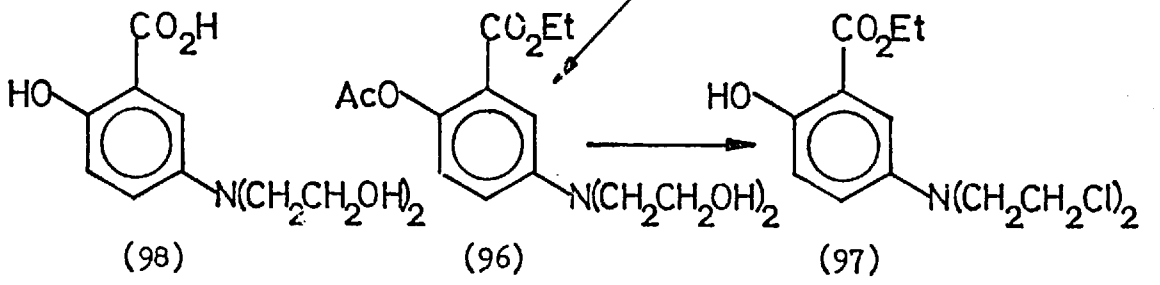
(90)



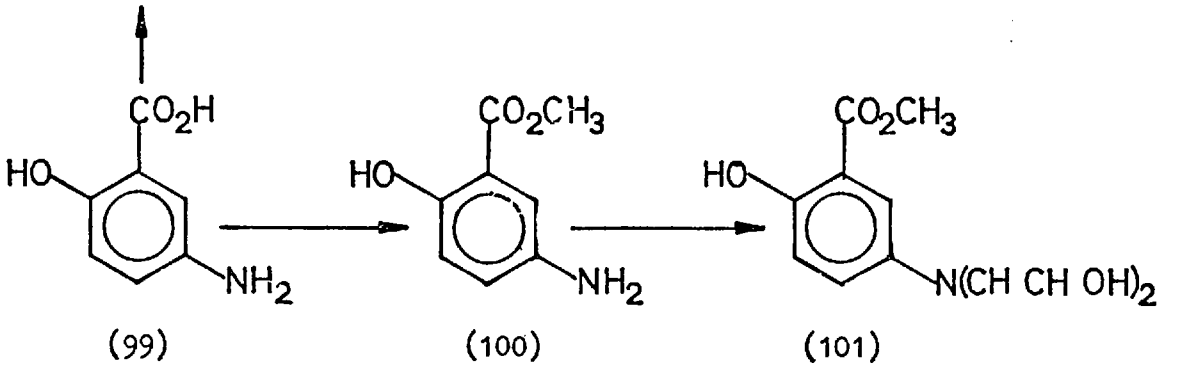
(91)



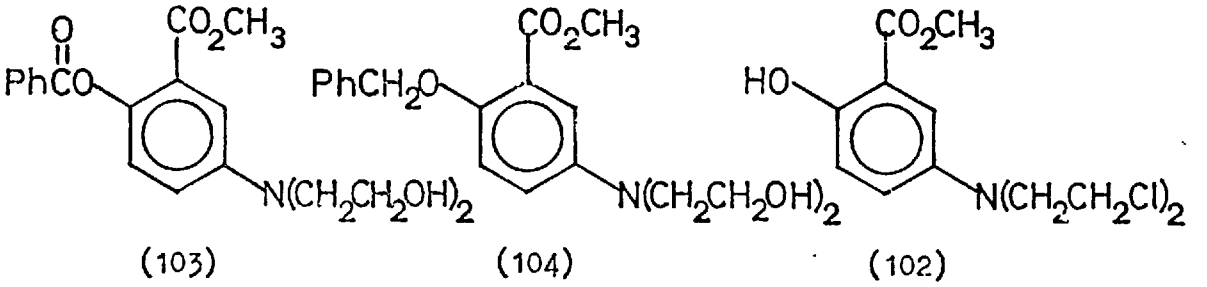
(93) (94) (95)



(98) (96) (97)



(99) (100) (101)



(103) (104) (102)

products (103) and (104) after chromatographic purification. White crystals of the O-benzoyl derivative (103) were obtained and identified from spectral and microanalytical data, while the O-benzyl derivative (104) was obtained as a yellow oil which, though identified from spectral data, could not be identified by microanalysis due to the great difficulty in removing last traces of solvents.

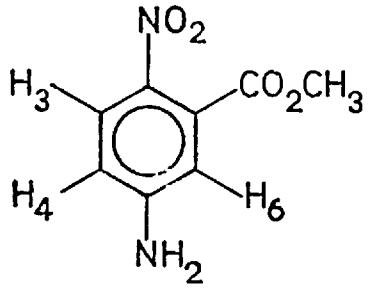
When the triol (101) was chlorinated by thionyl chloride in benzene, several products were obtained but very little, if any, mustard. However, when the triol (101) was dissolved in chloroform containing dry powdered zinc chloride, thionyl chloride added dropwise and the solution refluxed for 13 minutes, methyl 5-(NN-di-2-chloroethylamino)salicylate was obtained as needle-shaped crystals in a relatively good yield (52%). It was identified by microanalysis and by spectral data. Moreover the overall yield (48%) from the methyl ester (100) was much better than that for the corresponding 4-(NN-di-2-chloroethyl) isomer.

Aminophenyl Methoxycarbonyl Nitrogen Mustards

Bieksa and co-workers[523] have synthesized ethyl 2-amino-5-(NN-di-2-chloroethylamino)benzoate (109) and its corresponding acid (110) starting from anthranilic acid (105) via the route (105) to (108), obtaining an overall yield of 15%.

For the reasons explained earlier, it was desirable to synthesize the methyl ester (117) of this aminophenyl methoxycarbonyl mustard and 5-amino-2-nitrobenzoic acid (111) was chosen as starting material. It was esterified with methanol and dry hydrogen chloride, concentrated sulphuric acid, or boron trifluoride[507], obtaining very good yields of the crude ester (112). However, thin layer chromatography showed two components. A fractional crystallisation procedure (see Experimental) gave methyl 5-amino-2-nitrobenzoate (112) and a small amount of an isomeric ester which could not be identified. In both isomers, spectral data confirmed the formation of a methyl aminonitrobenzoate.

However, a literature search for all ten isomeric aminonitrobenzoic acids revealed no record of the methyl esters of three of the ten isomeric acids. Fig. 8 gives the normal commercial synthetic pathway for the preparation of 5-amino-2-nitrobenzoic acid (111), the most important step being the nitration of 3-N-acetylaminotoluene (122)[524]. Three mononitro isomers could possibly be formed which would give rise to 5-amino-2-nitrobenzoic acid (111), 3-amino-4-nitrobenzoic acid (126) and 3-amino-2-nitrobenzoic acid (128). These are precisely the three isomeric acids for which the methyl esters have not been reported.



(112)

The nitro group with a methoxycarbonyl substituent ortho to it is conjugated with an amino group para to it. As such, this nitro group is pulled towards coplanarity with the aromatic ring so that the methoxycarbonyl group has to take a greater share of the twisting necessary to avert steric conflict. Herrmann and Rae[526] demonstrated this effect, termed "electronic buttressing", in the n.m.r. spectrum of 5-substituted methyl 2-nitrobenzoates. Calculating the chemical shifts of the three aromatic protons in 5-amino-2-nitrobenzoic acid,

based on each substituent being considered as if it were monosubstituted[528], approximate values (τ) for H_3, H_4 and H_6 of 1.64, 3.02 and 2.49 were obtained, the nitro and carboxyl groups being anisotropically deshielding while the amine group is shielding. One would expect chemical shift values for the methyl ester to be all slightly upfield. However, in methyl 5-amino-2-nitrobenzoate, the effect of electronic buttressing on the methoxycarbonyl group results in relief of anisotropic deshielding on H_6 so that its chemical shift is moved upfield. Herrmann and Rae[526], in fact, found that the chemical shift for H_6 was the same as that for H_4 and equal to 3.35τ, while that for H_3 was 2.07τ. The chemical shifts measured for the main product obtained from the esterification of the commercial 5-aminosalicylic acid (111) agree quite closely with those quoted by Herrmann and Rae[526], being 2.03τ for H_3 and 3.27τ for H_4 and H_6. However, Herrmann and Rae did not report the complete n.m.r. spectrum of methyl 5-amino-2-nitrobenzoate (112) nor did they quote the melting point of the ester.

Thus, although spectral data and microanalysis strongly indicated that the main product of the esterification reaction was methyl 5-amino-2-nitrobenzoate (112) a more positive confirmation was required.

Rauhut and Bunnett[527] had synthesized methyl 5-N-acetylamino-2-nitrobenzoate (129) from methyl 3-acetamidobenzoate by nitration. A sample of the supposed ester (112) was acetylated and the product obtained had a melting point that was in agreement with that reported by Rauhut and Bunnett[527], thus confirming the structure (112).

Attempts to obtain methyl 5-(NN-di-2-hydroxyethylamino)-2-nitrobenzoate (115) by hydroxyethylation of the methyl ester (112) were mainly unsuccessful. Thus, when this amine was hydroxyethylated in a 3:1 mixture of glacial acetic acid-water only some starting material (40%) and the monohydroxyethylamino derivative (113) were obtained. Similar results were indicated when the hydroxyethylation was carried out in a sealed Carius tube at temperatures of 110° and 140°; however, considerable formation of tarry products was also obtained. This was not surprising as it has already been mentioned (pp 50 and 51) that hydroxyethylation of p-nitroaniline to the corresponding diol was only achieved with difficulty at temperatures of 150°. A very small amount of the diol (115) was in fact obtained during hydroxyethylation at room temperature but the yield was so low that it was hardly worthwhile to consider.

Methyl 5-(N-2-hydroxyethylamino)-2-nitrobenzoate (113) was there-

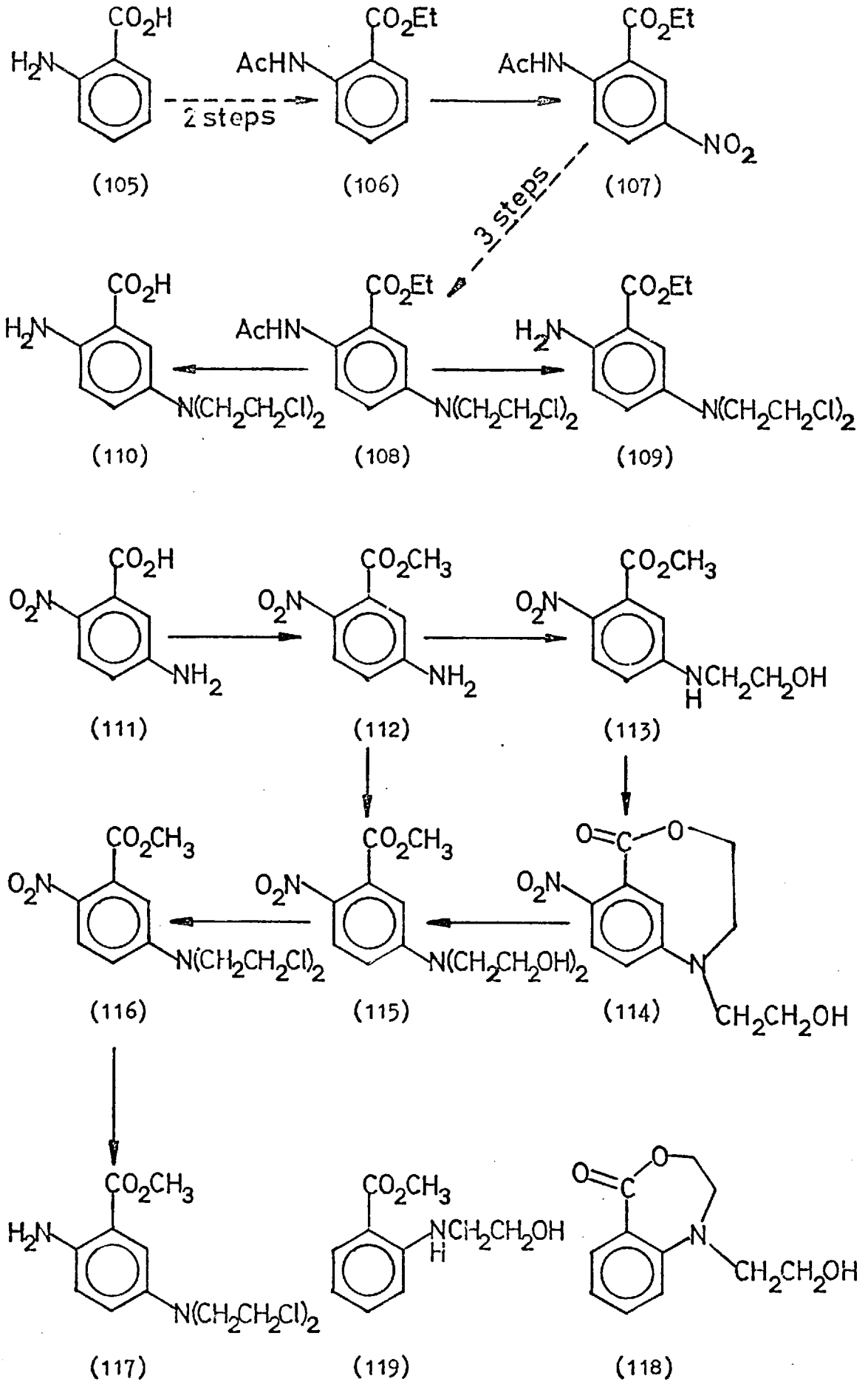
fore hydroxyethylated further in the hope of obtaining the diol. Hydroxyethylation at 140° for 16 hours, however, gave mainly starting material (33%) and a small amount (11%) of 1,2,3,5-tetrahydro-1-(2-hydroxyethyl)-5-keto-11-nitro-6,8-benz-1,4-oxazocine (114). A very small yield of the diol (115) was probably also obtained but was so viscous that great difficulty was encountered in drying it so that identification by microanalysis failed. The benzoxazocine derivative was identified from n.m.r. and mass spectra (see fig. 9) and by microanalysis. It was refluxed with methanol saturated with hydrogen chloride to give a product which had a low R_f and was identical in its spectral measurements to the "diol" obtained in the hydroxyethylation of (112) and (113). A similar cyclisation product was obtained by Everett and Ross[74] when they treated methyl anthranilate with ethylene oxide at elevated temperatures. In one hydroxyethylation reaction, they obtained the monohydroxyethyl derivative (119) but on repeating the reaction under identical conditions, they obtained 2,3,4,7-tetrahydro-4-(N-2-hydroxyethyl)-7-keto-5,6-benz-1,4-oxazepine (118) from which the corresponding diol (120) was obtained by ring cleavage on refluxing in methanolic hydrogen chloride.

The combined yields of methyl 5-(NN-di-2-hydroxyethylamino)-2-nitrobenzoate (115) obtained by the above-mentioned three preparations, were chlorinated in benzene with phosphoryl chloride. Methyl 5-(NN-di-2-chloroethylamino)-2-nitrobenzoate (116) was obtained in good yield but the total amount of this nitrogen mustard obtained was too little to attempt the final reduction stage.

Clearly, the proposed synthetic route was not adequate for large scale preparations and a modification of Bieksa and co-workers' route[519], would probably afford much better yields of the nitrogen mustard anilins (117) required.

The only other synthesis of an aminophenyl carboxyl nitrogen mustard recorded [530,531] was that of 4-amino-3-(NN-di-2-chloroethylamino)benzoic acid (130) and its ethyl ester (131).

Bieksa and Degutis[422] had prepared (NN-di-2-chloroethyl)-1,3-phenylenediamine (132) and Edwards[412] synthesized its p-methoxycarbonylphenyl carbamate. As there have been reports[615] that, in certain instances, a more favourable anti-tumour activity was obtained with meta substituted aromatic nitrogen mustards than with the corresponding ortho or para derivatives, it was decided to attempt the synthesis of a derivative of the meta substituted mustard diamine by introducing



(105) (106) (107) (110) (108) (109) (111) (112) (113) (116) (115) (114) (117) (119) (118)

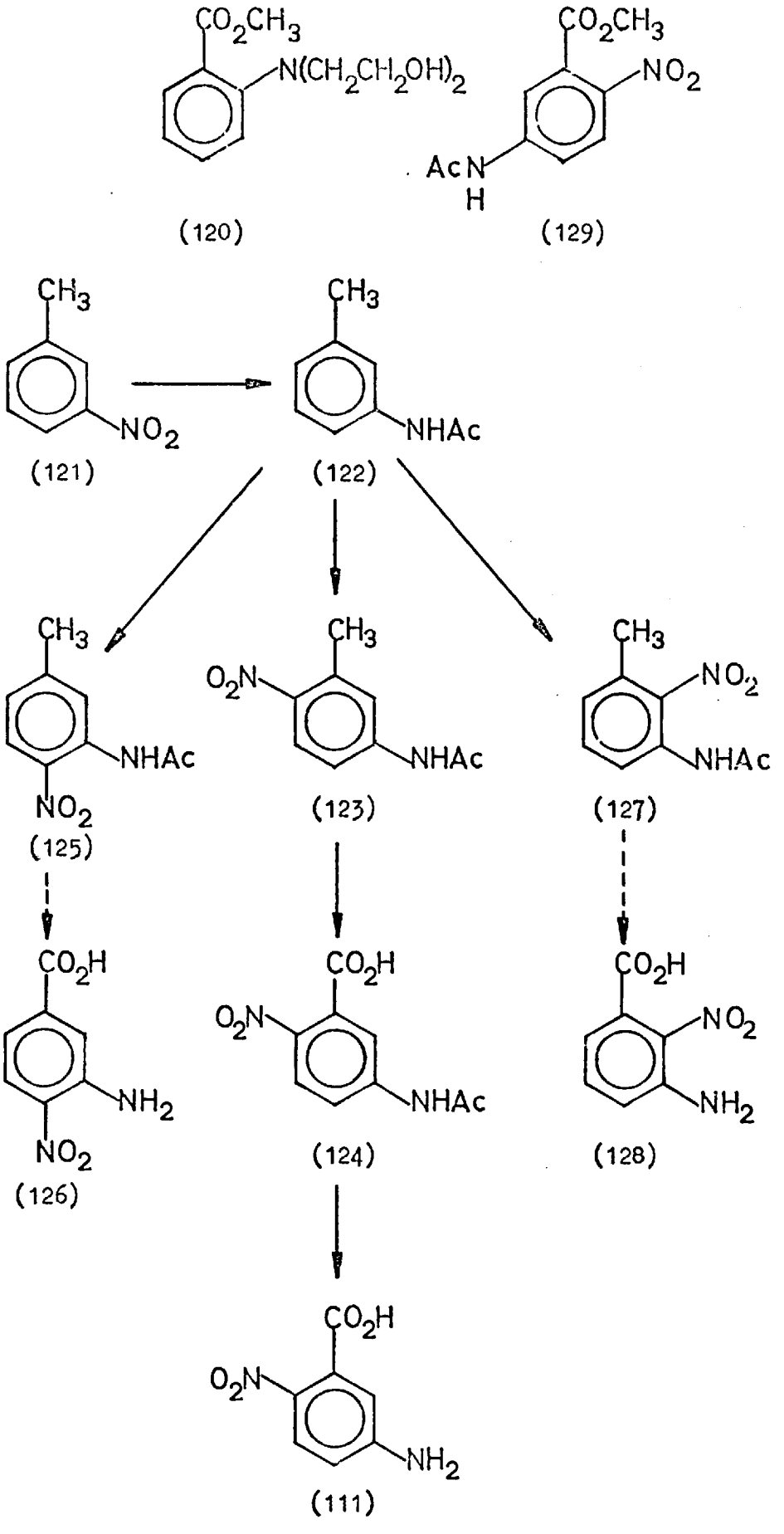

CO2CH3
N(CH2CH2OH)2
(120)
CO2CH3
NO2
AcN
H
(129)
CH3
NO2
(121)
CH3
NHAc
(122)
CH3
NHAc
NO2
(125)
CH3
O2N
NHAc
(123)
CH3
NO2
NHAc
(127)
CO2H
NH2
NO2
(126)
CO2H
O2N
NHAc
(124)
CO2H
NO2
NH2
(128)
CO2H
O2N
NH2
(111)

Fig. 8

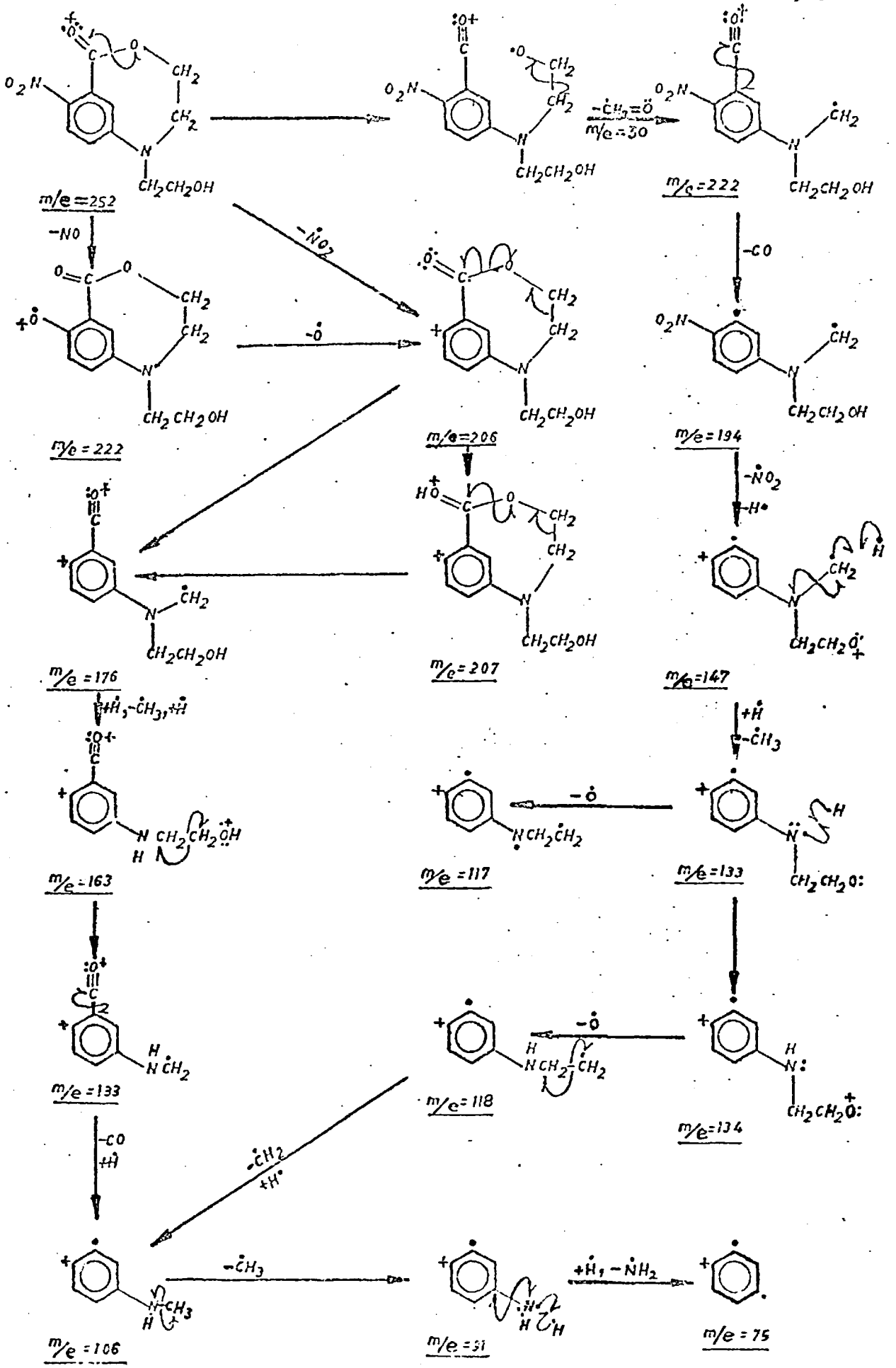

m/e=252
m/e=222
m/e=206
m/e=222
m/e=194
m/e=207
m/e=176
m/e=147
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m/e=51
m/e=75

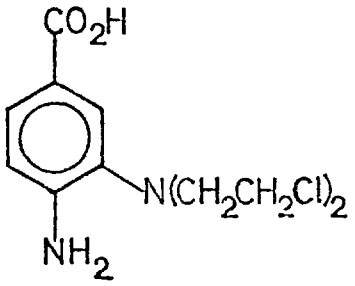
Fig.9

the methoxycarbonyl group in the same aromatic ring; and as Everett and Ross[74] had chlorinated the diol (120) (see above) to obtain methyl (NN-di-2-chloroethyl)anthranilate, these factors led to the idea of synthesizing methyl 4-amino-2(NN-di-2-chloroethylamino)benzoate (137) from 4-nitroanthranilic acid (133).

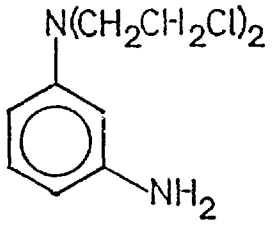
Consequently 4-nitroanthranilic acid (133) was esterified essentially as described by Wheeler and Barnes[532] to obtain the methyl ester (134). Six attempts at preparing methyl 4-nitro-(NN-di-2-hydroxyethyl)anthranilate (135) however, all failed. This was not surprising in the light of Everett and Ross's work[74] (see above) who could only obtain the monohydroxyethylation product of methyl anthranilate except in one instance when they obtained the corresponding N-2-hydroxyethyl benzoxazepine derivative (118). Hydroxyethylation of methyl 4-nitroanthranilate was attempted in various solvents and under various conditions. Prolonged hydroxyethylation in benzene at 165° (17 h) gave a charred product; in dioxan at 120° (16 h) and in dioxan (containing a catalytic amount of acetic acid) at room temperature only starting material was obtained. However in aqueous acetic acid at room temperature (48 h), in acetic acid-dioxan (1:1) at room temperature (72 h), and in dioxan at 150° in a Carius tube (16 h) minor yields (21-33%) of the corresponding monohydroxyethyl derivative (138) were obtained, the main product still being the starting material.

Table 2 (page 53) shows quite clearly that the ortho methoxycarbonyl group does not reduce the basic strength of the amine appreciably (pK_b of methylanthranilate : 2.23) nor does the meta nitro group (pK_b of m-nitroaniline : 2.47). m-Nitroaniline reacts fairly easily with ethylene oxide to give the diol [412,422]. The lack of reaction of methyl 4-nitroanthranilate (134) must therefore be due to considerable steric hindrance by the methoxycarbonyl group on the amine group ortho to it and to strong hydrogen bonding of the amine protons with the adjacent carbonyl group.

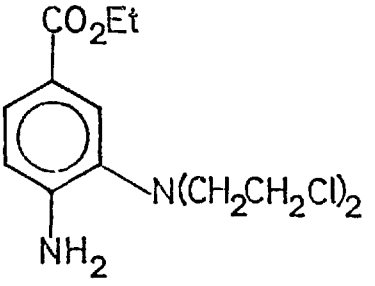
If however the 4-nitro group could be replaced by an electron donating group, it could hopefully activate the amino group enough, by increasing its pK_b, to form the diol or the oxazepine derivative. One such activating group could well be a protected amine group, but to effect specific protection of the reduced 4-nitro group, a bulky protecting group would have to be introduced. One such group could be a phthalimido derivative. A 4-phthalimido in preference to a 4-nitro group, it was reasoned, might be enough activating to overcome



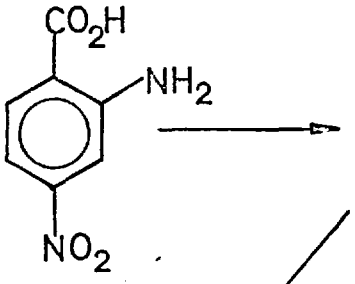
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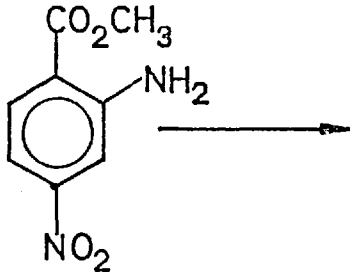
(132)



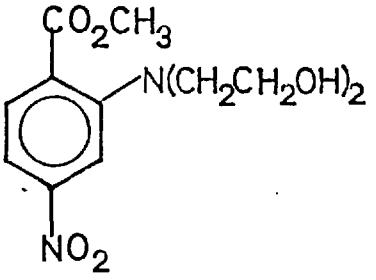
(131)



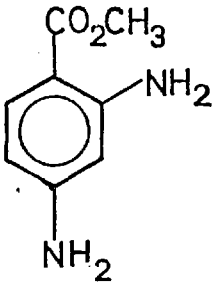
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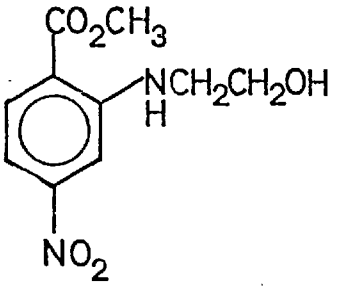
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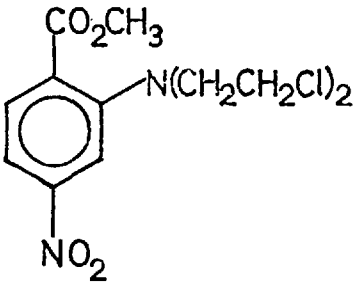
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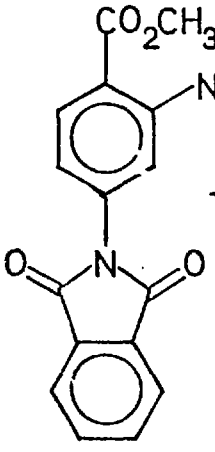
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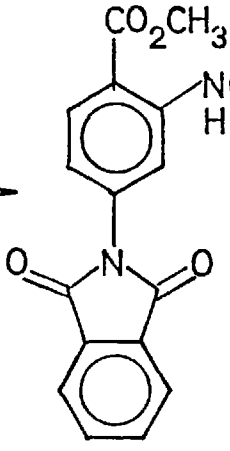
(138)



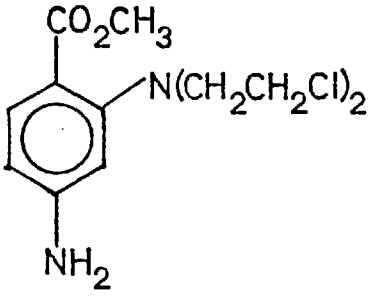
(136)



(140)



(141)



(137)

the steric interaction of the methoxycarbonyl group and form the diol which could then be chlorinated and deblocked to give the required diamine mustard (137).

Methyl 4-nitroanthranilate (134) was therefore reduced using stannous chloride and concentrated hydrochloric acid to obtain methyl 2,4-diaminobenzoate (139). Jaroslav and co-workers[534,535] reported using this diaminobenzoate to investigate its pyrolysis products and its electrophoretic spectrum (using paper electrophoresis) but did not give its method of preparation, melting point or spectral data.

This diamino derivative was selectively reacted with one equivalent of phthalic anhydride using the method developed by Bose and co-workers[536,537] obtaining a high yield of the 4-phthaloyl derivative (140). No trace of the 2-phthaloyl derivative was obtained; so the reaction was 100% selective, again high-lighting the strong steric interaction of the methoxycarbonyl group on the 2-amino group. Hydroxyethylation of the 4-phthalimido-2-aminobenzoate, however, only gave the monohydroxyethyl derivative (141) and starting material (140) even on prolonged heating in benzene at 150°. So the 4-phthalimido group was influencing adversely the reactivity of the anthranilate, and the required mustard (137) could not be obtained by the scheme proposed.

Methyl 4-nitroanthranilate (134) was therefore acetylated to give the 2-N-acetylamino derivative (142)[533] which was then hydrogenated over palladium-charcoal to give methyl 2-N-acetyl-2,4-diaminobenzoate (143). Sirks[538] mentioned this diaminobenzoate but reported no chemical identifications for it at all. This diaminobenzoate was hydroxyethylated in dioxan-acetic acid (1:1) at room temperature to afford some monohydroxyethyl derivative (148) and methyl 2-N-acetylamino-4-(NN-di-2-hydroxyethylamino)benzoate (144) in 58% yield. This diol was chlorinated with thionyl chloride in benzene, or with mesyl chloride in pyridine to give the N-acetyl nitrogen mustard (145).

Hydrolysis of (145) with 50% methanolic hydrogen chloride gave the aminophenyl methoxycarbonyl nitrogen mustard (146) as its hydrochloride. 2-Chlorophenyl chloroformate (149) was prepared essentially as described by Edwards[412] and this was reacted with the crude aminophenyl mustard (146) to yield the first ever recorded carbamate of an aminophenyl methoxycarbonyl nitrogen mustard, namely 2'-chlorophenyl N-[2-methoxycarbonyl-5-(NN-di-2-chloroethylamino)]phenylcarbamate (147).

Aminophenyl Methoxy Nitrogen Mustards

The only example in this group that has been synthesized, was prepared by Ross and co-workers[377] by nitrosation of (NN-di-2-chloroethyl)-3-methoxyaniline and subsequent reduction, thus obtaining (N^1N^1-di-2-chloroethyl)-3-methoxy-1,4-phenylenediamine (150).

In an effort to prepare isomers of (150), 2-methoxy-4-nitroaniline (151)[539] was treated with ethylene oxide in aqueous acetic acid (77%) or in acetic acid-dioxan (3:2) but only starting material and the N-2-hydroxyethyl derivative (152) were obtained. A similar result occurred when the reaction was effected in dioxan in a Carius tube at 150° for 16 hours. The result was rather surprising. One would expect the p-nitro group to lower the basicity of the amine nitrogen considerably (pK_b for 4-nitroaniline : 0.99 - table 2). However this would be offset by the electron donating methoxy group (pK_b for 2-methoxyaniline : 4.52). Bishydroxyethylation of 4-nitroaniline[423] and 2-methoxyaniline[420,434] has also been achieved so that from a basicity point of view there was no reason why bishydroxyethylation of 2-methoxy-4-nitroaniline should not be possible.

2-Methoxy-4-nitroaniline (151) was therefore acetylated in pyridine with acetic anhydride by stirring the solution overnight at room temperature followed by heating in boiling water for 6 hours. The product obtained was mainly the N-acetyl derivative (154). However, when in a second preparation, the reactants were heated immediately instead of stirred overnight at room temperature and then heated , the products obtained were the N-acetyl derivative (154) in major yield (44%) and the NN-diacetyl derivative (153) in minor yield (22%). These two products were separated by fractional crystallization (see experimental).

The N-acetyl derivative (154) had been previously obtained from acetic acid - acetic anhydride[539] or by direct reaction with acetic anhydride (4 hours)[540]. The diacetyl derivative (153) had not been prepared before. However, Lane and Williams[542] had prepared an isomer (157) by acetylation with acetyl chloride - acetic anhydride, while the diacetyl derivatives of 2-nitro-[543,544], 3-nitro-[543] and 4-nitro[545] aniline have also been prepared by various methods all involving acetic anhydride, except in one case involving acetyl chloride in pyridine and toluene[543].

N-Acetyl-2-methoxy-4-nitroaniline (154) was hydrogenated in an autoclave at 50° and 25 atmospheres but the product obtained turned

immediately very dark red while the catalyst was being removed by filtration. Hanford and Adams[540] had prepared the N-acetyl phenylenediamine derivative (155) by reduction with iron and water, but they reported that the product was very readily oxidized by air to a deep red solution and in their subsequent reactions they used the crude product directly.

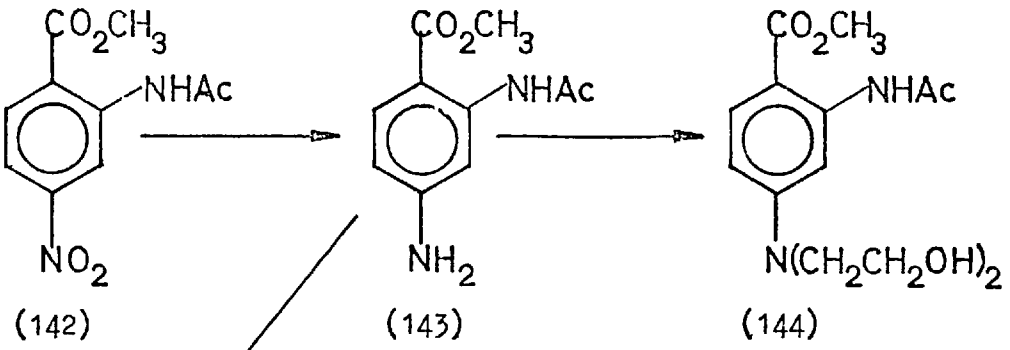
The crude product (155) was therefore hydroxyethylated in glacial acetic acid-dioxan at room temperature. The product obtained was however, only the monohydroxyethyl derivative (156) and again no bishydroxyethylation was achieved.

4-Methoxy-2-nitroaniline (158) was similarly hydroxyethylated. In aqueous acetic acid, the only product obtained was (N-2-hydroxyethyl)-4-methoxy-2-nitroaniline (159) but in dioxan at 150° (16 h) starting material (16%), monohydroxyethyl derivative (159) (18%), [N-2-(2-hydroxyethyl)oxyethyl]-4-methoxy-2-nitroaniline (160) (5%) and the corresponding diol (161) (2.8%) were obtained. When the latter reaction was kept at 150° for 3 days, the yield of the 2-(2-hydroxyethyl)oxyethyl derivative (160) and of the diol (161) was increased slightly but a higher yield of a tarry product was also obtained making separation of the diol from the tar extremely difficult. However when this tarry mixture (very low R_f) was treated with phosphoryl chloride in benzene, some corresponding aryl mustard (162) was obtained (high R_f) and identified from n.m.r. spectra.

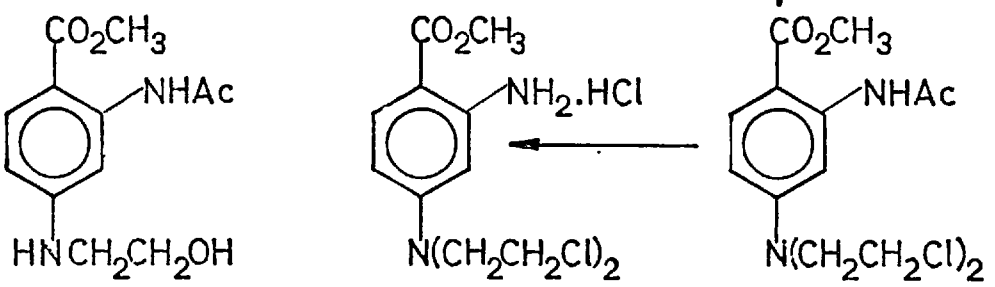
These experimental observations were rather surprising when compared to identical reactions with 2-methoxy-4-nitroaniline (151) which gave no bishydroxyethylation products.

As was explained on page 50, hydroxyethylation of 2-nitroaniline always gave starting material and the monohydroxyethyl product[424,430] due to the very low basicity of the starting material. Yet the introduction of a 4-methoxy group in 2-nitroaniline (to give 4-methoxy-2-nitroaniline) was sufficient to increase the basicity of the aniline nitrogen to a level permitting bishydroxyethylation, though in very small yields (see Table 2).

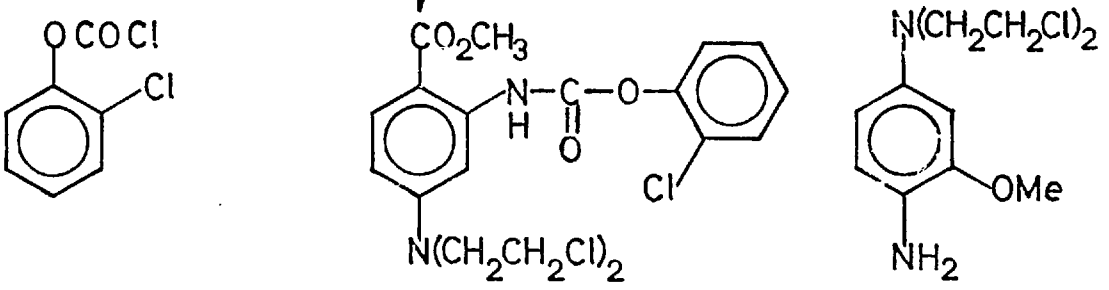
The hydroxyethylation at high temperatures of 4-methoxy-2-nitroaniline (158) was also interesting in that the formation of the 2-(2-hydroxyethyl)oxyethyl derivative (160) was indirectly predicted by Ross[74] when he stated that polymers of type (59) would be formed when greater than theoretical amounts of ethylene oxide were employed in hydroxyethylations at elevated temperatures, as was the case in the reaction discussed above.



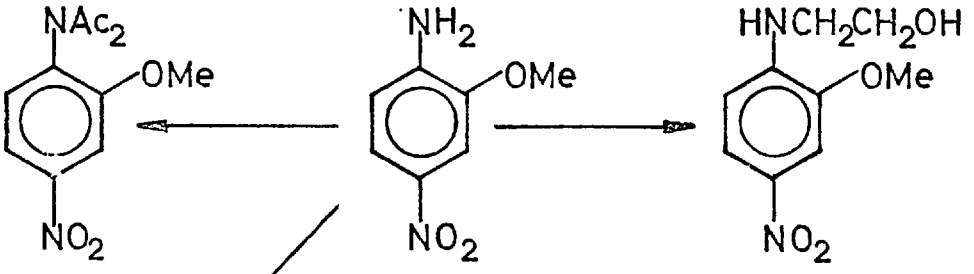
(142) (143) (144)



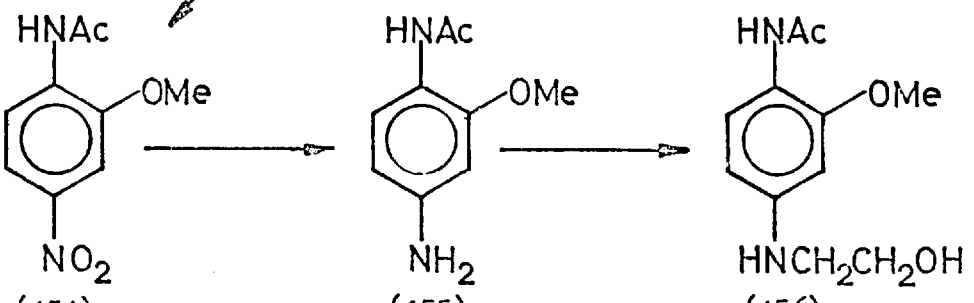
(148) (146) (145)



(149) (147) (150)



(153) (151) (152)



(154) (155) (156)

However, although the diol (161) was obtained, the yields were too low to make the reaction of any use in the large scale synthesis of the diol.

4-Methoxy-2-nitroaniline (158) was therefore acetylated in pyridine and acetic anhydride to give the N-acetyl derivative[547] (163) which was hydrogenated to give the corresponding N-acetylphenylene-diamine derivative[548] (164). However, when this diamine derivative (164) was hydroxyethylated in 50% aqueous acetic acid (48 hours), only starting material was obtained.

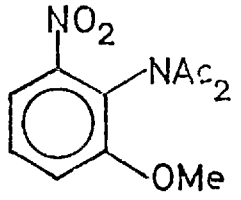
Again it is not known why bishydroxyethylation was not achieved especially as Bieksa and Degutis[424] obtained an 85% yield of the bis-hydroxyethyl derivative (169) when they reacted N^1-acetylphenylene-1,2-diamine (168) with ethylene oxide in 40% aqueous acetic acid overnight. One would expect that a methoxy group para to the N-acetylamino group would facilitate bishydroxyethylation.

A further attempt at preparing the diol (161) from the aromatic nucleophilic substitution of 4-chloro-3-nitroanisole (165) with diethanolamine again proved unsuccessful. When the two reactants were heated together for 77 hours, 2,2'-dichloro-5,5'-dimethoxyazobenzene[550] (167) and 2-chloro-4-methoxyaniline (166) were obtained and identified by their melting points and spectral data. This result was in agreement with Meltsner and co-workers' conclusion[470] (see page 62) that ethanolamines, apart from acting as nucleophiles, could also bring about reduction of the chloronitrobenzene to the respective azobenzene or aniline. In fact, it has already been mentioned on page 63 that Kremer and Bendich[475] had obtained only reduction products when diethanolamine was used as a nucleophile. However, when 4-chloro-3-nitroanisole (165) was refluxed with diethanolamine in propan-1-ol for 60 hours, a small amount of the diol (161) was obtained, identical in its n.m.r. spectrum to that obtained from 4-methoxy-2-nitroaniline (151). Some starting material (165) (66%) and the aniline reduction product (166) were also obtained.

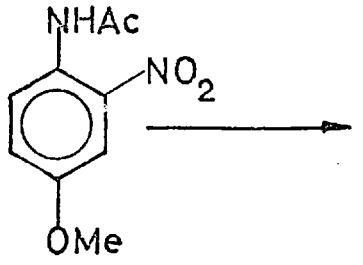
The diol (161) obtained from the two separate preparations was chlorinated with phosphoryl chloride in benzene giving the NN-di-2-chloroethyl derivative (162) in 56% yield as an oil. It was purified by t.l.c. and the spectral characteristics were in accordance with the structure, though microanalysis indicated that it was not entirely free from solvent (ether).

Although the nitrophenyl methoxy nitrogen mustard (162) was thus

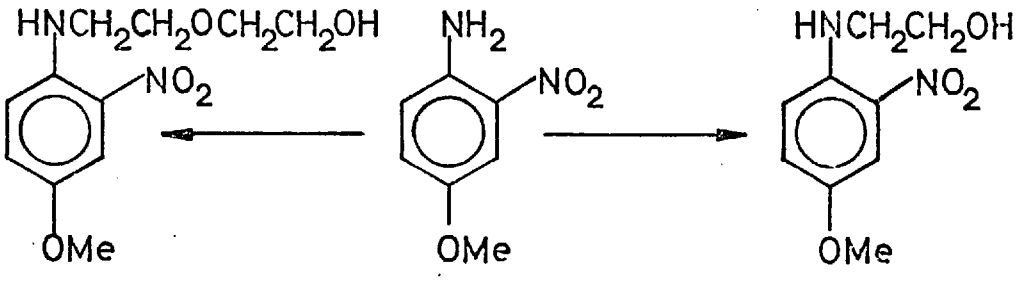
synthesized successfully, the yields of the intermediate diol (161), from both methods were very low and the amount of mustard (162) available was insufficient for reduction to the required amine.



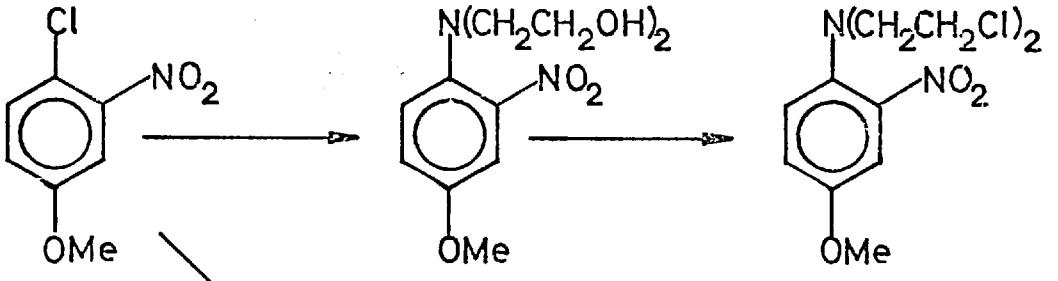
(157)



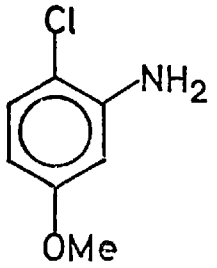
(163) (164)



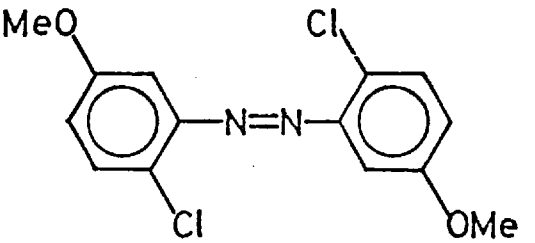
(160) (158) (159)



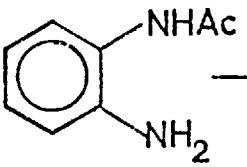
(165) (161) (162)



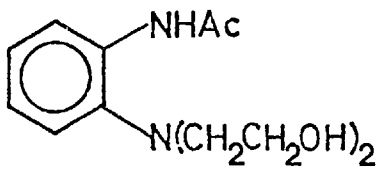
(166)



(167)



(168)



(169)

Aminophenyl chloro Nitrogen Mustards

No such compounds have, as yet, been synthesized. However, Schulze and Willitzer[606] have synthesized 3-chloro-4-nitroso-(NN-di-2-chloroethyl)aniline (170) which was just one step from the synthesis of an aminophenyl chloro mustard.

One possible route to the formation of aminophenyl chloro mustards is via the chloronitroanilines - hydroxyethylation, chlorination and reduction being intermediate steps.

Consequently 4-chloro-2-nitroaniline (171) was hydroxyethylated in 66% aqueous acetic acid by the usual method. However only starting material (74%) and the monohydroxyethylation product (172) (23%) were obtained, the latter being identified by its melting point, which correlated with that reported by Kremer and Bendich[474], and also from spectral data. In the light of Bieksa and Degutis' work [424] and on consideration of the results obtained from the hydroxyethylation of methoxynitroanilines (see above) it is not at all surprising that no diol was obtained, because the 4-chloro group decreases further the very low basicity of the 2-nitroaniline derivative (see Table 2).

Similarly, when 2-chloro-4-nitroaniline (173) was hydroxyethylated in 58% aqueous acetic acid at room temperature and also in dioxan at 140°, a high percentage of starting material plus a low yield of the monohydroxyethylation product (174) were obtained. As has already been mentioned (pp. 95 and Table 2), a 4-nitro group reduces considerably the basicity of a nitroaniline derivative and a 2-chloro group would probably reduce it even further.

Although Sera and co-workers[434] obtained the diol from 2-chloroaniline (via reaction with chlorohydrin) and Friefelder and Stone[420] obtained the monohydroxyethyl derivative (45%) on hydroxyethylation with ethylene oxide at 90° and the diol (90%) at 150°, one would expect that a 4-nitro group would reduce the basicity of the 2-chloroaniline to such an extent that formation of the diol of 2-chloro-4-nitroaniline (173) by hydroxyethylation, even at elevated temperatures, would become impossible. Such was in fact observed. These results could also be partly the result of steric inhibition as a result of the adjacent bulky chloro or nitro groups in ortho positions to the amine group.

2-Chloro-4-nitroaniline (173) was acetylated in pyridine and acetic anhydride in the hope of obtaining the N-acetyl derivative (175). However, as in the case of 2-methoxy-4-nitroaniline (see above), when

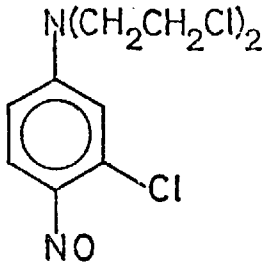
the reaction mixture was heated for 6 hours immediately after the reactants were mixed, the main, and in two preparations the only, product obtained was the diacetyl derivative (176), identified from spectral data and microanalysis. When the reactants were stirred at room temperature overnight prior to heating for 6 hours, a fair yield of the monoacetyl derivative (175) was also obtained.

It may be noteworthy to mention that Blanksma and co-workers[553] prepared the isomeric NN-diacetyl-2-chloro-5-nitroaniline by boiling N-acetyl-2-chloro-5-nitroaniline in acetic anhydride containing one drop of concentrated sulphuric acid. Taft and co-workers[544] investigated the acetylation of various anilines and concluded that an electron withdrawing substituent in an ortho position to the amino group greatly facilitated the formation of the diacetyl derivative while such a substituent in a para position retarded diacetylation. They reacted various anilines by boiling the aniline (1 mole) for 1 hour with 4 moles of acetic anhydride without the presence of a catalyst. Under these conditions, aniline gave 39.9% mono- and 48% diacetyl product. Similarly, 2-chloroaniline gave 10.5% and 77.7% respectively, 2-nitroaniline gave 30% and 65.6% respectively while 4-nitroaniline gave 92% and 6.2% respectively. These results are in agreement with those obtained for the acetylation of 2-chloro-4-nitroaniline (173) and 2-methoxy-4-nitroaniline (151) - a much higher yield of the diacetyl derivative was obtained in the acetylation of the 2-chloro derivative (173) than in the 2-methoxy derivative (151).

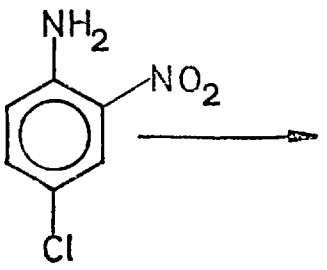
The NN-diacetyl-chloronitroaniline (176) was reduced by hydrogenation over palladium-charcoal but the resulting diamine derivative (177) was extremely unstable and on filtration (to remove the catalyst) its methanolic solution turned very dark red. It was therefore hydroxyethylated as the crude product in acetic acid-dioxan (1:1) but the results obtained were not conclusive.

To achieve bishydroxyethylation, it was therefore necessary to consider closely the relative positions of the chloro and nitro groups with respect to the amino group. From Table 2, it is clear that a 3-nitro group is essential and that the most basic of the three isomeric chloroanilines is 4-chloroaniline. So the combination of a chloronitroaniline that would afford the highest pK_b and hence the most basic amine would be 4-chloro-3-nitroaniline.

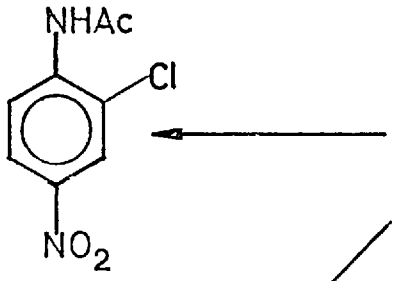
4-Chloro-3-nitroaniline (178) was therefore hydroxyethylated in aqueous acetic acid at room temperature. As was predicted the diol (179) was obtained (56%) and identified from spectral data and



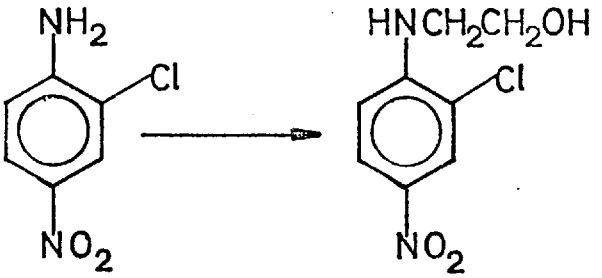
(170)



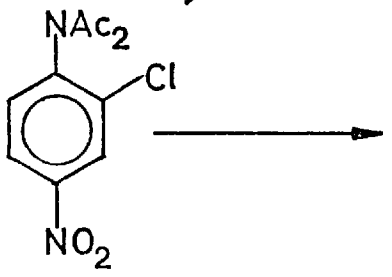
(171) (172)



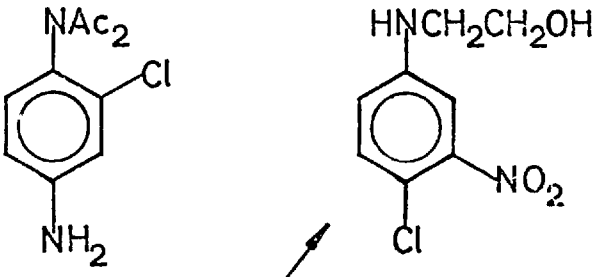
(175)



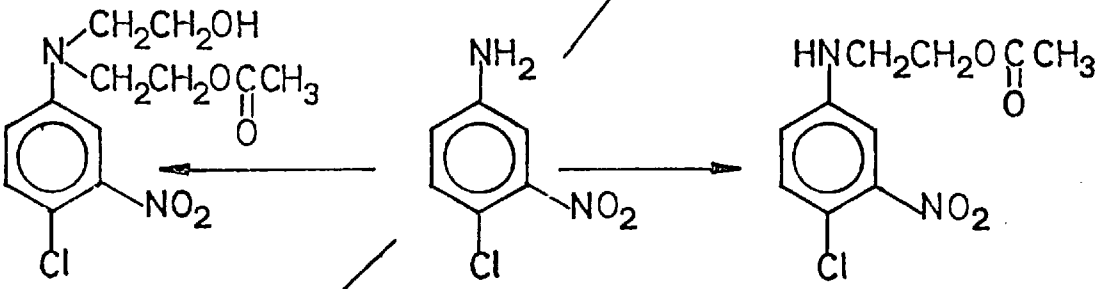
(173) (174)



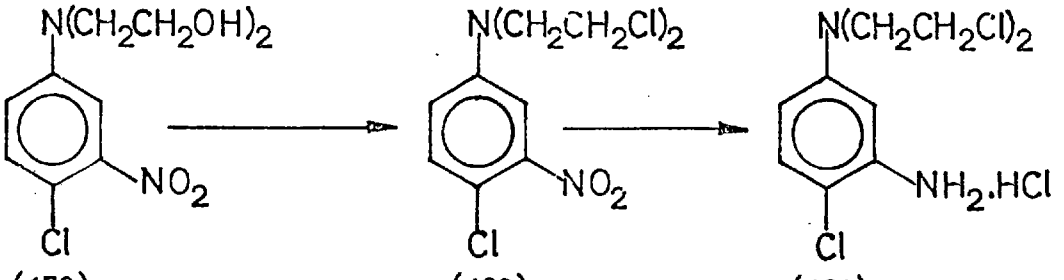
(176)



(177) (184)



(183) (178) (182)



(179) (180) (181)

microanalysis. However, three minor products were also obtained and identified. In order of decreasing R_f, these were: (N-2-acetoxyethyl)-4-chloro-3-nitroaniline (182) and a molecular 1:1 complex of (N-2-hydroxyethyl-N-2-acetoxyethyl)-4-chloro-3-nitroaniline (183) and (N-2-hydroxyethyl)-4-chloro-3-nitroaniline (184). This molecular complex was identified from spectral data and particularly by mass spectroscopy. Its fragmentation pattern is given in fig. 10. 4-Chloro-3-nitroaniline (178) was furthermore hydroxyethylated in dioxan at 100° obtaining a fairly high percentage of unreacted starting material (44%) as well as the monohydroxyethyl derivative (184) (38%) and the diol (179) (17%). Dioxan is probably inferior to benzene as a solvent for high temperature hydroxyethylation and its main advantage was its lower volatility and better solvent properties. A high percentage of Carius tube experiments using benzene led to the tube bursting during heating.

The diol derivative (179) was chlorinated with phosphoryl chloride in benzene, obtaining a reasonably good yield of the chloronitro mustard (180). This was reduced by hydrogenation over palladium-charcoal and the product was filtered immediately into a solution of hydrogen chloride in methanol. On condensing the solution the 3-aminophenyl-4-chloro nitrogen mustard was obtained as an unstable hydrochloride (181), the structure being confirmed spectroscopically.

It is quite clear from these results that, with the exception of 4-chloro-3-nitroaniline (and possibly 3-chloro-5-nitroaniline) the chloronitroanilines are not the optimum starting materials for the preparation of aminophenyl chloro mustards.

However, as has been discussed earlier, (see pp. 54 and 61-63), hydroxyethylation of an aminophenyl group is not the only method of preparing hydroxyethylamino derivatives. There have, in fact been a few cases, particularly with 2-nitro-chloro- or bromo-benzenes having a 4-nitro or a 4-sulphonamido group, where nucleophilic substitutions of the active halogen by diethanolamine have been successful[441,602,603].

To achieve aminophenyl chloro mustards via this method would necessitate the substitution of a series of dichloronitrobenzenes by diethanolamine - reactions that had been attempted by Kremer and Bendich[475] without any success whatsoever. In their attempted reactions, they had combined the reactants and heated them at 175° for two hours in or without the presence of a base , but the only products obtained were the result of reduction or hydrolysis.

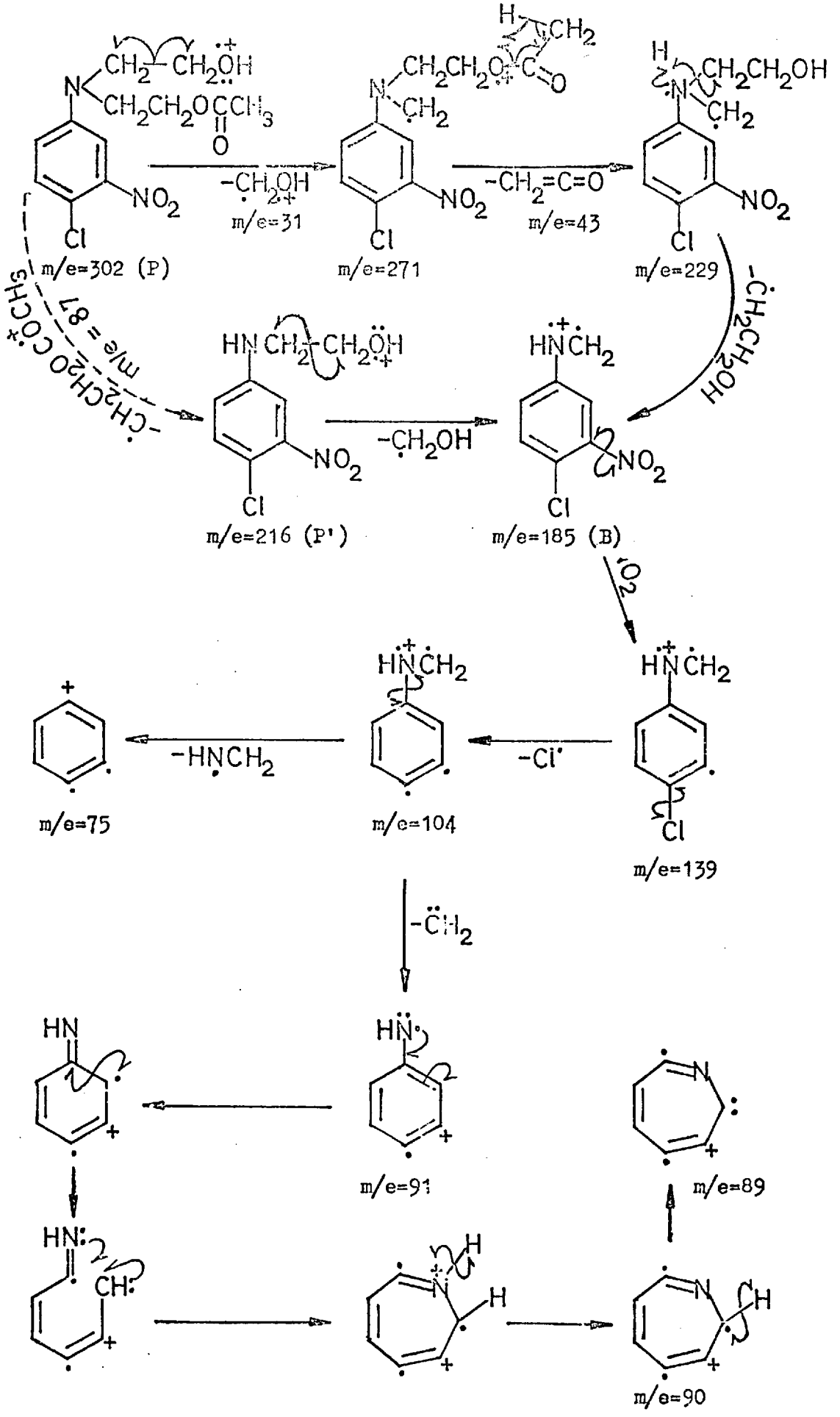

m/e=302 (P)
m/e=271
m/e=229
m/e=216 (P')
m/e=185 (B)
m/e=139
m/e=104
m/e=75
m/e=91
m/e=89
m/e=90
-CH2OH
m/e=31
-CH2=C=O
m/e=43
-·CH2CH2OH
-·CH2CH2O·C·CH3
m/e=87
-HNCH2
-Cl·
-·CH2

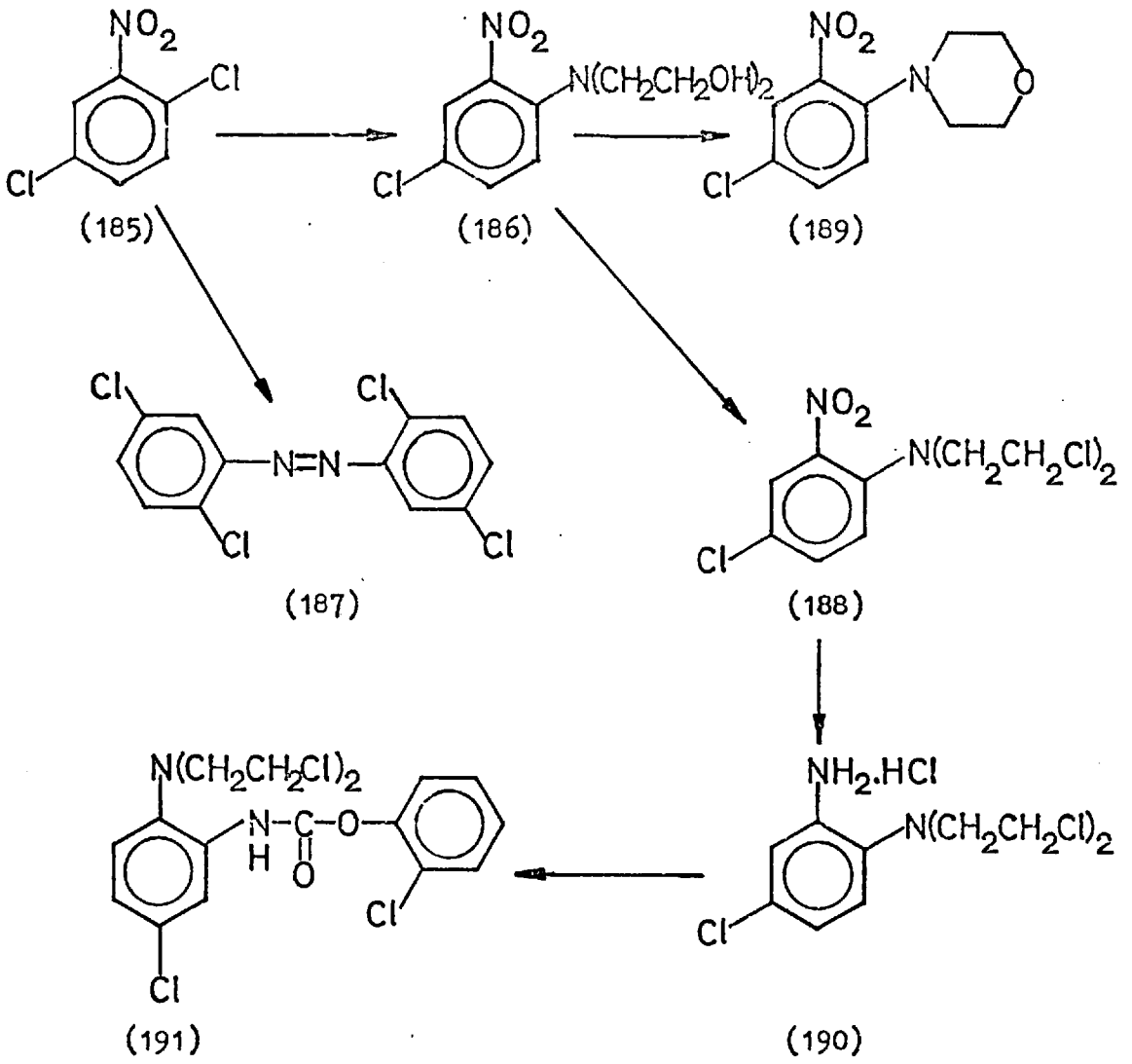
Fig. 10

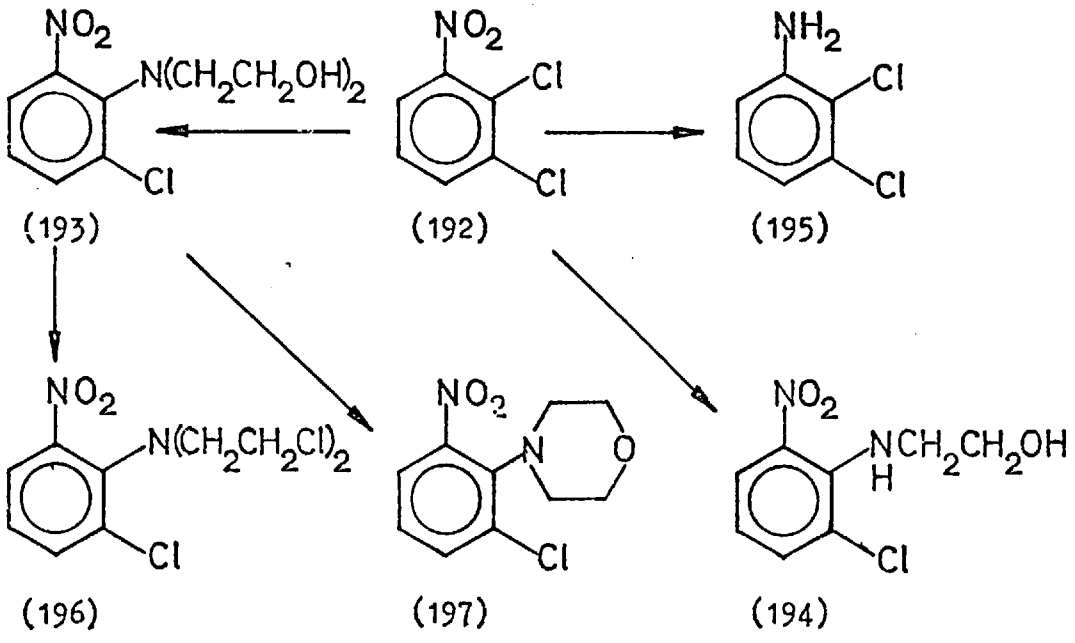
Notwithstanding Kremer and Bendich's failure to obtain the substitution products of various dichloronitrobenzenes with diethanolamine, it was decided to try precisely these reactions without using any base or solvent, and using lower temperatures. 2,5-Dichloronitrobenzene (185) was therefore treated with an excess of diethanolamine and heated at 110° for 3 hours. Spectral identification revealed that the main product (54%) was in fact the result of direct substitution, namely the required diol (186). Two by-products were also isolated and identified as the monohydroxyethyl derivative[474] (172) (1.6%) and the reduction product 2,2',5,5'-tetrachloroazobenzene[550,554] (187) (3.6%). The successful preparation of this diol (186) indicated the full potential of this method. Thus, while hydroxyethylation of 4-chloro-2-nitroaniline (171) gave poor yields of (172) and no bishydroxyethylation was achieved, this quick and simple method afforded the diol in good yield. Exploiting this reaction further, 2,3-dichloronitrobenzene (192) was similarly reacted at 115° for 5 hours. Once again the main product was the direct substitution product (193) (49%) - the by-products identified being the monohydroxyethyl derivative[474] (194) (4%) and 2,3-dichloroaniline (195) (3.5%). A fair amount of starting material (26%) was also re-obtained.

In both dichloronitrobenzenes mentioned above, the active chloro group, as explained on page 61, was the one ortho to the nitro group. But in 2,4-dichloronitrobenzene (200), both chloro groups are active. However, as explained on pages 61 to 63, the 2-chloro group is much more active than the 4-chloro group and one would predominantly expect substitution of the 2-chloro group.

This was borne out very clearly in the substitution of 2,4-dichloronitrobenzene (200) with diethanolamine at 108°. substitution of the 2-chloro group gave the expected diol (201) in 74% yield and a small amount of the monohydroxyethyl derivative[474] (202) (2.3%). Careful analysis of a small by-product however, revealed that substitution of the 4-chloro group had also been achieved, the respective diol (203) being isolated (5.6%). 4-Chloro substitution in 2,4-dichloronitrobenzene by a nucleophile has only once been reported before, the nucleophile being ammonia[476].

The question now arises whether the monohydroxyethyl by-products obtained during the diethanolamine condensations of dichloronitrobenzenes were the direct result of the reaction conditions employed or whether a small amount of monoethanolamine impurity was present in


NO2
Cl
Cl
(185)
NO2
N(CH2CH2OH)2
Cl
(186)
NO2
N
O
Cl
(189)
Cl
Cl
N=N
Cl
Cl
(187)
NO2
N(CH2CH2Cl)2
Cl
(188)
NH2.HCl
N(CH2CH2Cl)2
Cl
(190)
N(CH2CH2Cl)2
N
H
C
O
O
Cl
Cl
(191)


NO2
N(CH2CH2OH)2
Cl
(193)
NO2
Cl
Cl
(192)
NH2
Cl
Cl
(195)
NO2
N(CH2CH2Cl)2
Cl
(196)
NO2
N
O
Cl
(197)
NO2
NCH2CH2OH
H
Cl
(194)

commercial diethanolamine. A g.l.c. determination (on a Perkin Elmer F-11), using a 2 metre long 15% carbowax column, gave a 1½ min. retention time for monoethanolamine (2×10^3; 210°) but completely retained diethanolamine. A determination of commercial diethanolamine showed no trace of monoethanolamine under these conditions and at such a retention time thus indicating that the monohydroxyethyl derivatives obtained must be the direct result of dealkylation under the reaction conditions.

Condensation of diethanolamine with 3,4-dichloronitrobenzene (210) at 118° needed a much longer reaction time (24 hours) for complete reaction again indicating that the rate of substitution of the 4-chloro group is much smaller than that for the 2-chloro group. The products obtained were the expected diol (211) (61%), the monohydroxyethyl derivative (174) (2.3%) and the reduction product, 3,3',4,4'-tetrachloroazobenzene (212) (9.2%). Corbett and Holt[550] reported that Kremer and Bendich[475] had transposed the values of the melting points of 3,3',4,4'- and 3,3',5,5'-tetrachloroazobenzenes and as such quoted a melting point of 195.5° for (212) instead of the correct 158.5°.

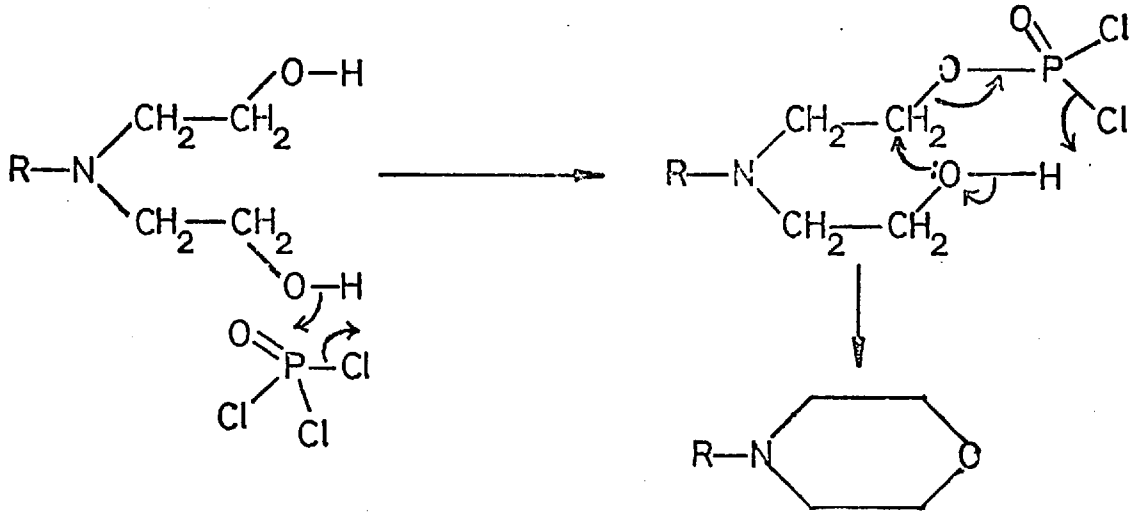
On page 62 it was mentioned that Holleman and co-workers[465] had found that in the reaction of dichloronitrobenzenes, increasing the temperature from 85° to 110°, had increased the rate and ease of reaction eightfold. It was decided to investigate the effect of temperature on the rate of reaction and the rate of formation of the diol from substitution of the 4-chloro group in 2,4-dichloronitrobenzene (200). At lower temperatures steric interference with coplanarity due to the introduction of a bulky diethanolamino group replacing the 2-chloro group was expected to be high while no such interference was expected to affect the substitution of the 4-chloro group. It was therefore expected that at lower temperature the yield of the 2-chloro-4-(bis-2-hydroxyethylamino)nitrobenzene (203) would be somewhat increased. But when the condensation of 2,4-dichloronitrobenzene (200) was attempted at 75°, the rate of reaction was found to be very slow - very little product was obtained after heating for 24 hours.

The variation in reactivity of the dichloronitrobenzenes with diethanolamine is given below:

Isomer	2,3-	2,4-	2,5-	3,4-
% Yield	49	84	54	61

These figures are in agreement with those obtained by Holleman and co-workers[465] and are comparable with those of Kremer and Bendich[474] for the condensation reactions with monoethanolamine except that their reported rate of reactivity of the 2,5-isomer was higher than the 3,4-isomer (see page 63).

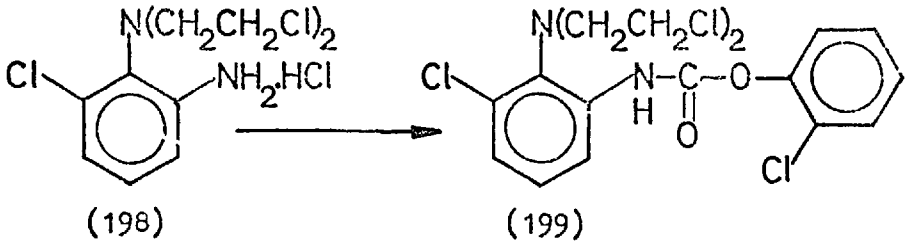
The diols from the above condensation reactions were chlorinated using phosphoryl chloride in benzene, obtaining the respective nitrogen mustards in good yields plus small amounts of the respective morpholino derivatives, with R_f values almost equal to those of nitrogen mustards. A possible mechanism for their formation is given below:



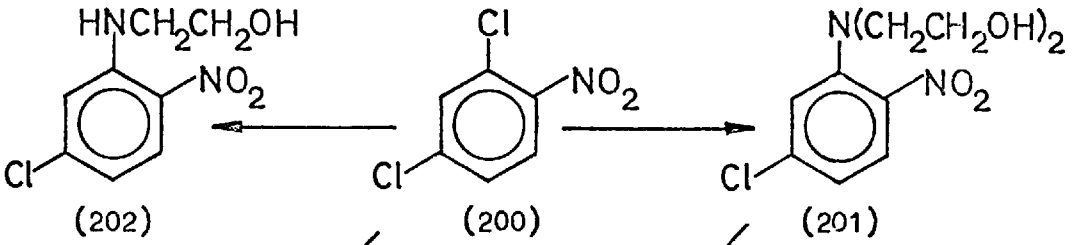
Thus the diol (186) gave the 4-chloro-2-nitrophenyl mustard (188) (87%) plus a small amount of the morpholino derivative (189) (3.7%). Reduction of this nitrophenyl mustard (188) with hydrogen at atmospheric pressure using palladium-charcoal catalyst gave the required 2-amino-phenyl 4-chloro mustard hydrochloride (190). It was however found unstable, slowly losing hydrogen chloride to form the free amine (see microanalysis) as well as oxidising in air. It was therefore characterized as its 2-chlorophenyl carbamate (191).

The diol (193) was similarly chlorinated to get the nitrogen mustard (196) (52%) and the morpholine derivative (197) (18%). The 6-chloro-2-nitrophenyl mustard (196) was reduced to the unstable 2-aminophenyl derivative (198) which was immediately characterized as its 2-chlorophenyl carbamate (199).

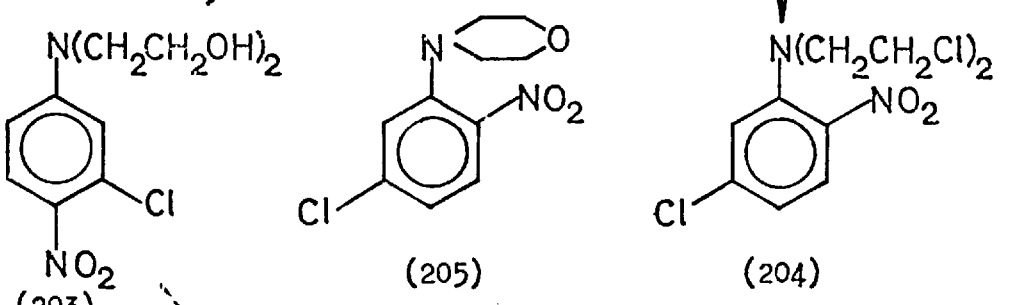
Diol (201) was likewise chlorinated obtaining the respective mustard (204) (74.5%) and the morpholine derivative (205) (6.2%). Similarly, chlorination of the isomeric diol (203) gave the mustard (208) and the morpholino isomer (209). Mustard (204) was hydrogenated to give the corresponding aminophenyl mustard hydrochloride (206)



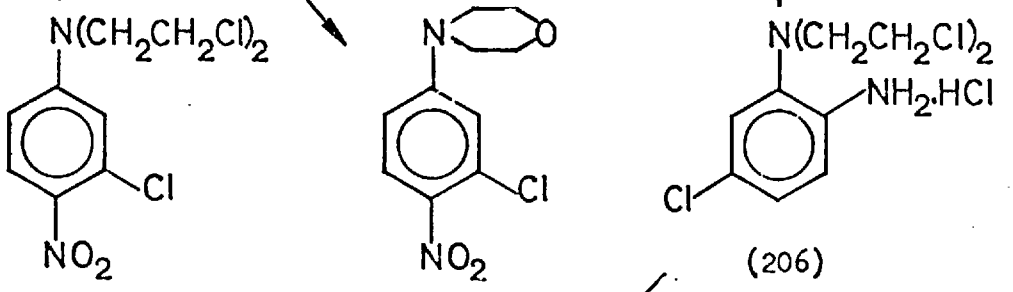
(198) (199)



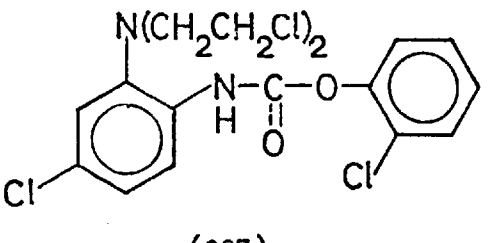
(202) (200) (201)



(203) (205) (204)



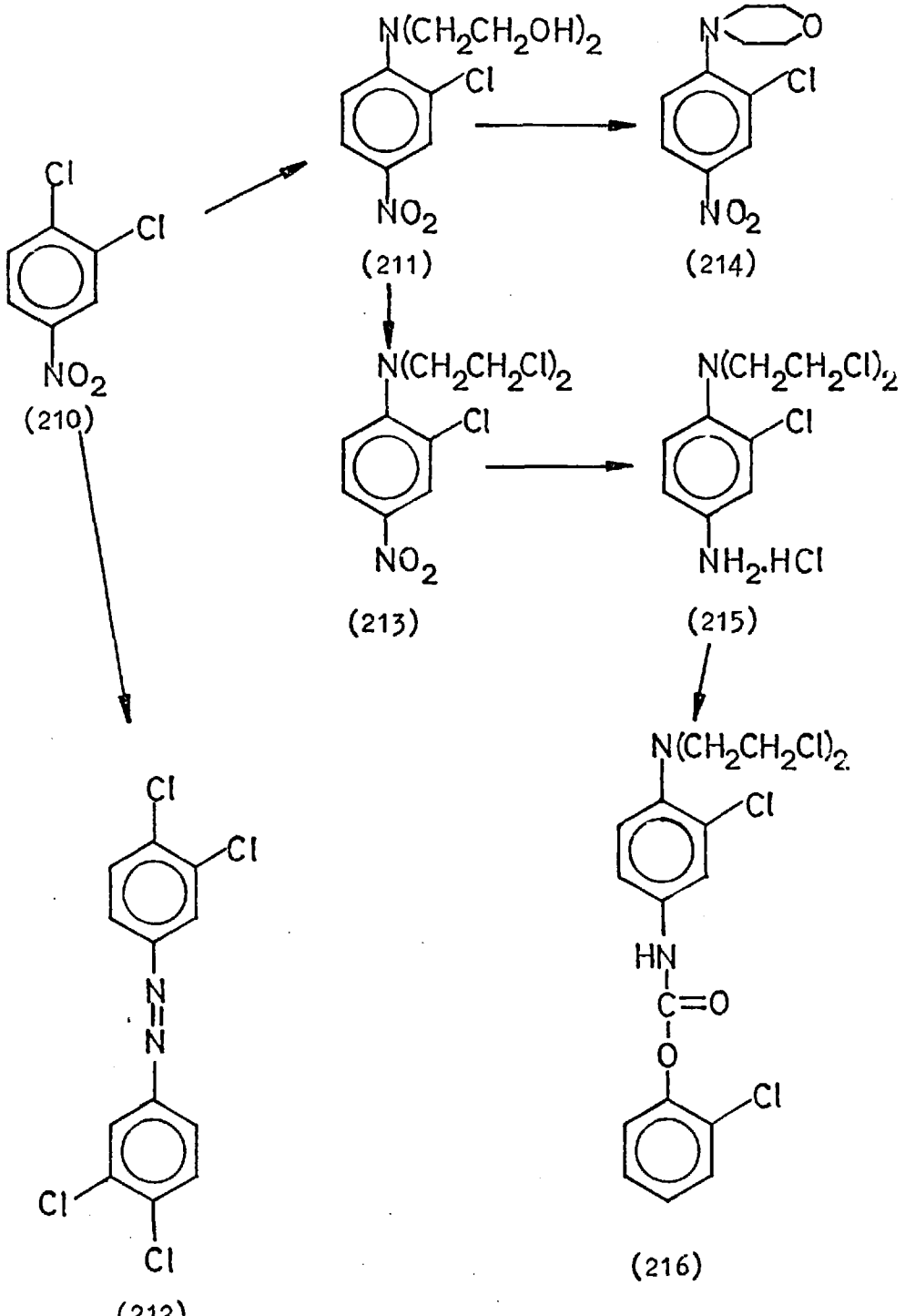
(208) (209) (206)



(207)

which was converted to the 2-chlorophenyl carbamate (207) which was however very unstable, decomposing within 18 hours to a dark brown oil, microanalysis of which was inconsistent with the molecular formula for the carbamate.

Finally the isomeric diol (211), on chlorination gave the corresponding nitrophenyl mustard (213) (72%) and the respective isomeric morpholino derivative (214) (4.3%). Hydrogenation of (213) gave the unstable aminophenyl hydrochloride (214) which as with similar isomers was characterized as its 2-chlorophenyl carbamate (216).



Conclusion

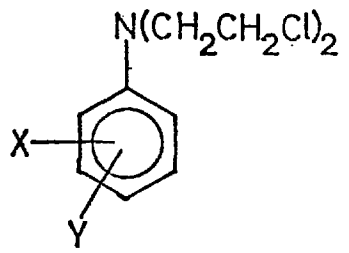
In the introduction to Chapter 3, the need for synthesizing more phenolic and aminophenyl trisubstituted nitrogen mustards was established. This has now been achieved. No carbamates of such phenolic and aminophenyl mustards have hitherto been reported. Synthesis of a carbamate of a phenolic mustard has now been carried out as well as the synthesis of five aminophenyl mustards. Part of the work involved studies on the hydroxyethylation of several ring-substituted anilines and considerable variations in reactivity have been revealed; these results can be explained by electronic and steric factors.

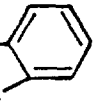
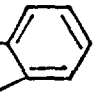
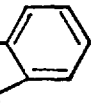
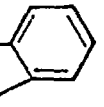
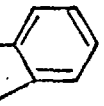
It has also been found that conversion of the di-2-hydroxyethyl compounds into nitrogen mustards is sometimes best accomplished with zinc chloride - catalysed thionyl chloride in benzene or with mesyl chloride in pyridine, good yields being obtained.

The use of diethanolamine condensations on dichloronitrobenzenes has now been established as a source of the intermediate diols, thereby facilitating preparations of mustards carrying additional amino-substituents.

Table 4 shows all the ring-substituted nitrogen mustards synthesized during the course of this work and therefore supplements Table 3. (see page 69).

Table 4. Summary of all ring substituted nitrogen mustards, of the type shown below, synthesized during the course of this work:



X	Y	X	Y
2-OH	5-CO_2Me	2-O.CO.NH-	5-CO_2Me
3-OH	4-CO_2Me		
4-OH	3-CO_2Me	3-NH.CO.O- Cl	4-CO_2Me
3-NH_2	4-CO_2Me		
3-NH_2	4-Cl	2-NH.CO.O- Cl	6-Cl
2-NH_2	6-Cl		
2-NH_2	5-Cl	2-NH.CO.O- Cl	5-Cl
2-NH_2	4-Cl		
4-NH_2	2-Cl	2-NH.CO.O- Cl	4-Cl
3-NHAc	4-CO_2Me		
4-NO_2	3-CO_2Me	4-NH.CO.O- Cl	2-Cl
2-NO_2	4-OMe		
3-NO_2	4-Cl		
2-NO_2	6-Cl		
2-NO_2	5-Cl		
2-NO_2	4-Cl		
4-NO_2	2-Cl		
4-NO_2	3-Cl		

CHAPTER 4

EXPERIMENTAL

The figures in parentheses, shown throughout this chapter, refer to pages in laboratory notebooks.

Infra-red spectra were recorded on a Perkin Elmer 700 spectrophotometer using a nujol mull, unless otherwise stated, and are quoted in cm^{-1}. In general, only characteristic peaks are mentioned. U.V. spectra were recorded on a Pye Unicam SP 1800 spectrophotometer, using 99.9% ethanol as solvent. Values are quoted in nm and the logarithm of their respective extinction coefficients are given in parentheses. N.m.r. spectra were recorded on a Varian T60 spectrometer using, unless otherwise stated, deuterochloroform as solvent, tetramethylsilane as internal reference and, where necessary, deuterium oxide as exchange agent. All coupling constants, J, are quoted in Hertz, Hz.

Thin layer chromatograms, preparative or otherwise, were carried out using Kieselgel GF_{254} (E. Merck Ltd.), unless otherwise stated. Column chromatography, including the dry column technique[511], was carried out by using Grade 3 neutral alumina, prepared from Brockmann Grade 1 active neutral alumina (BDH Chemicals Ltd.) by the usual method. When necessary, Fluorescent Indicator F_{254} (E. Merck Ltd.) was added.

Unless otherwise stated, saturated aqueous sodium hydrogen carbonate was used for neutralization of acidic solutions. Organic solutions were dried with magnesium sulphate, except where otherwise stated. Petroleum refers to the fraction of b.p. 40-60° unless stated otherwise while ether refers specifically to diethylether.

Melting points were determined using a Kofler block and were uncorrected.

The following abbreviations are used in quoting i.r. spectra: d, doublet; s, strong; sh., shoulder; v, very; w, weak. The following abbreviations are used in quoting n.m.r. spectra: br, broad; d, doublet; dd, double doublet; $J_{o,m}$, $J_{ortho,meta}$; m, multiplet; q, quadruplet; s, singlet; t, triplet; v, very.

PHENOLIC METHOXYCARBONYL NITROGEN MUSTARDS

Methyl 3-nitro-4-benzyloxybenzoate (171, 187, 314)

Methyl 3-nitro-4-hydroxybenzoate (9.85 g, 50 mmol) was dissolved in dry methanol (20 ml) with heating. Potassium (2 g, 50 mmol) in methanol (25 ml) was added, the solution first turning from yellow to orange, then coagulating into an orange-red solid. A further amount of methanol (80 ml) was added followed by benzyl chloride (6.33 g, 50 mmol). The mixture was refluxed for 24 h, a homogeneous solution being achieved after 2 h. On cooling, an inorganic salt precipitated out. This was filtered off and the filtrate was concentrated leaving an orange solid. This was washed repeatedly with 5% aqueous potassium hydroxide until no further orange solution was formed. The pale yellow residue was finally washed with water and petroleum, and dried to afford the title compound (0.9 g, 6%), m.p. 106.5-107.5° (lit.,[489] m.p. 107-108°), $\nu_{max.}$: 1710 (CO_2CH_3), 1615, 1532 (NO_2), 1442 (sh.), 1352, 1304 (NO_2), 1025, 745. τ: 1.4 (1H, d, aromatic), 1.73 (1H, dd, aromatic), 2.53 (5H, br s, aromatic), 2.77 (1H, d, aromatic), 4.63 (2H, s, $PhCH_2O$), 6.03 (3H, s, OCH_3). T.l.c. (ethyl acetate) on the orange solid showed that it was composed mainly of starting material.

Methyl 3-nitro-4-benzoyloxybenzoate (316)

Methyl 3-nitro-4-hydroxybenzoate (2 g) was dissolved in dry pyridine (10 ml). Benzoyl chloride (3 ml) was added, resulting in the formation of a white precipitate. The mixture was stirred at room temperature for 1 h, then filtered. The filtrate was concentrated, redissolved in dichloromethane, washed successively with 5% aqueous potassium hydroxide (10 ml) and water (3 x 10 ml), dried and evaporated slowly at room temperature giving white needles of the title compound (2.55 g, 83%), m.p. 84-87°. Three successive recrystallizations from dichloromethane gave chromatographically pure white needles, m.p. 88.5-89° (lit.,[490] m.p. 95°), $\nu_{max.}$: 1735 (C=O), 1540 (NO_2), 1370 (NO_2), 1300 (C-O), 1260, 1240 (C-O), 1122 (OCH_2), 1065, 715. τ: 1.17 (1H, d, J_m= 2.5, aromatic), 1.4-2.0 (3H, m, aromatic), 2.17-2.67 (4H, m, aromatic), 6.0 (3H, s, OCH_3).

Methyl 3-nitro-4-hydroxybenzoate

It was obtained from Cambrian Chemicals Ltd. $\nu_{max.}$: 3250 (OH) (3200 in $CHCl_3$), 1720 (CO_2Me), 1623, 1540 (NO_2), 1445, 1310 (sh.), 1300 (NO_2), 1175, 775, 700. τ: -0.96 (1H, s, exchanged, OH), 1.14 (1H, d, J_m= 2, aromatic), 1.67 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 2.7 (1H, d, J_o= 9, aromatic), 6.00 (3H, s, CO_2CH_3).

Methyl 3-amino-4-hydroxybenzoate (1, 45, 81, 118)

Methyl 3-nitro-4-hydroxybenzoate (19.8 g) was dissolved in dry ethanol (300 ml). Catalytic amounts of freshly prepared active Raney nickel were added and hydrogen (8.2 l) at atmospheric pressure was added over a period of 2½ h. The Raney nickel was removed by filtration (with usual precautions) to give a reddish-brown solution which, on decolourization with activated charcoal, gave a yellow solution. Evaporation to dryness under reduced pressure gave a cream-white solid which was recrystallized from aqueous ethanol to give chromatographically pure white needles of the title compound (14.6 g, 82%), m.p. 141.5-143° (lit.,[491] m.p. 142-144°), mixed m.p. with orthocaine (authentic sample of title compound) gave m.p. 142-144°.

Another preparation on an equal scale yielded 98% of recrystallized pale brown crystals, m.p. 141.5-143.5°. A further preparation was carried out at room temperature but in an autoclave at 100 atmospheres overnight; a 79% yield of white needles, m.p. 143-143.5° (from aq. EtOH) was obtained. $\nu_{max.}$: 3360/3330 (NH_2), 3060 (OH), 1687 (CO_2Me), 1605, 1445, 1310, 1218, 920, 780. τ(DMSO-d_6): 2.56 (1H, s, aromatic), 2.60 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 3.17 (1H, d, J_o= 9, aromatic), 4.27 (3H, v br, exchanged, NH_2, OH), 6.17 (3H, s, OCH_3).

Methyl 4-hydroxy-3-(di-2-hydroxyethylamino)benzoate (7, 100, 120, 142)

Methyl 3-amino-4-hydroxybenzoate (10.02 g, 60 mmol) was suspended, with stirring, in aqueous acetic acid (1:2, 225 ml), cooled in ice-water and liquid ethylene oxide (50 ml) was added. Stirring at around 1° was continued for 2 h when a further amount of liquid ethylene oxide (10 ml) was added. Stirring in ice-water was continued for a further 7 h and then the temperature was allowed to rise overnight to room temperature. All excess solvents were removed under reduced pressure, the residue neutralized and extracted into ethyl acetate (3 x 100 ml).

The combined extracts were dried and evaporated to give an orange-coloured oil which was crystallized from benzene-petroleum to give crude cream-white crystals of the diol (10.7 g, 70%), m.p. 69-73°. Repeated recrystallization from chloroform-petroleum afforded white crystals of the title compound, m.p. 73.5-75.5° (lit.,[439] m.p. 79°), soluble in chloroform, benzene and ethyl acetate but insoluble in ether. T.l.c. ($CHCl_3$) indicated a minor trace of impurity but i.r. and n.m.r. spectra, as compared to those of an authentic sample, were identical. $\nu_{max.}$: 3350 (phenolic OH), 3200 (aliphatic OH), 1710 (CO_2Me), 1445, 1310, 1080, 780. τ: 2.12 (1H, s, aromatic), 2.20 (1H, dd, $J_{o,m}$ = 9, 2, aromatic), 3.04 (1H, d, J_o = 9, aromatic), 4.78 (3H, br s, exchanged, OH), 6.12 (3H, s, OCH_3), 6.35 (4H, t, $C-CH_2-O$), 6.83 (4H, t, $N-CH_2-C$).

In one experiment, crystallization could not be achieved. Distillation of the oil yielded a colourless viscous distillate, b.p. 55° at 3mmHg, $\nu_{max.}$(film): 3400 (OH), 2950/2890 (CH_2), 1725 (C=O), 1440, 1380, 1250, 1090, 1060, 960, 900. τ: 5.78 (2H, q, $C-CH_2-O$), 6.2 (2H, q, $CO-O-CH_2-C$), 6.4 (2H, br s, exchanged, OH), 7.9 (3H, s, $COCH_3$). The residue crystallized to the title compound and was then recrystallized.

Methyl 4-hydroxy-3-(di-2-chloroethylamino)benzoate

(1) Preparation using thionyl chloride in benzene (11, 29, 112, 124, 130, 158, 167)

Methyl 4-hydroxy-3-(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 refluxed for 20 min after which, on cooling, a precipitate was obtained. This was filtered giving a deep-red filtrate A, and a pink residual solid B, which became light pink on drying. This solid, B, was neutralized with ammonia (d 0.88) (100 ml), extracted into chloroform (3 x 50 ml), and the extracts were combined, washed with water (3 x 50 ml), dried and concentrated affording a yellowish oil. (In one trial purification using column chromatography over a period of 11 days, most of the product decomposed on the column). Trituration with dry analar acetone -petroleum gave white crystals (0.873 g, 22%), m.p. 69-73°. Repeated recrystallization from acetone-petroleum gave colourless rhombic crystals, m.p. 70-73°. Final purification by preparative t.l.c. ($CHCl_3$) and recrystallization gave the title compound, m.p. 75-78° (lit., [439]

m.p. 76°). $\nu_{max.}$: 3270 (OH), 1715 (CO_2Me), 1445, 1300, 1210, 1120, 790. τ: 2.0 (1H, s, aromatic), 2.07 (1H, dd, $J_{o,m}$ = 9, 2, aromatic), 2.22 (1H, br s, exchanged, OH), 2.93 (1H, d, J_o = 9, aromatic), 6.10 (3H, s, OCH_3), 6.57 (8H, t, NCH_2CH_2Cl). A mixed m.p. with an authentic sample was undepressed.

The deep-red filtrate, A, was neutralized and the benzene layer was washed with water, dried and concentrated to an orange-coloured oil, $\nu_{max.}$ 1760 and 1700. Column chromatography (benzene) of this oil afforded fast-moving eluates which crystallized from acetone-petroleum to yellow crystals, m.p. 130-131° (1.24 g). Lassaigne's sodium fusion test indicated the presence of sulphur, nitrogen and chlorine. Preparative t.l.c. ($CHCl_3$) gave several bands. A minor yellow band gave an oil, low R_f, $\nu_{max.}$($CHCl_3$): 3450, 2900, 1760, 1700, 1600, 1580, 1440, 1360, 1280. The major band (70%), colourless but u.v. active, at approximately R_f 0.6, gave white crystals (26%), m.p. 131° (from dichloromethane-petroleum) $\nu_{max.}$: 1700. Recrystallization of the remainder of the yellow crystals from dichloromethane-petroleum also yielded the same compound, m.p. 131°.

A further preparation gave white crystals (from acetone-petroleum fraction b.p. 60-80°) of <u>6-methoxycarbonyl-4-N-2'-chloroethyl-2,3-dihydro-1,4-benzoxazine</u>, m.p. 74-74.5° (Found: C, 56.26; H, 5.50; N, 5.49; Cl, 14.11. $C_{12}H_{14}ClNO_3$ requires C, 56.37; H, 5.52; N, 5.48; Cl, 13.86%), $\nu_{max.}$: 1702 (CO_2Me), 1602, 1582 (ring carbons), 1518, 1445, 1357 (C-N), 1333, 1301, 1281 (C-O), 1252 (Ar-O-C), 1218, 1100, 780. τ: 2.55 (1H, dd, $J_{o,m}$ = 9, 2, aromatic), 2.63 (1H, s, aromatic), 3.17 (1H, d, J_o = 9, aromatic), 5.7 (2H, t, Ph-O-CH_2-C), 6.1 (3H, s, OCH_3), 6.27 (4H, s, NCH_2CH_2Cl), 6.5 (2H, t, Ph-O-C-CH_2-N). These spectral characteristics and the R_f value were identical to the white crystals with m.p. 131°. A mixed m.p. with an authentic sample of the difunctional nitrogen mustard (m.p. 75-78°) was 50-69°.

(2) <u>A much improved preparation using thionyl chloride in benzene in the presence of catalytic zinc chloride</u> (500)

Methyl 4-hydroxy-3-(di-2-hydroxyethylamino)benzoate (3.4 g) was dissolved in dry benzene (25 ml). Powdered dry zinc chloride (approx. 0.25 g) was added and this mixture refluxed with stirring while dropwise addition of thionyl chloride (12 ml) in benzene (10 ml) was effected. On complete addition of thionyl chloride (30 min), the

mixture, resulting from the precipitation occurring during the addition of the thionyl chloride, was further refluxed for 20 min. Considerable frothing occurred and the mixture turned dark red. The mixture was then filtered and the residue neutralized with ammonia (d 0.88), extracted into dichloromethane, washed with water, dried and partly concentrated. It was then chromatographed using preparative t.l.c. (CH_2Cl_2) and the band of high R_f extracted with ether, dried, and condensed to give a yellow oil (2.238 g, 58%) of the title compound which crystallized on trituration with acetone-petroleum to very light pink crystals, m.p. 72-75° (lit.,[439] m.p.76°). An n.m.r. spectrum of the oil was identical to that of an authentic sample of the title compound.

2-(Di-2-chloroethylamino)-4-methoxycarbonylphenyl N-phenylcarbamate (37, 42, 136, 619)

Methyl 4-hydroxy-3-(di-2-chloroethylamino)benzoate (501 mg, 1.7 mmol), phenyl isocyanate (214 mg, 1.8 mmol), and triethylamine (5 drops) were sealed in a small tube and stirred very slowly for 48 h at 66-68°. The resulting dark-red oil was chromatographed (CH_2Cl_2 - petroleum, 2:1) giving two colourless but u.v. active bands. The one with high R_f gave a dark yellow oil crystallizing slowly from dichloromethane-petroleum to large colourless crystals of 6-methoxycarbonyl-4-N-2'-chloroethyl-2,3-dihydro-1,4-benzoxazine (177 mg), m.p. 131-135°. τ identical to that of an authentic sample. The other band, of lower R_f, gave an almost colourless oil (507 mg, 72%) which on cooling for a few hours crystallized from benzene-petroleum, m.p. 56-61°. Recrystallizations from ether-petroleum gave white crystals of the title product, m.p. 89.5-90.5° (Found: C, 55.55; H, 4.81; N, 6.78; Cl, 16.96. $C_{19}H_{20}Cl_2N_2O_4$ requires C, 55.49; H, 4.90; N, 6.81; Cl, 17.24%). $\nu_{max.}$($CHCl_3$): 3415 (NH), 3015, 2960 (CH_2), 1755 (N.C=O.O), 1720 (CO_2Me), 1605, 1530, 1505, 1445, 1302, 1275, 1202, 1180, 1125, 1010, 998, 689. τ: 2.08-3.18 (9H, m, aromatic & NH), 6.10 (3H, s, OCH_3), 6.53 (8H, s, NCH_2CH_2Cl).

4-Aminosalicylic acid (98)

It was obtained in 99% purity from Ralph Emanuel Ltd. It was soluble in ethyl acetate but insoluble in dichloromethane. $\nu_{max.}$: 3465/3365 (NH_2), 3200-2750 (s, intramolecularly H-bonded OH), 2600-2550 (w. d, overtones, CO_2H), 1650 (sh.)/1645 (CO_2H), 1620 (δNH), 1450, 1300 (C-O, C-N), 1238/1207/1179 (C-O, C-N, δO-H), 890/830 (δCH, out-of-plane, 1,2,4-trisubstitution), 780, 738. τ (acetone-d_6): 2.33 (1H, d, J_o = 8, aromatic), 2.8 (4H, v br, exchanged, CO_2H, OH, NH_2), 3.73 (1H, dd, $J_{o,m}$ = 8, 2.5, aromatic), 3.79 (1H, s, aromatic).

Methyl 4-aminosalicylate

(a) Using hydrogen chloride catalyst (24, 25, 40, 47)

4-Aminosalicylic acid (30.6 g, 0.2 mol) was suspended in dry methanol (300 ml) saturated with dry hydrogen chloride and refluxed for 22 h. A heavy deposit of white crystals still persisted in the reaction mixture. A sample was dried giving white crystals of 4-aminosalicylic acid monohydrochloride, m.p. 218-221° (lit.,[508] m.p. 223-225°). A further amount of dry methanol (500 ml) was added to the bulk of the reaction mixture and this was re-saturated with dry hydrogen chloride, refluxed with stirring (48 h) and the resulting homogeneous solution was concentrated under reduced pressure to give a solid which was neutralized, extracted into ethyl acetate (3 x 150 ml) (ether was used in one experiment), dried and condensed to give highly impure cream-white crystals (18.8 g), m.p. 94-118°, R_f ($CHCl_3$): 0.46, 0.33 (ester), 0.05 (3-aminophenol), 0.00 (starting material). Recrystallization from methanol gave predominantly white crystals of 3-aminophenol (67%), m.p. 120-121° (lit.,[529] m.p. 123-124°). Mixed m.p. with an authentic sample of 3-aminophenol gave m.p. 119-123°, R_f ($CHCl_3$): 0.05, $\nu_{max.}$: 3350/3285 (NH_2), 3100-2800 (br, OH), 2720/2625, 1600 (δNH), 1597 (ring carbons), 1310 (δO-H), 1266 (C-O), 1180 (C-N), 1160, 920 (δNH), 858, 790, 705 (δC-C). This i.r. spectrum was completely identical to that of an authentic sample of 3-aminophenol. $\lambda_{max.}$: 216 (3.94), 235 (3.71), 285 (3.20). Again, this u.v. spectrum was identical to an authentic spectrum of 3-aminophenol. The remaining mother liquor was collected and a sample was chromatographed (preparative t.l.c. - $CHCl_3$) giving a further small amount of 3-aminophenol and also methyl 4-aminosalicylate as cream-white crystals, m.p. 116-118°, R_f($CHCl_3$): 0.33. A mixed m.p. with 3-aminophenol gave m.p. 94-105°.

Recrystallization from benzene-petroleum gave cream-white crystals of the ester, m.p. 118-119.5° (lit.,[500] m.p. 120-121°; [504] m.p. 123-125°) $\nu_{max.}$: 3460/3370 (NH_2), 3250 (OH), 1660 (sh.)/1645 (CO_2Me), 1625 (δNH), 1580/1520 (ring carbons), 1445 (ring C=C), 1365, 1350 (sh.), 1305 (sh.), 1290 (C-O-C), 1238, 1210/1205, 1166 (C-O-C), 1122, 1095, 986, 970, 860, 800, 763, 720. $\lambda_{max.}$: 241 (3.95), 289 (4.19), 307 (4.26). This u.v. spectrum was identical to that recorded by Drain and co-workers[500] for methyl 4-aminosalicylate. τ: -0.98 (1H, s, exchanged, OH), 2.33 (1H, d, J_o= 9.5, aromatic), 3.82 (1H, d, J_m= 2.5, aromatic), 3.84 (1H, dd, $J_{o,m}$= 9.5, 2.5, aromatic), 5.87 (2H, br, exchanged, NH_2), 6.12 (3H, s, OCH_3).

(b) Using sulphuric acid catalyst (87, 96)

4-Aminosalicylic acid (10 g, 65 mmol) was dissolved in dry methanol (280 ml) and concentrated sulphuric acid (16 ml); a white precipitate of the sulphate being initially formed which redissolved after addition of part of the concentrated sulphuric acid. Esterification was accomplished following refluxing for 8½ h. Removal of the excess solvent followed by neutralization, precipitated the crude ester (8.56 g, 79%), m.p. 114-120°, containing a small amount of 3-aminophenol. Recrystallization from methanol-water (charcoal) gave still impure methyl ester (7.95 g, 73%), m.p. 116.5-119°. Recrystallization from hot benzene-petroleum (b.p. 60-80°), however, gave chromatographically pure cream-white crystals of the title compound (6.7 g, 62%), m.p. 118-119.5°. Further recrystallization of a small sample from benzene-petroleum gave crystals of the pure ester, m.p. 119.5-121°. Spectral measurements were identical to those of an authentic sample of the methyl ester.

(c) Using boron trifluoride catalyst (83, 94, 150)

4-Aminosalicylic acid (80 g, 0.523 mol) was suspended in anhydrous methanol (525 ml) and boron trifluoride ethereate complex (300 ml, 4.5 mol) was added slowly with stirring below 40°. The resulting clear solution was refluxed for 24 h, then left standing at room temperature for 5 days. The solution was then condensed to a thick slurry, redissolved in distilled water (150 ml) and neutralized with 10 M aqueous sodium hydroxide, with cooling, resulting in the formation

of a precipitate. Following filtration, the residue was redissolved in dilute hydrochloric acid, neutralized, extracted into ethyl acetate and this organic layer was washed with water, dried and condensed giving crude methyl ester. Recrystallization from benzene-petroleum (charcoal) gave cream-white crystals of the title compound (67 g, 77%) m.p. 117-120°. A small scale preparation using this method gave the ester (94%), m.p. 120.5-121.5°. Another preparation using identical conditions, with the exception that the reaction mixture was not refluxed but left standing at room temperature for 15 days, gave the ester (73%), m.p. 117-119.5°. In every preparation, the product was identified by its m.p. and its spectral characteristics.

Methyl 4-(di-2-hydroxyethylamino)salicylate (104, 162, 195)

Methyl 4-aminosalicylate (18.9 g, 113.2 mmol) was dissolved in glacial acetic acid (200 ml) and water (50 ml), cooled to around 0° with stirring, and liquid ethylene oxide (25 ml) was added. It was stirred for 6 h, then the temperature was allowed to rise to room temperature and stirring was continued for a total of 48 h. The solvents were removed under reduced pressure giving an oil which was neutralized, extracted into ether (4 x 100 ml), washed, dried (charcoal), and condensed again, affording a yellow oil which crystallized from dichloromethane-petroleum, giving the crude product (24.3 g, 84%). Recrystallization from hot benzene gave white crystals of the triol (15.3 g, 53%), m.p. 97-100.5°. Further recrystallization from benzene of a small sample gave chromatographically pure white needles, m.p. 101-103° (lit.,[421] m.p. 97-98°). R_f (ethyl acetate): 0.38. $\nu_{max.}$: 3580-3120 (aliphatic OH), 3300 (phenolic OH), 1660 (CO_2Me), 1625 (δNH), 1572, 1525, 1450 (sh.), 1345, 1318, 1292 (C-O-C), 1265, 1205, 1180 (C-O-C), 1060, 1010, 968, 907, 862, 790. $\lambda_{max.}$: 252 (3.94), 298.5 (4.22), 318.5 (4.45). τ: -0.94 (1H, s, exchanged, phenolic OH), 2.32 (1H, d, J_o= 9, aromatic), 3.78 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 3.84 (1H, s, aromatic), 6.10 (3H, s, OCH_3), 6.12 (4H, t, C-CH_2-O), 6.39 (4H, t, N-CH_2-C), 6.57 (2H, br, exchanged, aliphatic OH).

Attempted preparation of methyl 2-O-benzoyl-4-(di-2-hydroxyethylamino)-salicylate (183, 210)

Methyl 4-(di-2-hydroxyethylamino)salicylate (12.3 g, 48 mmol) was dissolved in 1,2-dimethoxyethane (100 ml). Sodium methoxide (2.64 g, 49 mmol) was added and the mixture stirred at around 50° for 1 h. It was then treated (30 min) with benzoyl chloride (6.75 g, 48 mmol) in 1,2-dimethoxyethane (15 ml) and stirred for a further 1 h, then evaporated giving an oil which was soluble in dichloromethane (200 ml). After washing successively with 1 M sodium hydroxide (aq.) (3 x 50 ml) and water (100 ml), it was dried and concentrated under reduced pressure affording a brown viscous oil (8.6 g). Dry column chromatography (ethyl acetate) gave three main bands. The band of highest R_f was further purified by t.l.c. (ethyl acetate), crystallized to a white solid, m.p. 114-115°, and recrystallized from dichloromethane-petroleum to give white crystals of methyl 2-O-benzoyl-4-(di-2-benzoyloxyethylamino)salicylate (500 mg), m.p. 115-116°. (Found: C, 69.91; H, 5.06; N, 2.24. $C_{33}H_{29}NO_8$ requires C, 69.83; H, 5.15; N, 2.47%). $\nu_{max.}$: 1738 (sh.), 1722 (Ph.C=O), 1702 (CO_2Me), 1618, 1458, 1440, 1300 (sh.), 1280, 1260 (sh.), 1200, 1165, 1130 (C-O), 1098, 1040, 725. τ: 1.6-3.4 (18H, m, aromatic), 5.41 (4H, t, C-CH_2-O), 6.11 (4H, t, N-CH_2-C), 6.33 (3H, s, OCH_3). The other two bands yielded impure viscous oils. The n.m.r. spectrum of the band with second-highest R_f was indicative of the title compound.

Chlorination of methyl 4-(di-2-hydroxyethylamino)salicylate with zinc chloride-catalysed thionyl chloride (546, 581)

Methyl 4-(di-2-hydroxyethylamino)salicylate (1.24 g) was dissolved in dry benzene (15 ml). Powdered anhydrous zinc chloride (under benzene) was added and the reaction mixture was vigorously stirred and refluxed. Thionyl chloride (4.4 ml) in dry benzene (4 ml) was added dropwise (20 min) - the reaction mixture turning dark blue-green. Refluxing was continued for 45 min, then the solution was cooled and neutralized with aqueous sodium carbonate, extracted into dichloromethane, dried and condensed to give a reddish oil (1.4896 g). T.l.c. (CH_2Cl_2) gave a band of highest R_f which afforded a yellow oil (525 mg). It was further purified to give a chromatographically pure, very light-yellow oil of a

1:1 molecular complex of methyl 3-chloro-4-(di-2-chloroethylamino)-salicylate and methyl 5-chloro-4-(di-2-chloroethylamino)salicylate (Found: C, 43.99; H, 4.34; N, 4.53; Cl, 30.74. $C_{12}H_{14}NCl_3O_3$ requires C, 44.00; H, 4.32; N, 4.29; Cl, 32.56%). $\nu_{max.}$ (film): 3130 (br, OH), 2960/2860 (CH_2), 1673 (CO_2Me), 1615, 1562, 1497, 1445, 1340, 1272, 1260, 1240, 1208, 1180, 1128, 1089 (Ar-Cl), 1018, 980, 912, 860, 798, 748, 735, 670. τ: -1.55 ($\frac{1}{2}$H, s, exchanged, OH), -0.72 ($\frac{1}{2}$H, s, exchanged, OH), 2.18 ($\frac{1}{2}$H, s, aromatic), 2.29 ($\frac{1}{2}$H, d, J_o= 9, aromatic), 3.27 ($\frac{1}{2}$H, d, J_o= 9, aromatic), 3.29 ($\frac{1}{2}$H, s, aromatic), 6.05 (3H, s, OCH_3), 6.37 (8H, s, NCH_2CH_2Cl).

Methyl 4-(di-2-chloroethylamino)salicylate (607, 675)

Methyl 4-(di-2-hydroxyethylamino)salicylate (4 g) was dissolved in pyridine (25 ml) and methanesulphonyl chloride (5.3 ml) in pyridine (5 ml) was added. An exothermic reaction followed and the mixture was stirred and further heated at 100° for 20 min. On slight cooling, a precipitate was obtained. This was filtered off and washed with petroleum and dichloromethane to give white crystals of pyridine methanesulphonate (5.8 g), m.p. 174°, soluble in water. The filtrate was washed with dilute hydrochloric acid, aqueous sodium carbonate and finally water, dried and condensed giving an orange oil which was chromatographed by preparative t.l.c. (CH_2Cl_2) giving a major band of high R_f which afforded an orange-yellow oil of the crude nitrogen mustard (3.34 g, 73%). Trituration in petroleum-ether gave a white solid which was recrystallized from ether-petroleum to give light-pink crystals of the title compound (2.95 g, 65%), m.p. 61.5-62.5° (lit.,[421] m.p. 60.5-61°). $\nu_{max.}$(film): 3150-3090 (OH), 2955/2870 (CH_2), 1660 (CO_2Me), 1625, 1568, 1520, 1445, 1352, 1280, 1255, 1205, 1175, 1118, 840, 780, 748-704 (C-Cl) (lit.,[421] $\nu_{max.}$: 1669, 1193, 1166, 741-719), $\nu_{max.}$($CHCl_3$): same as for $\nu_{max.}$(film) above 1300. below: 1268, 1172, 897, 839, 700. τ: -0.98 (1H, s, exchanged, OH), 2.30 (1H, d, J_o= 9.5, aromatic), 3.78 (1H, dd, $J_{o,m}$= 9.5, 2.5, aromatic), 3.81 (1H, d, J_m= 2.5, aromatic), 6.1 (3H, s, OCH_3), 6.28 (8H, s, NCH_2CH_2Cl).

5-Aminosalicylic acid

5-Aminosalicylic acid was obtained from Ralph Emanuel Ltd. It was insoluble in dichloromethane, ethanol, methanol or water but was soluble in dimethyl sulphoxide. $\nu_{max.}$: 3000(sh.)/2550 (OH), 1645 (CO_2H), 1620, 1580, 1490, 1360, 1320, 1275, 1250, 1200, 1142, 822, 786. τ(DMSO-d_6): 2.57 (4H, br, exchanged, NH_2, OH, CO_2H), 2.72 (1H, d, J_m= 3, aromatic), 3.00 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 3.27 (1H, d, J_o= 9, aromatic).

Methyl 5-aminosalicylate (19, 21, 89, 199)

5-Aminosalicylic acid (30.6 g, 0.2 mol) was suspended in dry methanol (500 ml) with stirring. The mixture was saturated with dry hydrogen chloride resulting in a dark homogeneous solution which was refluxed for 20 h. The excess solvent was then removed under reduced pressure and the resulting solid was neutralized, extracted into ether (3 x 200 ml) and the organic solution was dried and concentrated, affording the title compound which was recrystallized from methanol to give brown crystals of the ester (28.4 g, 85%), m.p. 94.5-95.5° (lit.,[522] m.p. 96°). In one preparation, recrystallization from methanol (charcoal) gave chromatographically pure, light-yellow crystals of the ester, m.p. 95.5-96.5°. R_f (ethyl acetate): 0.68. $\nu_{max.}$: 3390/3310 (NH_2), 3070 (OH), 1672 (CO_2Me), 1495, 1445, 1345, 1310, 1280, 1240, 1220, 1093, 980, 900, 845-840 (d), 800, 730. $\lambda_{max.}$: 228 (4.18), 356 (3.42). τ: -0.34 (1H, br, OH), 2.73 (1H, d, J_m= 2.5, aromatic), 2.80-3.17 (2H, m, aromatic), 6.03 (3H, s, OCH_3), 6.6 (2H, br, exchanged, NH_2).

Methyl 5-(di-2-hydroxyethylamino)salicylate (49, 55, 90, 192, 201)

Methyl 5-aminosalicylate (3.34 g, 20 mmol) was dissolved in a mixture of water (15 ml) and glacial acetic acid (50 ml) with stirring and the solution was cooled in ice. Liquid ethylene oxide (16 ml) was added and stirring was continued in ice-water for 1 h. A further amount of liquid ethylene oxide (8 ml) was added and the solution was stirred in ice-water for 23 h, letting the temperature rise gradually to room temperature. The excess solvents were removed under reduced pressure leaving a red oil which was neutralized, extracted into ether (3 x 50 ml), washed successively with aqueous

sodium hydrogen carbonate (50 ml) and water (50 ml), dried, decolourized with activated charcoal and condensed giving yellow crystals of the crude title compound (4.7 g, 92%) which were recrystallized from chloroform-petroleum (b.p. 60-80°) affording bright canary-yellow crystals of the title compound, m.p. 110-111.5° (Found: C, 56.34; H, 6.66; N, 5.34. $C_{12}H_{17}NO_5$ requires C, 56.47; H, 6.67; N, 5.49%). R_f (EtOH): 0.63, R_f (ethyl acetate): 0.33. $\nu_{max.}$: 3245 (OH), 1680 (CO_2Me), 1618, 1502, 1445, 1295, 1265, 1240, 1220, 1192, 1092, 1058, 1032, 1002, 900, 828, 799, 762, 730. $\nu_{max.}$ ($CHCl_3$): 3245 (OH), 2910/2850 (CH_2), 1720/1670 (CO_2Me). ν_{max}: 235 (4.27), 267 (3.84), 385 (3.43). τ: -0.28 (1H, s, exchanged, phenolic OH), 2.65-3.0 (3H, m, aromatic), 6.05 (3H, s, OCH_3), 6.20 (4H, t, $C\text{-}CH_2\text{-}O$), 6.55 (4H, t, $N\text{-}CH_2\text{-}C$), 6.87 (2H, br, exchanged, aliphatic OH). In solution, the triol fluoresced with a blue-green colour particularly at the surface of the solution. A final recrystallization gave yellow crystals of the triol, m.p. 113-115°.

Methyl 2-O-benzoyl-5-(di-2-hydroxyethylamino)salicylate (61, 77, 191, 306)

Methyl 5-(di-2-hydroxyethylamino)salicylate (2.05 g, 8 mmol) was dissolved in 1,2-dimethoxyethane (24 ml) and treated with sodium methoxide (0.44 g, 8 mmol). The mixture was stirred at 50° for 1 h, then treated over 30 min with redistilled benzoyl chloride (1.125 g, 8 mmol) in 1,2-dimethoxyethane (2.5 ml). Stirring was continued for 1 h, then the excess solvents were removed under reduced pressure and the residue was extracted into chloroform (50 ml), washed successively with 1 M sodium hydroxide (3 x 25 ml) and water (2 x 25 ml), dried, and condensed leaving a yellow oil which partly crystallized (from toluene) on cooling in a refrigerator for 2 days, affording, on washing with ether, white crystals (504 mg), m.p. 92-98°. The uncrystallized oily filtrate and the ether washings were combined. Column chromatography (silica - ether) of this gave a red oil (2.03 g) which was further purified by preparative t.l.c. (CH_2Cl_2) giving two bands. The higher R_f band (R_f = 0.66) gave an oil, τ: -0.33 (phenolic OH). The lower R_f band (R_f = 0.44) after further chromatographic purification gave a yellow oil which crystallized from ether-petroleum (b.p. 60-80°) to white crystals (0.33 g), m.p. 106-108°. Recrystallization of the combined solids gave white crystals of the benzoyl

derivative (880 mg, 30.5%), m.p. 107-108°. (Found: C, 63.47; H, 6.00; N, 3.77. $C_{19}H_{21}NO_6$ requires C, 63.51; H, 5.89; N, 3.90%). $\nu_{max.}$: 3225 (OH), 1730 (sh., PhCOO), 1725 (CO_2Me), 1610, 1505, 1440 (sh.), 1367 (sh.), 1280, 1260, 1220, 1090, 1070, 722. $\nu_{max.}$($CHCl_3$): 3320 (OH), 2925/2865 (CH_2), 1720 (PhCOO, CO_2Me). τ: 1.67 (1H, d, J_m= 2.5, aromatic), 1.79 (1H, d, J_m= 2.5, aromatic), 2.27-3.30 (6H, m, aromatic), 6.29 (3H, s, OCH_3), 6.0-6.6 (8H, m, NCH_2CH_2O), 6.0-7.3 (2H, br, exchanged, OH). In one n.m.r. spectrum, the two aliphatic protons were shown as a broad signal at 5.57τ.

Methyl 2-O-benzyl-5-(di-2-hydroxyethylamino)salicylate (144)

Methyl 5-(di-2-hydroxyethylamino)salicylate (2.55 g, 10 mmol) was dissolved in dry methanol (25 ml) in which potassium hydroxide (565.5 mg, 9.92 mmol) was dissolved. This solution was warmed and benzyl bromide (1.71 g, 10 mmol) in methanol (5 ml) was added dropwise. It was then refluxed until the pH fell from 12 to 4 (4 h) when some inorganic salt crystallized out. The solution was decanted and the solvent evaporated to give an orange oil. Ice-cold water was added and the mixture was extracted with chloroform. The organic layer was washed with water, dried and concentrated to give an oil which was chromatographed (preparative t.l.c. - ethyl acetate) affording a yellow oil which was purified several times by preparative t.l.c. to give the chromatographically pure benzyloxy derivative as a light yellow oil containing a small amount of solvent (Found: C, 67.9?; H, 7.33; N, 3.91. $C_{19}H_{23}NO_5$ requires C, 66.07; H, 6.71; N, 4.06%) $\nu_{max.}$(film): 3330 (OH), 2950/2875 (CH_2), 1710 (CO_2Me), 1501 (vs), 1442, 1360, 1300, 1255, 1080, 816, 745, 707. τ: 2.4-3.27 (8H, m, aromatic), 4.9 (2H, s, CH_2Ph), 6.1 (3H, s, OCH_3), 6.23 (4H, t, $C-CH_2-O$), 6.53 (4H, t, $N-CH_2-C$), 5.3-7.6 (2H, br, exchanged, OH). Attempts at making a crystalline derivative of 3,5-dinitrobenzoyl chloride were unsuccessful.

Methyl 5-(di-2-chloroethylamino)salicylate (57, 520, 522)

Methyl 5-(di-2-hydroxyethylamino)salicylate (1 g) was dissolved in chloroform (12 ml). Catalytic amounts of dry zinc chloride powder (kept under benzene) were added followed by dropwise addition of thionyl chloride (3.6 ml) in chloroform (3 ml) with stirring at room temperature. Initially a precipitate was formed but it redissolved affording a slightly reddish solution on complete addition of the thionyl chloride solution (45 min). This solution was refluxed for 13 min and then, the excess solvents were removed under reduced pressure, the residue was neutralized, extracted into dichloromethane (40 ml), washed with water and dried giving an orange solution (charcoal) with a slightly bluish-green fluorescent effect. Preparative t.l.c. (CH_2Cl_2) gave a high R_f, u.v. active band, which afforded a yellow oil (593 mg) that crystallized on trituration with petroleum to cream-yellow crystals, m.p. 79-81°. Recrystallization from dichloromethane-petroleum gave light-yellow crystals of the title compound (52%), m.p. 65-66°. A mixed m.p. with the crude product gave an appearance of melting at 79° but reformed needle-shaped crystals, m.p. 84-85° (Found: C, 49.19; H, 5.22; N, 4.56; Cl, 24.22. $C_{12}H_{15}Cl_2NO_3$ requires C, 49.33; H, 5.17; N, 4.79; Cl, 24.27%). $\nu_{max.}$: 3150 (OH), 1675 (CO_2Me), 1615, 1495, 1445, 1365, 1310, 1300, 1260, 1215, 1145, 1090, 950, 830, 790, 750. τ: -0.28 (1H, s, exchanged, OH), 2.7-3.1 (3H, m, aromatic), 6.07 (3H, s, OCH_3), 6.12 (8H, s, NCH_2CH_2Cl).

AMINOPHENYL METHOXYCARBONYL NITROGEN MUSTARDS

5-Amino-2-nitrobenzoic acid (208)

It was obtained in 70% form (moist) from Aldrich Chemical Co. Inc. It was dried over phosphorus pentoxide and recrystallized from methanol to give orange-brown crystals, m.p. 235° (dec. 229°) (lit.,[525] m.p. 235°). $\nu_{max.}$: 3450/3350 (NH_2), 3220 (OH), 2720-2550 (CO_2H overtones), 1690 (CO_2H), 1625 (δNH), 1598, 1580, 1500 (sh., NO_2), 1485, 1322 (NO_2), 1300, 1265, 1157, 1078, 880, 850, 762.

Methyl 5-amino-2-nitrobenzoate (26, 65, 140, 206, 241, 244, 262)

Recrystallized and dry 5-amino-2-nitrobenzoic acid (30.36 g, 166.7 mmol) was dissolved in dry methanol (200 ml) saturated with dry hydrogen chloride. The solution was refluxed, with slow addition of hydrogen chloride, for 16 h and then further refluxed for 24 h. The excess methanol was then removed under reduced pressure, and the resulting oil was neutralized, when a dark brown solid was precipitated. It was filtered, redissolved in ether, washed with aqueous sodium hydrogen carbonate (100 ml) and water (2 x 100 ml), dried, decolourized with active charcoal and condensed giving golden-yellow crystals (27 g, 84%), m.p. 86-94°. T.l.c. (ethyl acetate) gave two bands at R_f 0.63 and R_f 0.70. On attempting to redissolve this crude product in methanol, a small amount was partly insoluble. This was filtered off, washed with petroleum (b.p. 60-80°) and dried giving orange-red needles m.p. 193-199°, which, on recrystallization from methanol gave red needles, m.p. 204-206° (At 170° the crystal structure changed from needle to prism shaped). The bulk of the crude ester dissolved in methanol was purified by partly concentrating the hot methanol solution followed by addition of distilled water to the hot solution. A small amount of red oil separated out. This was removed by a pipette and the solution was further concentrated. More distilled water was added, again resulting in a further precipitation of a red oil. This sequence was repeated until the solution turned from orange to bright yellow and no further red oil was precipitated and removed. Then the yellow solution was allowed to cool in ice, yellow crystals being obtained in good yield, m.p. 97.5-100.5°. R_f (ethyl acetate): 0.63 plus a trace impurity at 0.70. A sample was purified by preparative

t.l.c. (benzene : ether : dichloromethane - 4:4:1) extracting the fraction at R_f 0.42 giving the pure title compound, m.p. 98-100° (Found: C, 48.98; H, 4.30; N, 14.17. $C_8H_8N_2O_4$ requires C, 48.99; H, 4.11; N, 14.28%). The bulk of the ester obtained from the first recrystallization was further recrystallized slowly from dichloromethane giving yellow prisms, m.p. 100.5-104.5°. Further purification was achieved by dissolving the ester in dichloromethane and passing dry hydrogen chloride to saturation. The white precipitate of the ester hydrochloride was collected and dissolved in methanol giving a yellow solution from which the chromatographically pure ester was obtained. $\nu_{max.}$: 3460/3353 (NH_2), 3220, 1725 (CO_2Me), 1635, 1607, 1580, 1501 (NO_2), 1480, 1320 (NO_2), 1265, 1143, 1075, 985, 878, 840, 765. $\nu_{max.}$($CHCl_3$): 3460/3360 (NH_2), 1725 (CO_2Me), 1620, 1588, 1445, 1325 (NO_2), 1260-1190 (br). τ(acetone-d_6): 2.02 (1H, d, J_o= 9.8, aromatic), 3.22 (2H, dd, $J_{o,m}$= 9.5, 3, aromatic), 3.57-5.63 (2H, br, exchanged, NH_2) (in other spectra, this band moved to 3.88), 6.13 (3H, s, OCH_3). τ: 2.03 (1H, d, J_o= 9.8, aromatic), 3.27 (1H, dd, $J_{o,m}$= 9.8, 3, aromatic), 3.27 (1H, d, J_m= 3, aromatic), 5.42 (2H, br, exchanged, NH_2), 6.07 (3H, s, OCH_3). [lit.,[526] τ for aromatic protons only: 2.07 (1H), 3.35 (2H)]. It was furthermore characterized as its N-acetyl derivative (see below).

The small amount of red oil precipitated during the initial recrystallization of the title compound, solidified and was recrystallized from methanol giving further amountss of the title compound plus red needles of an isomeric unknown ester, m.p. 194-195° (dec.). R_f (ethyl acetate): 0.72. (Found: C, 49.30; H, 4.17; N, 14.43. $C_8H_8N_2O_4$ requires C, 48.99; H, 4.11; N, 14.28%). A small sample was further crystallized to give needles, m.p. 204-206°. $\nu_{max.}$: 3470/3350 (NH_2), 3160 (overtone δNH), 1720 (CO_2Me), 1635 (δNH), 1600, 1578, 1440, 1500/1330 (NO_2), 1250 (C-N), 1165 (δC-H), 1050 (C-O), 848 (δC-H), 748. τ(100MHz, DMSO-d_6): 1.98 (1H, d, J= 9, aromatic), 2.41 (1H, dd, J= 14, 2, aromatic), 2.97 (1H, dd, J= 9, 2, aromatic), 1.8-2.8 (2H, br, NH_2), 6.16 (3H, s, OCH_3). τ(acetone-d_6): 7.4 (2H, br, exchanged, NH_2).

Esterification using boron trifluoride - diethyletherate complex or concentrated sulphuric acid as catalysts instead of hydrogen chloride gave identical results though possibly slightly improved yields.

Methyl 5-N-acetylamino-2-nitrobenzoate (376)

Methyl 5-amino-2-nitrobenzoate (500 mg) was dissolved in glacial acetic acid (2 ml) and acetic anhydride (1 ml) and heated on a steam bath for 5 h. It was then poured on ice forming cream crystals of the crude title compound. It was redissolved in ether, neutralized, washed with water, dried and condensed to give an oil which was purified by preparative t.l.c. (CH_2Cl_2) giving an oil which on being seeded with crystals of the crude product gave cream crystals (353 mg) of the N-acetyl derivative, m.p. 108.5-109.5°. Recrystallization from hot benzene gave white platelets m.p. 108.5-109° (lit.,[527] m.p. 111-112°). $\nu_{max.}$: 3295 (NH), 1735 (NCOMe), 1687 (CO_2Me), 1595, 1560, 1515 (NO_2), 1445, 1355 (NO_2), 1300, 1270, 1245, 1145, 1080, 990, 920, 865/855, 825, 775, 725. τ: 1.6-2.3 (1H, br, exchanged, NH), 1.9-2.2 (3H, m, aromatic), 6.03 (3H, s, OCH_3), 7.77 (3H, s, $NCOCH_3$).

Hydroxyethylation of methyl 5-amino-2-nitrobenzoate (108, 154, 164, 227, 310, 530, 552, 595)

Chromatographically pure methyl 5-amino-2-nitrobenzoate (2 g) was dissolved in 75% aqueous acetic acid (40 ml). Liquid ethylene oxide (3 ml) was added and the solution was stirred in a sealed flask at room temperature for 24 h. Then further liquid ethylene oxide (1 ml) was added and stirring was continued for another 24 h. The solvents were removed under reduced pressure leaving a red oil which was neutralized, extracted into ether, dried and condensed, leaving a brownish-yellow oil which darkened on standing. Dry column chromatography (ether) gave three bands. The fastest moving band gave yellow prisms of starting material (800 mg, 40%), m.p. 100-101°. The second fastest band gave, after further purification by t.l.c. (ethyl acetate), an orange-yellow viscous oil of methyl 5-(2-hydroxyethylamino)-2-nitrobenzoate (Found: C, 49.79; H, 5.22; N, 11.39. $C_{10}H_{12}N_2O_5$ requires C, 50.00; H, 5.04; N, 11.66%). $\nu_{max.}$(film): 3450 (OH), 3355 (NH), 2950/2880 (C-H), 1725 (CO_2Me), 1603, 1595, 1530 (NO_2), 1500, 1380 (NO_2), 1326, 1265, 1145, 1070 (C-O), 985, 880, 855, 770. τ: 2.02 (1H, d, J_o= 10, aromatic), 3.40 (1H, d, J_m= 3, aromatic), 3.40 (1H, dd, $J_{o,m}$= 10, 3, aromatic), 4.57 (1H, br, exchanged, NH), 6.07 (3H, s, OCH_3), 6.22 (2H, t, $C\text{-}CH_2\text{-}O$), 6.63 (2H, t, $N\text{-}CH_2\text{-}C$), 7.35 (1H, br, exchanged, OH).

The slow moving band was still impure. Preparative t.l.c. (ethyl acetate) gave further monohydroxyethyl derivative plus a yellow-brown oil which was purified several times by t.l.c. to give a very small amount of a yellow-orange oil of methyl 5-(di-2-hydroxyethylamino)-2-nitrobenzoate. $\nu_{max.}$ (film): 3645-3095 (br, OH), 2950/2895 (CH_2), 1732 (CO_2Me), 1595, 1578, 1500 (NO_2), 1445, 1320 (NO_2), 1270, 1140, 1060, 740. τ: 2.07 (1H, d, aromatic), 3.21-3.47 (2H, m, aromatic), 5.94-7.17 (2H, br, exchanged, OH), 6.10 (3H, s, OCH_3), 6.14-6.37 (8H, m, NCH_2CH_2O). Identification by microanalysis was unsuccessful due to the great difficulty in separating the diol from traces of impurities, particularly of the monohydroxyethyl derivative and of the condensation product of acetic acid and ethylene oxide. It was however characterized as the di-2-chloroethylamino derivative (see below).

In several preparations a high excess of liquid ethylene oxide was used and resulted in the condensation of this excess with acetic acid, making purification of the hydroxyethylated products extremely difficult. This condensation product was only partially removed by distillation of the oily products giving a clear non-aromatic distillate, b.p. 58° at 5×10^{-3} mmHg.

Hydroxyethylation of methyl 5-amino-2-nitrobenzoate in dioxan or benzene in a sealed Carius tube at 140° and 110° respectively for over 24 h gave some monohydroxyethylamino derivative but mainly a red oil which when chlorinated in benzene with phosphoryl chloride afforded only traces of the nitrogen mustard.

Hydroxyethylation of methyl 5-(2-hydroxyethylamino)-2-nitrobenzoate (432, 448, 482)

Methyl 5-(2-hydroxyethylamino)-2-nitrobenzoate (1 g) was dissolved in dioxan (3 ml) and freshly redistilled liquid ethylene oxide (3 ml) was added. The solution was placed in a Carius tube which was then sealed and carefully heated at 140° for 16 h, after which it was cooled, opened and the contents condensed and chromatographed (preparative t.l.c. - ether) to give starting material (328 mg, 33%), a small amount of diol (118 mg, 10%) identified from spectral data, and a fraction of highest R_f which gave, on further purification by t.l.c. (dichloromethane), and recrystallization from dichloromethane-petroleum, orange-yellow crystals of 1,2,3,5-tetrahydro-1-(2-hydroxy-

ethyl)-5-keto-11-nitro-6,8-benz-1,4-oxazocine (110 mg, 10.5%), m.p. 143-144° (Found: C, 52.69; H, 4.69; N, 11.07. $C_{11}H_{12}N_2O_5$ requires C, 52.38; H, 4.80; N, 11.11%). $\nu_{max.}$: 3375 (OH), 1735 (CO), 1610, 1588, 1500 (NO_2), 1325 (NO_2), 1298, 1275, 1195, 1140, 1115, 1070, 975, 945, 870, 847, 815, 805, 760. τ: 1.90 (1H, d, J_o= 9.8, aromatic), 3.52 (1H, d, J_m= 3, aromatic), 3.52 (1H, dd, $J_{o,m}$= 9.8, 3, aromatic), 5.03 (2H, s, N-C-CH_2-O-CO), 5.73 (2H, t, C-CH_2-O), 6.03 (2H, s, N-CH_2-C-O-CO), 6.08 (1H, br, exchanged, OH), 6.47 (2H, t, N-CH_2-C). m/e: 252(P), 251, 222, 207, 206, 194, 176, 163, 147, 134, 133, 118, 117, 106, 91, 89, 75. A sample was refluxed in methanol saturated with hydrogen chloride for 1 h, then condensed, neutralized and chromatographed giving a low R_f band with spectral features identical to those of the diol. Microanalysis of this diol, however, failed once more to give acceptable figures.

Methyl 5-(di-2-chloroethylamino)-2-nitrobenzoate (503)

The various small amounts of methyl 5-(di-2-hydroxyethylamino)-2-nitrobenzoate obtained from the hydroxyethylation reactions and from the ring cleavage of the benzoxazocine derivative were combined (0.5 g) and dissolved in benzene (3 ml). Phosphoryl chloride (1 g) in benzene (3 ml) was added dropwise and the mixture refluxed until no more evolution of gas was noticed. The solution was then neutralized with ammonia (d 0.88), extracted with ether, washed, dried and chromatographed (preparative t.l.c. - dichloromethane). The fraction of highest R_f gave a yellow solid which was recrystallized from dichloromethane-petroleum giving yellow crystals of the title compound, m.p. 89-91° (Found: C, 44.79; H, 4.52; N, 8.45. $C_{12}H_{14}Cl_2N_2O_4$ requires C, 44.88; H, 4.39; N, 8.72%). τ: 1.93 (1H, d, J_o= 10, aromatic), 3.25 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 3.30 (1H, d, J_m= 3, aromatic), 6.06 (3H, s, OCH_3), 6.22 (8H, q, NCH_2CH_2Cl).

4-Nitroanthranilic acid (250)

4-Nitroanthranilic acid was obtained from Aldrich Chemical Co. Inc. in 97% purity, m.p. 264° [lit.,[527] m.p. 264°(dec.)]. $\nu_{max.}$: 3490/3380 (NH_2), 3070 (br, OH), 2730-2550 (CO_2H overtones), 1678 (CO_2H), 1572, 1525/1362 (NO_2), 1322, 1260 (s), 898, 838, 745. τ(DMSO-d_6): 2.03 (1H, d, J_o= 9, aromatic), 2.30 (1H, d, J_m= 2.5, aromatic), 2.74 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 3.06 (3H, v br 1.5-4.7, exchanged, OH, NH_2).

Methyl 4-nitroanthranilate (251, 263, 265)

4-Nitroanthranilic acid (35 g) was dissolved in methanol (300 ml) and concentrated sulphuric acid (35 ml) was added slowly. The resulting solution was refluxed for 24 h, then concentrated under reduced pressure, neutralized, extracted into ether, washed, dried and condensed to give the ester as an orange solid (35.6 g, 96%). Recrystallization from dichloromethane-petroleum gave fine orange needles of the title compound, m.p. 156-157° (lit.,[532] m.p. 156-157°) R_f (ethyl acetate) 0.78. Very soluble in 1,4-dioxan and DMSO, moderately soluble in methanol and acetic acid, but only slightly soluble in dichloromethane, chloroform, ether or benzene and insoluble in petroleum. $\nu_{max.}$: 3460/3360 (NH_2), 1700 (CO_2Me), 1640 (δNH), 1625, 1588, 1520/1355 (NO_2), 1310 (C-N), 1260 (δC-H), 1200 (C-O), 1095, 880, 835 (δNH), 745 (δC-H). τ(acetone-d_6): 2.03 (1H, d, J_o= 9, aromatic), 2.30 (1H, d, J_m= 2, aromatic), 2.73 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 3.13 (2H, br, exchanged, NH_2), 6.1 (3H, s, OCH_3).

Hydroxyethylation of methyl 4-nitroanthranilate

(a) In aqueous acetic acid at room temperature (255, 273)

Methyl 4-nitroanthranilate (2 g) was partly dissolved in a mixture of glacial acetic acid (50 ml) and water (25 ml), cooled to 0°, liquid ethylene oxide (25 ml) added, and the solution was stirred vigorously for 48 h. The excess solvents were removed under reduced pressure and the residue was neutralized giving an orange precipitate which was filtered and chromatographed by the dry column technique (ether). The highest R_f band gave orange crystals of the starting material (1 g, 50%), m.p. 145-150°.

$\nu_{max.}$ and τ identical to that of an authentic sample. The second highest R_f band gave orange-yellow crystals, m.p. 108-111°. Recrystallization from dichloromethane gave orange-yellow needles of methyl (N-2-hydroxyethyl)-4-nitroanthranilate (600 mg, 25%), m.p. 112-113.5° (Found: C, 50.20; H, 5.30; N, 11.62. $C_{10}H_{12}N_2O_5$ requires C, 50.00; H, 5.03; N, 11.66%). $\nu_{max.}$: 3360 (NH), 3450-3200 (H-bonded OH), 1700 (CO_2Me), 1540/1360 (NO_2), 1290 (C-N), 1270 (δC-H), 1240 (C-O), 840 (δ N-H), 760 (δ C-H). τ: 1.87 (1H, br, exchanged, NH), 1.93 (1H, d, J_o= 9, aromatic), 2.43 (1H, d, J_m= 2, aromatic), 2.63 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 6.07 (2H, m, C-CH_2-O), 6.10 (3H, s, OCH_3), 6.57 (2H, t, N-CH_2-C), 7.73 (1H, br, exchanged, OH).

(b) In acetic acid-dioxan at room temperature (300)

Methyl 4-nitroanthranilate (2 g) was dissolved in glacial acetic acid - dioxan (1:1, 40 ml), liquid ethylene oxide (10 ml) was added, and the solution stirred at room temperature for 72 h. The reaction products were then worked up as above to give starting material (1.35 g, 67%), m.p. 155-157°, spectral data identical to that of an authentic sample; and the monohydroxyethyl product (810 mg, 33%), m.p. 111-113°, $\nu_{max.}$ and τ identical to those of an authentic sample.

(c) In dioxan at 150° (330)

Methyl 4-nitroanthranilate (2 g) and dioxan (5 ml) were placed in a Carius tube, liquid ethylene oxide (2 ml) was added and the tube was sealed and heated at 150-155° for 16 h. On cooling, the dark-red liquid was condensed and chromatographed (dry column - dichloromethane) to give starting material, recrystallized from dichloromethane-petroleum, m.p. 155-157°; and yellow-orange needles of the N-2-hydroxyethyl derivative (500 mg, 21%), m.p. 108-110° (dichloromethane-petroleum). The n.m.r. spectra of the above products corresponded to those of authentic samples.

Methyl 2,4-diaminobenzoate (333, 340)

Methyl 4-nitroanthranilate (10 g) was added to a vigorously stirred, cooled solution of stannous chloride (35 g) and concentrated hydrochloric acid (50 ml). After a few minutes, the orange colour of the nitro compound disappeared. It was stirred for 2 h, then heated on a steam bath, cooled, neutralized with 10 M aqueous sodium hydroxide (60 ml) followed by aqueous sodium hydrogen carbonate, and extracted with ether. The ethereal layer was washed, dried and condensed to give an off-white solid. Recrystallization from ether-petroleum gave fine white needles of the title compound (7.4 g, 87%), m.p. 110-111° (Found: C, 58.00; H, 6.10; N, 16.94. $C_8H_{10}N_2O_2$ requires C, 57.82; H, 6.07; N, 16.86%). $\nu_{max.}$: 3410/3320/3220 (NH_2 groups - one more H-bonded), 1660 (CO_2Me), 1625 (δNH), 1585, 1558, 1445, 1320 (C-N), 1270 (C-O), 1202, 1175, 1122, 867/853, 792. τ: 2.32 (1H, d, J_o= 9, aromatic), 4.03 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 4.13 (1H, d, J_m= 2, aromatic); 5.3 (4H, br s, exchanged, NH_2), 6.18 (3H, s, OCH_3).

Methyl 4-phthalimidoanthranilate (350, 360)

Methyl 2,4-diaminobenzoate (1.66 g, 10 mmol) and phthalic anhydride (1.48 g, 10 mmol) were partly dissolved in toluene (20 ml) and triethylamine (0.5 ml) was added. The mixture was refluxed in a Dean and Stark apparatus for 2¼ h, removing the water produced, resulting in a homogeneous solution which was cooled, depositing tan-coloured crystals (2.05 g) m.p. 205-208°, insoluble in cold ethyl acetate, acetone, methanol or petroleum but soluble in DMSO and hot dichloromethane, chloroform or benzene. Recrystallization from hot chloroform-petroleum gave cream-white needles of the title compound (70%), m.p. 207-208° (Found: C, 64.65; H, 4.19; N, 9.44. $C_{16}H_{12}N_2O_4$ requires C, 64.86; H, 4.08; N, 9.46%). $\nu_{max.}$: 3442/3342 (NH_2), 1725/1688 (phthalimide C=O), 1670 (CO_2Me), 1620, 1585, 1445, 1380, 1310 (C-N), 1255 (δC-H), 1205, 1120 (C-O), 1095, 1075, 898, 880, 790, 730. τ: 1.9-2.3 (5H, m, aromatic), 3.0-3.3 (2H, m, aromatic), 4.5 (2H, br, exchanged, NH_2), 6.09 (3H, s, OCH_3).

Hydroxyethylation of methyl 4-phthalimidoanthranilate

(a) In acetic acid-dioxan mixture at room temperature (356)

Methyl 4-phthalimidoanthranilate (458 mg) was partly dissolved in glacial acetic acid (10 ml) and dioxan (10 ml). Liquid ethylene oxide (2 ml) was added, the flask sealed and the contents stirred vigorously at room temperature for 48 h, resulting in a homogeneous solution which was condensed, neutralized and extracted with dichloromethane. The organic layer was washed with aqueous sodium hydrogen carbonate and water, dried, and condensed to give an orange-yellow solid. Recrystallization from hot dichloromethane-petroleum gave golden yellow needles (266 mg), m.p. 180-182°. They were redissolved in dichloromethane (charcoal) and further recrystallized to give chromatographically pure cream-yellow needles of methyl 2-(2-hydroxyethylamino)-4-phthalimidobenzoate (51%), m.p. 185-186°. (Found: C, 63.40; H, 4.60; N, 8.11. $C_{18}H_{16}N_2O_5$ requires C, 63.53; H, 4.74; N, 8.23%). $\nu_{max.}$: 3450 (OH), 3340 (NH), 1715 (phthalimide C=O), 1680 (CO_2Me), 1580, 1520, 1445, 1380, 1274, 1130, 1090, 1075, 895, 855, 780, 730. τ: 1.7 - 2.4 (5H, m, aromatic), 3.0-3.4 (2H, m, aromatic), 6.09 (3H, s, OCH_3), 6.13 (2H, t, $C\text{-}CH_2\text{-}O$), 6.57 (2H, t, $N\text{-}CH_2\text{-}C$), 8.27 (2H, br, exchanged, NH, OH).

(b) In benzene at 150° (370)

Methyl 4-phthalimidoanthranilate (740 mg) was dissolved in dry benzene (3 ml) and sealed in a Carius tube with liquid ethylene oxide (1 ml). The tube was heated slowly at 150° for 16 h. The contents of the tube were then dissolved in dichloromethane, filtered and crystallized to give only starting material, identified from its R_f and its identical n.m.r. spectrum to that of an authentic sample.

Methyl 2-N-acetylamino-4-nitrobenzoate (367, 388, 464)

Methyl 4-nitroanthranilate (25 g) was partly dissolved in pyridine (100 ml) and acetic anhydride (70 ml) and the mixture was stirred and heated on a steam bath for 5 h. The resulting clear solution was condensed under reduced pressure, redissolved in dichloromethane (300 ml), washed with aqueous sodium carbonate (300 ml) and water, dried, decolourized (charcoal) and partly condensed.

Addition of petroleum precipitated yellow crystals (12.31 g) identified as the title compound. The filtrate was condensed to give a dark brown oil (16.6 g) which was treated with 10% aqueous hydrochloric acid, then extracted into ether, neutralized, washed with water, dried and partly condensed to give more tan-yellow crystals (8 g). Recrystallization from dichloromethane-petroleum gave yellow crystals of the title compound (67%), m.p. 145-147° (lit.,[533] m.p. 144°). $\nu_{max.}$: 3265 (NH), 3120, 1710/1695 ($COCH_3$, CO_2Me), 1602, 1542 (NO_2), 1430, 1358 (NO_2), 1330, 1280, 1265, 1240, 1155, 1115, 1080, 1022, 960, 910, 835, 805/798, 755.

Methyl 2-N-acetyl-2,4-diaminobenzoate (427)

Methyl 2-N-acetylamino-4-nitrobenzoate (12 g) was dissolved in methanol (300 ml), 10% palladium-charcoal (1.2 g) was added and the mixture hydrogenated in an autoclave at 25 atm. and 50° but the temperature was allowed to drop slowly overnight, resulting in the deposition of white crystals on the sides of the vessel (2.2 g), m.p. 213-218° (very slightly contaminated with palladium-charcoal). The methanolic filtrate was condensed to give slightly yellowish crystals (7.15 g) identical in their i.r. spectrum to the white crystals. Recrystallization from boiling chloroform gave very light yellow translucent crystals of the title compound (87%), m.p. 215-216° (dec.) (Found: C, 57.54; H, 5.63; N, 13.48. $C_{10}H_{12}N_2O_3$ requires C, 57.69; H, 5.81; N, 13.45%). $\nu_{max.}$: 3420/3340 (NH_2), 3235 (NH), 1688/1660 (COMe), 1650 (CO_2Me), 1620, 1582, 1532, 1440, 1350, 1292, 1280, 1200, 1162, 1098, 1025, 970, 885, 795. τ(DMSO-d_6): -1.34 (1H, br, not exchanged, NH strongly H-bonded), 1.96 (1H, d, J_m= 2.5, aromatic), 2.16 (1H, d, J_o= 9, aromatic), 3.64 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 6.10 (3H, s, OCH_3), 6.42 (2H, br, exchanged, NH_2), 7.8 (3H, s, $NCOCH_3$).

Methyl 2-N-acetyl-4-(NN-di-2-hydroxyethyl)-2,4-diaminobenzoate (441, 487)

Methyl 2-N-acetyl-2,4-diaminobenzoate (1.03 g) was suspended in dioxan (15 ml) and acetic acid (15 ml) in a flask; freshly redistilled liquid ethylene oxide (4 ml) was added, the flask sealed and the contents stirred vigorously for 4 days. The excess solvents were then removed under reduced pressure, and the residue was

neutralized, extracted into dichloromethane, washed with water, dried and chromatographed (preparative t.l.c. - ethyl acetate) to give three bands. The fraction of highest R_f gave a yellow oil which crystallized and was recrystallized from dichloromethane-petroleum giving cream-white crystals of an unknown compound (46.4 mg) m.p. 125-127°. The fraction of medium R_f gave a cream-white solid, m.p. 126-129° which was recrystallized from dichloromethane-petroleum affording white crystals of methyl 2-N-acetyl-4-(N-2-hydroxyethyl)-2,4-diaminobenzoate (401.2 mg, 32%), m.p. 130-131° (Found: C, 55.18; H, 6.01; N, 10.27. $C_{12}H_{16}N_2O_4.\frac{1}{6}CH_2Cl_2$ requires C, 55.19; H, 6.13; N, 10.44%). $\nu_{max.}$: 3392(NH), 3320 (NH), 3150 (br, OH), 1675 (sh.) (CO_2Me), 1655 (NCOMe), 1630, 1555, 1440, 1360, 1305, 1278, 1260, 1195, 1165, 1110, 1060, 970, 890, 852, 815. τ: -1.38 (1H, br, not exchanged, NH), 1.92 (1H, d, J_m= 2.5, aromatic), 2.12 (1H, d, J_o= 9, aromatic), 3.80 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 6.12 (3H, s, OCH_3), 6.22 (2H, t, C-CH_2-O), 6.63 (2H, t, N-CH_2-C), 7.77 (3H, s, C-CH_3). The fraction of low R_f gave an oil which crystallized to a cream-white solid (854 mg). Recrystallization from dichloromethane-petroleum gave white needles, m.p. 111-114°. Purification by preparative t.l.c. and recrystallization gave white needles of the title compound (58%), m.p. 118-120° (Found: C, 56.88; H, 6.60; N, 9.41. $C_{14}H_{20}N_2O_5$ requires C, 56.75; H, 6.80; N, 9.45%). $\nu_{max.}$: 3275 (br, OH), 3220 (NH), 1680 (CO_2Me), 1658 (NCOMe), 1620, 1578, 1530, 1440 (sh.), 1340, 1310, 1295, 1280, 1240, 1200, 1165, 1125, 1085, 1060, 1010, 960, 855, 840, 830, 780, 730, 710. τ: -1.3 (1H, br, unexchanged, NH), 1.93 (1H, d, J_m= 2.5, aromatic), 2.17 (1H, d, J_o= 9, aromatic), 3.67 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 5.9 (2H, br, exchanged, OH), 6.14 (3H, s, OCH_3), 6.17-6.4 (8H, m, NCH_2CH_2O), 7.83 (3H, s, C-CH_3).

Methyl 2-N-acetyl-4-(NN-di-2-chloroethyl)-2,4-diaminobenzoate

(a) Chlorination with thionyl chloride in benzene (470)

Methyl 2-N-acetyl-4-(NN-di-2-hydroxyethyl)-2,4-diaminobenzoate (250 mg) was dissolved in hot benzene (3 ml) and refluxed. Thionyl chloride (1 ml) in dry benzene (2 ml) was added dropwise (7 min) and the solution refluxed for a further 1 h. The benzene solution was then diluted with dichloromethane (50 ml), the solution was neutralized, washed with water (2 x 50 ml), dried and condensed giving an oil which was purified by preparative t.l.c. (ether). The highest R_f band

gave a yellow oil (132.3 mg) which was purified further by t.l.c. (ether) to give a very light yellow oil (110 mg) which crystallized on trituration with petroleum to white crystals, m.p. 59-63°. Recrystallization from hot petroleum gave white crystals of the title compound (39%), m.p. 72-73° (Found: C, 50.51; H, 5.35; N, 8.25; Cl, 21.56. $C_{14}H_{18}Cl_2N_2O_3$ requires C, 50.46; H, 5.44; N, 8.41; Cl, 21.28%). $\nu_{max.}$ ($CHCl_3$): 3280 (NH), 3002/2960 (CH_2), 1680 (sh.) (CO_2Me), 1665 (NCOMe), 1620, 1570, 1525, 1488, 1460, 1440, 1398, 1360, 1340, 1280, 1200, 1173, 1113, 900 (s), 862, 708, 656. τ: -1.28 (1H, br, not exchanged, NH), 1.74 (1H, d, J_m= 2.5, aromatic), 2.08 (1H, d, J_o= 9, aromatic), 3.63 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 6.12 (3H, s, OCH_3), 6.22 (8H, t, NCH_2CH_2Cl), 7.78 (3H, s, $NCOCH_3$).

(b) Chlorination with mesyl chloride - pyridine (645)

Methyl 2-N-acetyl-4-(NN-di-2-hydroxyethyl)-2,4-diaminobenzoate (1.5936 g) was dissolved in dry pyridine (12 ml) and methanesulphonyl chloride (1.2 ml) was added. After a few minutes a fairly vigorous reaction was obtained and on subsiding, the mixture was refluxed for 20 min, then poured on ice (100 ml) and extracted with dichloromethane. The organic layer was washed with dilute hydrochloric acid, then neutralized, washed with water and dried to give a yellow solution (charcoal) which was condensed to give an orange-red oil. Preparative t.l.c. (CH_2Cl_2) gave a low R_f band which gave slightly pink crystals of the title compound (790 mg, 44%), m.p. 73-75°. I.r. and n.m.r. spectra were identical to those of an authentic sample.

Methyl 4-(NN-di-2-chloroethyl)-2,4-diaminobenzoate (480, 647)

The above-mentioned N-acetyl nitrogen mustard (527 mg) was refluxed in 50% w/w methanolic hydrogen chloride for 1 h under pressure. This was achieved by fixing a balloon partly filled with dry nitrogen at the open neck of the reflux apparatus. The hydrogen chloride released on heating, further inflated the balloon, thus keeping a steady pressure of hydrogen chloride during the reaction. The methanol and excess hydrogen chloride were then removed under reduced pressure leaving a white solid insoluble in dichloromethane but soluble in methanol or water. This was filtered and dried. Recrystallization from methanol-ether gave fine white needles of

the hydrochloride of the title compound (620 mg), partly melting at 97-100° with a change of crystalline shape and remelting at 135-137°. A further determination after 1 h gave m.p. 88-95° with remelting at 111-123°. $\nu_{max.}$: 3375 (associated NH_3^+), 2655-2450 (overtones NH_3^+), 1702 (CO_2Me), 1635 (δNH_3^+), 1605, 1565, 1525 (δNH_3^+), 1442, 1362, 1335 (C-N), 1302, 1280, 1205, 1182, 1115, 843, 780, 747 (C-Cl). τ(methanol-d_4): 1.82-1.92 (2H, m, aromatic), 2.92-3.13 (1H, m, aromatic), 4.85 (s, exchanged, NH_2, HCl plus OH in methanol), 6.03 (s, OCH_3), 6.10 (t, NCH_2CH_2Cl). Characterized as the N-2'-chlorophenylcarbamate (see below).

2-Chlorophenyl chloroformate (518)

2-Chlorophenol (18.42 g, 144 mmol) was dissolved in dry benzene (50 ml) and quinoline (18.72 g, 144 mmol) was added. This solution was added dropwise to an ice-cooled solution of excess phosgene in benzene (100 ml) with stirring. Stirring was maintained for 3 h. Nitrogen was then passed through the solution to remove any excess phosgene, and the solution was filtered to remove the quinoline hydrochloride. The filtrate was condensed to give a yellow oil which was distilled to give the title compound as a colourless liquid (10.43 g, 38%), b.p. 61-62° at 0.3mmHg, n_D^{15} 1.5318 (lit.,[412] b.p. 94° at 15mmHg, n_D^{23} 1.5238). $\nu_{max.}$(film): 3520 (small), 1782 (OCOCl), 1600/1588, 1480, 1458, 1220, 1147, 1122 (Ar-Cl), 1075, 885, 850 (C-Cl), 758, 710, 695/685. τ: 1.87-3.37 (m, aromatic, strong singlet at 2.65).

2'-Chlorophenyl N-[2-methoxycarbonyl-5-(di-2-chloroethylamino)]-phenylcarbamate (657)

Methyl 4-(NN-di-2-chloroethyl)-2,4-diaminobenzoate hydrochloride (610 mg) was suspended in benzene (10 ml) and dry pyridine (1 ml). 2-Chlorophenyl chloroformate (380 mg) was added, resulting in a vigorous reaction which soon subsided; the mixture was then refluxed for 2 h. The pyridine hydrochloride formed was filtered off and the filtrate was washed with dilute hydrochloric acid, aqueous sodium carbonate, and water, dried and condensed to give an oil which crystallized from ether-petroleum to white crystals, m.p. 110-112°. Purification by t.l.c. (CH_2Cl_2) and recrystallization gave white crystals of the title compound (500 mg), m.p. 111-112.5° (Found:

C, 51.44; H, 4.23; N, 6.04; Cl, 24.16. $C_{19}H_{19}Cl_3N_2O_4$ requires C, 51.20; H, 4.30; N, 6.28; Cl. 23.86%). τ: -0.93 (1H, br, NH), 2.16 (1H, d, J_o= 9, aromatic), 2.28 (1H, d, J_o= 9, aromatic), 2.47-3.10 (4H, m, aromatic), 3.69 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 6.13 (3H, s, OCH_3), 6.27 (8H, t, NCH_2CH_2Cl).

AMINOPHENYL METHOXY NITROGEN MUSTARDS

2-Methoxy-4-nitroaniline

It was obtained from Ralph N. Emanuel Ltd. $\nu_{max.}$: 3450/3330 (NH_2), 1622 (δNH), 1582, 1520/1318 (NO_2), 1300, 1280 (C-N), 1240 (C-O-C), 1162 (C-N), 1112, 1085, 1040 (C-O-C), 950, 879 (C-H), 825 (C-N), 802 (C-H), 762, 740.

Hydroxyethylation of 2-methoxy-4-nitroaniline

(a) In aqueous acetic acid at room temperature (302, 322)

2-Methoxy-4-nitroaniline (2 g) was suspended in a mixture of water (8 ml) and glacial acetic acid (26 ml), liquid ethylene oxide (4 ml) was added, the container was sealed and the mixture vigorously stirred for 52 h. The solvents were then removed and the residual oil was neutralized, extracted into ethyl acetate, washed, dried, decolourized (charcoal) and condensed to give a yellowish brown oil. Trituration with cold dichloromethane-petroleum gave a sticky solid which was filtered off. This was redissolved in dichloromethane but addition of petroleum precipitated a red oil. Petroleum was added till no more red oil was precipitated. The petroleum solution was then left standing when orange crystals, m.p. 85-87°, were obtained. Repeated recrystallization from dichloromethane-petroleum gave orange crystals of 2-methoxy-4-nitro-(N-2-hydroxyethyl)aniline (500 mg, 20%), m.p. 87-88° (Found: C, 50.86; H, 5.80; N, 13.17. $C_9H_{12}N_2O_4$ requires C, 50.94; H, 5.70; N, 13.20%). $\nu_{max.}$: 3360 (OH), 3250(NH), 1600, 1542 (NO_2), 1500, 1480 (sh.), 1440 (sh.), 1340 (NO_2), 1300, 1260, 1240, 1215, 1160, 1105, 1090, 1070 (C-O), 1035, 875, 810, 760, 740. τ: 2.08 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 2.36 (1H, d, J_m= 2.5, aromatic), 3.46 (1H, d, J_o= 9, aromatic), 4.60 (1H, br, exchanged, NH), 6.06 (3H, s, OCH_3), 6.09 (2H, t, C-CH_2-O), 6.57 (2H, t, N-CH_2-C), 7.93 (variable) (1H, br, exchanged, OH).

Using glacial acetic acid-dioxan (3:2) as solvent and stirring for 60 h gave, on working up the products as above, the starting material (approx. 60%) and the monohydroxyethyl derivative (32%), m.p. 85-87°.

(b) In dioxan at 150° (477)

2-Methoxy-4-nitroaniline (1 g) was dissolved in dioxan (3 ml) in a Carius tube. Freshly redistilled liquid ethylene oxide (2 ml) was added; the tube was sealed and heated at 150° for 16 h. The tube was then opened and the contents condensed and chromatographed (preparative t.l.c.-dichloromethane). The high R_f fraction gave yellow crystals of starting material (718 mg, 72%), m.p. 137-139° (lit.,[539] m.p. 139-140°). The low R_f fraction gave an orange oil which crystallized to orange crystals of the monohydroxyethyl derivative (311 mg, 25%), m.p. 86-88°. Recrystallization from dichloromethane-petroleum gave crystals, m.p. 88-89°. A mixed m.p. with the product obtained from the hydroxyethylation reaction at room temperature was undepressed.

N-Acetyl-2-methoxy-4-nitroaniline (383, 463, 468)

2-Methoxy-4-nitroaniline (40 g) was dissolved in pyridine (100 ml) and acetic anhydride (100 ml) and heated in boiling water for 6 h. The excess solvents were then removed under reduced pressure and the residue washed with 10% dilute hydrochloric acid (200 ml), extracted with dichloromethane, separated, and the organic layer was neutralized, then washed with water (2 x 250 ml), decolourized (charcoal), dried, and condensed to give orange-tan crystals. Recrystallization from dichloromethane-petroleum gave orange-tan crystals (48 g), m.p. 147-152°. T.l.c. (ethyl acetate) gave two spots. $\nu_{max.}$: 1700/1665 (NCOMe). τ: 5.98/6.03 (two singlets, OCH_3), 7.72 (s, $NCOCH_3$). The singlet at 7.72τ had 1.43 times the number of protons as the two singlets at around 6τ combined. Slow recrystallization (12 h) from a very concentrated solution in dichloromethane gave salmon-coloured platelets of pure N-acetyl-2-methoxy-4-nitroaniline (22 g, 44%), m.p. 155-157° (lit.,[540] m.p. 157°). R_f (petroleum-dichloromethane, 2:1): 0.37. $\nu_{max.}$: 3260 (NH), 1675 (NCOMe), 1625 (w), 1590, 1552 (NO_2), 1515, 1495, 1417, 1365 (sh.), 1345 (NO_2), 1295, 1260, 1235, 1200 (w), 1145, 1110, 1030, 1015, 975 (w), 935 (w), 885, 840 (w), 810, 750, 735 (w). τ: 1.34 (1H, d, J_o= 9, aromatic), 1.95 (1H, br, exchanged, NH), 2.07 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 2.23 (1H, d, J_m= 2.5, aromatic), 5.98 (3H, s, OCH_3), 7.73 (3H, s, $NCOCH_3$).

The dichloromethane mother-liquor was evaporated to dryness and

methanol was added. A dark-coloured solution was obtained which immediately precipitated yellowish-white crystals (17.5 g), m.p. 115-130°. Recrystallization from dichloromethane gave slightly yellowish prisms of NN-diacetyl-2-methoxy-4-nitroaniline (14 g, 22%), m.p. 136-137°. R_f(petroleum-dichloromethane, 2:1) : 0.54. A small sample was purified by t.l.c. and recrystallized to give white needles of the diacetyl derivative, m.p. 136-137° (Found: C, 52.27; H, 4.82; N, 11.00. $C_{11}H_{12}N_2O_5$ requires C, 52.38; H, 4.80; N, 11.11%). $\nu_{max.}$: 3100 (sharp), 1705 (vs)(NCOMe), 1622 (w), 1595, 1540 (NO_2), 1500, 1450, 1420, 1378, 1358 (NO_2), 1330, 1308, 1275 (sh.), 1258 (vs), 1220, 1135, 1108, 1032/1027, 960, 890, 812, 740. τ: 2.01 (1H, dd, $J_{o,m}$= 8.5, 2.5, aromatic), 2.08 (1H, s, aromatic), 2.65 (1H, d, J_o= 8.5, aromatic), 6.03 (3H, s, OCH_3), 7.72 (6H, s, $NCOCH_3$).

In one preparation the nitroaniline was dissolved in pyridine and acetic anhydride and stirred overnight at room temperature prior to heating in boiling water for 6 h. The product obtained was mainly the monoacetyl derivative (94%), m.p. 153-154° but an i.r. spectrum indicated trace amounts of the diacetyl derivative.

N^1-Acetyl-2-methoxy-1,4-phenylenediamine (452)

N-Acetyl-2-methoxy-4-nitroaniline (20 g) was dissolved in methanol (300 ml), 10% palladium-charcoal (2 g) was added and the mixture hydrogenated in an autoclave at 50° and 25 atmospheres overnight. The contents were then filtered and condensed to give a dark red oil of the title compound (15 g) (lit.,[540] m.p. 119-120°,[541] m.p. 72°). It was used directly for the next stage.

Hydroxyethylation of N^1-acetyl-2-methoxy-1,4-phenylenediamine (453)

The product from the above reaction (approximately 15 g) was dissolved in glacial acetic acid (100 ml) and dioxan (60 ml). Freshly redistilled liquid ethylene oxide (37 ml) was added and the solution stirred at room temperature in a sealed container for 36 h. The excess solvents were removed under reduced pressure, neutralized, washed dried and condensed to give an oil which deposited crystals on standing for 4 weeks. These were filtered and recrystallized from ethanol giving white crystals m.p. 123-125°. Preparative t.l.c. (acetone)

gave white crystals of N^1-acetyl-N^4-(2-hydroxyethyl)-2-methoxy-1,4-phenylenediamine, m.p. 220-222° (Found: C, 59.26; H, 7.13; N, 12.61. $C_{11}H_{16}N_2O_3$ requires C, 58.98; H, 7.19; N, 12.49%). $\nu_{max.}$: 3350 (br, OH), 3260 (NH), 1670 (sh.), 1658 (NCOMe), 1618, 1540, 1425, 1305, 1280 (C-N), 1260 (C-O), 1205, 1180, 1140, 1070, 1030, 970, 940, 880, 845, 820, 795, 730. τ(acetone-d_6): 2.07 (1H, d, J_o= 9, aromatic), 2.9-3.9 (2H, m, aromatic), 5.3 (2H, br, exchanged, NH), 6.12 (3H, s, OCH_3), 6.15-6.60 (4H, two t, NCH_2CH_2O), 6.15-6.6 (1H, br, exchanged, OH), 7.83 (3H, s, $NCOCH_3$).

4-Methoxy-2-nitroaniline

It was obtained from Aldrich Chemical Co. Inc. (lit.,[546] m.p. 125-126°). $\nu_{max.}$: 3455/3350 (NH_2), 1641 (δNH_2), 1595, 1572, 1508 (NO_2), 1423, 1342 (NO_2), 1285, 1258, 1220, 1187 (sh.), 1165, 1100, 1030, 895 (br), 850, 825, 792, 767. τ: 2.4 (1H, d, J_m= 3, aromatic), 2.89 (1H, dd, $J_{o,m}$= 9.5, 3, aromatic), 3.2 (1H, d, J_o= 9.5, aromatic), 4.03 (2H, br, exchanged, NH_2), 6.2 (3H, s, OCH_3).

Hydroxyethylation of 4-methoxy-2-nitroaniline

(a) In aqueous acetic acid at room temperature (286, 290, 320)

4-Methoxy-2-nitroaniline (2 g) was dissolved in glacial acetic acid (26 ml) and water (8 ml) and liquid ethylene oxide (2 ml) was added. The container was sealed and the contents stirred vigorously at room temperature for 60 h. The solvents were then removed under reduced pressure and the residual red oil was neutralized, extracted into ethyl acetate, dried and condensed to give a red oil which solidified to a red solid. Recrystallization from dichloromethane-petroleum gave fine orange needles of (N-2-hydroxyethyl)-4-methoxy-2-nitroaniline (2.25 g, 89%), m.p. 89-91°. Further recrystallization gave needles m.p. 90-92° (Found: C, 51.00; H, 5.90; N, 13.07. $C_9H_{12}N_2O_4$ requires C, 50.94; H, 5.70; N, 13.20%). $\nu_{max.}$: 3350 (NH), 3200 (OH), 1580, 1530 (NO_2), 1410, 1355 (NO_2), 1322, 1300, 1280, 1242, 1222, 1162, 1070, 1055, 925, 910, 861, 838, 822, 790, 778, 740. τ: 1.8 (1H, br, exchanged, NH), 2.35 (1H, d, J_m= 2.5, aromatic), 2.77 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 3.03 (1H, d, J_o= 9, aromatic), 6.03 (2H, t, C-CH_2-O), 6.20 (3H, s, OCH_3), 6.43 (2H, t, N-CH_2-C), 7.95 (1H, br, exchanged, OH).

A larger scale preparation gave some starting material as well, making crystallization possible only by seeding with authentic crystals of monohydroxyethyl derivative.

(b) In dioxan at 150° (337, 400)

4-Methoxy-2-nitroaniline (5 g) was placed in a Carius tube and dioxan (5 ml) and liquid ethylene oxide (5 ml) were added and the tube sealed and heated at 150° for 16 h. The contents were then condensed and chromatographed (dry column-ether). In order of decreasing R_f, the products obtained were starting material (791 mg, 16%), m.p. 124-126° (lit.,[546] m.p. 125-126°), orange red needles of the N-2-hydroxyethyl derivative (1.145 g, 18%), m.p. 88-91°. $\nu_{max.}$ and τ identical to those of an authentic sample; a scarlet-red oil of [N-2-(2-hydroxyethyl)oxyethyl]-4-methoxy-2-nitroaniline (380 mg, 5%) (Found: C, 51.37; H, 6.27; N, 11.00. $C_{11}H_{16}N_2O_5$ requires C, 51.56; H, 6.29; N, 10.93%). $\nu_{max.}$(film): 3420 (br, OH), 3360 (NH), 2945/2890 (CH_2), 1642 (w, δNH), 1575 (vs, aryl C-C), 1523 (vs, NO_2), 1460, 1418, 1358 (NO_2), 1320, 1290, 1235, 1160, 1137, 1070, 1050, 935, 900, 865, 820, 775. τ: 1.87 (1H, br, exchanged, NH), 2.42 (1H, d, J_m= 3, aromatic), 2.83 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 3.17 (1H, d, J_o= 9, aromatic), 6.20 (3H, s, OCH_3), 6.07-6.70 (8H, m, $NCH_2CH_2OCH_2CH_2O$), 7.5 (1H, br, exchanged, OH); a scarlet-red oil of (NN-di-2-hydroxyethyl)-4-methoxy-2-nitroaniline (210 mg, 2.8%) (Found: C, 51.16; H, 5.95; N, 10.46. $C_{11}H_{16}N_2O_5$ requires C, 51.55; H, 6.29; N, 10.93%). R_f (ether): very low. $\nu_{max.}$(film): 3350 (br, OH), 2945/2890 (CH_2), 1612, 1572, 1526 (NO_2), 1504, 1460/1446, 1412, 1352 (NO_2), 1290, 1230, 1155/1130, 1060, 1040, 912, 850 (w), 805, 737 (s). τ: 2.57 (1H, d, J_o= 9, aromatic), 2.80 (1H, s, aromatic), 2.91 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 6.13 (3H, s, OCH_3), 6.38 (4H, t, C-CH_2-O), 6.77 (4H, t, N-CH_2-C), 7.10 (2H, br, exchanged, OH); and a brown-black tar (2.25 g) of zero R_f.

When a similar reaction was left at 150° for a much longer period of time (3 days) a higher yield of the [N-2-(2-hydroxyethyl)oxyethyl] derivative was obtained. The main product, however was an intractable tar (3.114 g). Chlorination of this mixture with phosphoryl chloride gave a small amount of the nitrogen mustard (n.m.r. spectrum identical to an authentic sample - see below).

N-Acetyl-4-methoxy-2-nitroaniline (379, 381, 387, 465)

4-Methoxy-2-nitroaniline (30 g) was dissolved in pyridine (60 ml) and acetic anhydride (60 ml) and heated in boiling water for 7 h. The solvents were then removed under reduced pressure and the residue washed with 10% dilute hydrochloric acid, neutralized, extracted into dichloromethane, washed with aqueous sodium hydrogen carbonate and water, dried and condensed to give orange-yellow crystals of the title compound (36.4 g, 97%), m.p. 114-116° (lit.,[547] m.p. 117°). $\nu_{max.}$: 3360 (NH), 3090 (aryl C-H), 1700 (NCOMe), 1630 (w,δNH), 1580, 1520 (NO_2), 1430 (sh.), 1350, 1322 (NO_2), 1300, 1260, 1250 (sh.), 1228, 1200, 1160, 1087, 1045 (s), 1010, 940, 890, 860/852, 810, 778, 690.

N^1-Acetyl-4-methoxy-1,2-phenylenediamine (391)

N-Acetyl-4-methoxy-2-nitroaniline (20 g) was dissolved in dry methanol (400 ml) and 10% palladium-charcoal (2 g) was added. The mixture was hydrogenated in an autoclave at 20 atm. and 50° overnight. The resulting colourless solution was filtered and condensed to give a white solid (16.9 g), m.p. 146-148°. Recrystallization from boiling methanol gave white crystals of the title compound (98%), m.p. 148.5-149° (lit.,[548] m.p. 145°). $\nu_{max.}$: 3430/3345 (NH_2), 3215 (NH), 1630 (s, NCOMe), 1604, 1540, 1505, 1435 (sh.), 1327, 1292, 1260, 1225, 1212, 1174, 1075, 1030, 970, 955, 842, 787, 724 (br).

4-Chloro-3-nitroanisole

It was obtained from Aldrich Chemical Co. Inc., m.p. 45° (lit.,[549] m.p. 45°). τ: 2.50 (1H, d, J_o= 9, aromatic), 2.57 (1H, d, J_m= 2.5, aromatic), 2.90 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 6.10 (3H, s, OCH_3).

Nucleophilic substitution of 4-chloro-3-nitroanisole

(a) Reactants only at 116° (561)

4-Chloro-3-nitroanisole (5 g) was melted and dissolved in diethanolamine (10 ml), and this solution was stirred at 116° in a stoppered container for 77 h. On opening the container, ammonia was detected; the reaction mixture was poured into water (100 ml)

and extracted into dichloromethane (200 ml), then washed, dried, and partly condensed to give orange-red crystals (1.924 g). Recrystallization from hot dichloromethane gave orange needles of 2,2'-dichloro-5,5'-dimethoxyazobenzene, m.p. 174.5-176° (lit.,[550] m.p. 172°), almost insoluble in cold dichloromethane, chloroform or acetone. τ(DMSO-d_6): 2.23-2.97 (6H, m, aromatic), 6.07 (6H, s, OCH_3).

The remaining dark liquid was chromatographed by t.l.c. (CH_2Cl_2) to give 2-chloro-4-methoxyaniline (1.293 g) as a red oil. $\nu_{max.}$(film): 3450/3350 (NH_2), 3200 (w), 2950 (CH_3), 2840 (w), 1620 (vs), 1600 (sh.), 1500 (vs), 1470, 1455, 1440 (sh.), 1340, 1315, 1275, 1220 (vs), 1180 (vs), 1160, 1110, 1070, 1020, 957, 825, 785, 730. τ: 2.85 (1H, d, J_o= 9, aromatic), 3.67 (1H, d, J_m= 2.5, aromatic), 3.75 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 5.97 (2H, br, exchanged, NH_2), 6.29 (3H, s, OCH_3).

(b) In refluxing propan-1-ol (601)

4-Chloro-3-nitroanisole (2 g) was dissolved in propan-1-ol (20 ml), diethanolamine (6 ml) was added and the solution was refluxed for 60 h, the solution turning to a deep red colour. It was then partly condensed and the residue was poured into water, extracted into dichloromethane and condensed to give a red oil (2.0537 g). Preparative t.l.c. (dichloromethane) gave, in order of decreasing R_f: orange crystals of starting material (1.3177 g, 66%), m.p. 42-44°, mixed m.p. 40-43°; a brown-red oil of 2-chloro-4-methoxyaniline (234.9 mg), identical to that quoted above; and, after repeated purification by preparative t.l.c. (methanol), a scarlet-red oil of (NN-di-2-hydroxyethyl)-4-methoxy-2-nitroaniline (119.4 mg), τ identical to that of a sample obtained during hydroxyethylation of 4-methoxy-2-nitroaniline at high temperatures.

(NN-Di-2-chloroethyl)-4-methoxy-2-nitroaniline (655)

(NN-Di-2-hydroxyethyl)-4-methoxy-2-nitroaniline (112 mg) was dissolved in hot benzene (10 ml), phosphoryl chloride (1 ml) was added, and the solution refluxed for 3 h. It was then neutralized, extracted into dichloromethane, washed, dried, and condensed to give a dark red oil which was chromatographed (t.l.c.) affording a high R_f band (from dichloromethane) which gave an orange oil of the title compound (72 mg, 56%) (Found: C, 45.67; H, 4.96; N, 9.24; Cl, 23.41. $C_{11}H_{14}Cl_2N_2O_3 \cdot \frac{1}{10}C_4H_{10}O$ requires C, 45.56; H, 5.03; N, 9.32; Cl, 23.59%). $\nu_{max.}$ (film): 2960/2850 (CH_2), 1620 (w), 1535 (NO_2), 1508, 1462, 1448, 1375 (sh.), 1360 (NO_2), 1305, 1240, 1197, 1160, 1035, 920, 860, 840, 810, 750. τ: 2.56 (1H, d, J_o= 9, aromatic), 2.72 (1H, d, J_m= 3, aromatic), 2.87 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 6.11 (3H, s, OCH_3), 6.51 (8H, s, NCH_2CH_2Cl).

AMINOPHENYL CHLORO NITROGEN MUSTARDS

4-Chloro-2-nitroaniline

It was obtained from Aldrich Chemical Co. Inc. in 99% purity, m.p. 117-119°. $\nu_{max.}$: 3450/3337 (NH_2), 1635 (δNH), 1602, 1560, 1505 (NO_2), 1410, 1350 (NO_2), 1310, 1280, 1257, 1175, 1122, 915, 892, 835, 780, 740. τ: 1.91 (1H, d, J_m= 2.5, aromatic), 2.70 (1H, dd, $J_{o,m}$= 8.8, 2.5, aromatic), 3.25 (1H, d, J_o= 8.8, aromatic), 3.92 (2H, br, exchanged, NH_2).

Hydroxyethylation of 4-chloro-2-nitroaniline (604)

4-Chloro-2-nitroaniline (1.023 g) was dissolved in glacial acetic acid (20 ml) and water (10 ml) and liquid ethylene oxide (5 ml) was added. The solution was stirred in a sealed container for 120 h, then condensed and neutralized affording an orange precipitate which was filtered to give orange crystals (988 mg), m.p. 88-103°. The filtrate was extracted with dichloromethane and the organic layer was dried and condensed to give more orange crystals (70 mg). Preparative t.l.c. (CH_2Cl_2) on a sample of the crude product (100 mg) gave mainly the starting material (74%) m.p. 116-118°, and orange crystals of 4-chloro-(N-2-hydroxyethyl)-2-nitroaniline (23%), m.p. 107-108° (lit.,[474] m.p.107.5°). $\nu_{max.}$($CHCl_3$): 3365 (NH), 3030/2940 (CH_2), 1625 (δNH), 1565, 1515 (NO_2), 1465, 1430/1418, 1360 (NO_2), 1275, 1240-1210, 1170, 1140, 1075, 930, 900, 850, 810, 765 (C-Cl). τ: 1.81 (1H, d, J_m= 3, aromatic), 1.87 (1H, br, exchanged, NH), 2.59 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 3.13 (1H, d, J_o= 9, aromatic), 6.10 (2H, t, C-CH_2-O), 6.47 (2H, t, N-CH_2-C), 8.05 (1H, br, exchanged, OH).

2-Chloro-4-nitroaniline

It was obtained from Koch-Light Laboratories Ltd., m.p. 105-107°. $\nu_{max.}$: 3455/3352 (NH_2), 1625 (δNH), 1590, 1580 (sh.), 1505 (sh., NO_2), 1482, 1340 (NO_2), 1310, 1270, 1137, 1040 (w), 905, 837/827, 760, 737, 655. τ: 1.74 (1H, d, J_m= 3, aromatic), 1.95 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 3.18 (1H, d, J_o= 9, aromatic), 5.06 (2H, br, exchanged, NH_2).

Hydroxyethylation of 2-chloro-4-nitroaniline

(a) In aqueous acetic acid at room temperature (280, 326)

2-Chloro-4-nitroaniline (2 g) was suspended in glacial acetic acid (35 ml) and water (25 ml), liquid ethylene oxide (10 ml) was added, and the solution was vigorously stirred in a sealed flask kept at room temperature for 4 days. The solvents were then removed under reduced pressure, and the residue was neutralized, extracted into ether, washed, dried and condensed to give a yellow oil. Fractional crystallization from dichloromethane-petroleum with overnight cooling initially affording yellow crystals (500 mg), m.p. 101-114° followed by yellow crystals of starting material (1 g 50%), m.p. 103-106° (lit.,[551] m.p. 106.5-107.5°). The yellow crystals, m.p. 101-114°, were recrystallized twice from dry acetone-petroleum to give yellow needles of 2-chloro-(N-2-hydroxyethyl)-4-nitroaniline (18%), m.p. 115-117° (lit.,[474] m.p.120°) (Found: C,44.48, H,4.39, N,12.74. Calc. for $C_8H_9ClN_2O_3$: C, 44.36; H, 4.19; N, 12.93%). $\nu_{max.}$: 3400-3150 (OH), 3340 (NH), 1595, 1535 (NO_2), 1485 (sh.), 1325 (NO_2), 1305, 1155 (C-O), 1133, 1085/1075 (Ar-Cl), 1055 (C-O), 935 (w), 910 (sh.), 898, 828/817, 760, 735 (C-Cl). τ(DMSO-d_6): 1.78 (1H, d, J_m= 3, aromatic), 1.95 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 3.25 (1H, d, J_o= 9, aromatic), 4.00 (1H, br, exchanged, NH), 6.17 (2H, t, C-CH_2-O), 6.58 (2H, t, N-CH_2-C), 6.6 (1H, br, exchanged, OH).

Using a glacial acetic acid-dioxan mixture (1:1) as solvent, starting material was re-obtained in quantitative yields even after 60 h reaction time.

(b) In dioxan at 140° (419)

2-Chloro-4-nitroaniline (5 g) was placed in a Carius tube, dioxan (5 ml) and freshly redistilled liquid ethylene oxide (5 ml) were added and the tube was sealed and heated at 140° for 16 h. Then the tube was opened and the contents were condensed to give an oil which partly crystallized from dichloromethane-petroleum with cooling giving yellow crystals of starting material (760 mg, 15%), τ identical to that of an authentic sample, recrystallized to give m.p. 104-106°. The remaining product was recondensed to give an oil which was chromatographed (dry column-CH_2Cl_2), to give in order of decreasing R_f: more starting material (2.23 g, 45%), m.p. 103-105° (lit.,[551] m.p. 106-107°) and

orange crystals of 2-chloro-(N-2-hydroxyethyl)-4-nitroaniline (700 mg, 15%), m.p. 115-117°. τ identical to that of an authentic sample.

N-Acetyl-2-chloro-4-nitroaniline (385,459,461,462,496)

2-Chloro-4-nitroaniline (25 g) was dissolved in pyridine (50 ml) and acetic anhydride (50 ml) and heated in boiling water for 6 h. It was then condensed, washed with 10% dilute hydrochloric acid, extracted into dichloromethane, neutralized, washed with water (2 x 200 ml), decolourized (charcoal), dried and condensed to give an oil. Ethanol (150 ml) was added resulting in crystallization affording cream-white crystals (28 g) m.p. 99-100°. The crude product was fractionally recrystallized (methanol) obtaining cream-white crystals, m.p. 100-104°. Further concentration of the mother liquor afforded a very small amount of yellow crystals, m.p. 139-142°. The cream-white crystals, m.p. 100-104° were further recrystallized to give cream-white crystals (24 g), m.p. 109-112°. A small sample was purified by preparative t.l.c. (dichloromethane) and recrystallized to give white crystals of NN-diacetyl-2-chloro-4-nitroaniline, m.p. 111-113.5°. (Found: C, 46.55; H, 3.56; N, 10.89; Cl, 13.94. $C_{10}H_9ClN_2O_4$ requires C, 46.80; H, 3.53; N, 10.92; Cl, 13.81%). $\nu_{max.}$: 3098 (w, aryl C-H), 1718 (sh.)/ 1712 (NAc_2), 1610 (w), 1593 (w), 1534 (NO_2), 1374, 1354 (NO_2), 1312, 1254 (s), 1231, 1157 (w), 1132 (w), 1074 (w), 1053/1043 (w), 1026, 979 (w), 961 (w), 918, 881 (w), 846 (w), 771, 756 (C-Cl), 723. τ: 1.58 (1H, d, J_m= 3, aromatic), 1.68 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 2.49 (1H, d, J_o= 9, aromatic), 7.67 (6H, s, C-CH_3). The small amount of yellow crystals (500 mg), m.p. 139-142° were recrystallized from dichloromethane-petroleum to give yellow needles of N-acetyl-2-chloro-4-nitroaniline, m.p. 143-144° (lit.,[552] m.p. 143°). $\nu_{max.}$: 3280 (NH), 3098 (w, aryl C-H), 1690 (NCOMe), 1605, 1595, 1540, 1506 (NO_2), 1400, 1360 (NO_2), 1320, 1285, 1260, 1240, 1160, 1140, 1058, 1010, 910, 850, 838 (w), 760 (sh.)/752 (C-Cl), 720, 680. τ: 1.30 (1H, d, J_o= 9, aromatic), 1.70 (1H, d, J_m= 3, aromatic), 1.86 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 2.03 (1H, br, exchanged, NH), 7.68 (3H, s, $NCOCH_3$).

In one preparation, the pyridine-acetic anhydride solution was stirred overnight at room temperature prior to heating the solution. The products obtained were the same as for the above preparation but the yield of the monoacetyl product was higher than that of the diacetyl derivative.

4-Chloro-3-nitroaniline

It was obtained from Ralph N. Emanuel Ltd., m.p. 101-103°. τ: 2.68 (1H, d, J_o= 9, aromatic), 2.81 (1H, d, J_m = 3, aromatic), 3.18 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 6.03 (2H, br, exchanged, NH_2).

Hydroxyethylation of 4-chloro-3-nitroaniline

(a) In aqueous acetic acid at room temperature (393, 424, 485)

4-Chloro-3-nitroaniline (2 g) was suspended in glacial acetic acid (20 ml) and water (10 ml) and freshly redistilled liquid ethylene oxide (4 ml) was added. The container was sealed and the mixture stirred vigorously at room temperature for 5 days. The resulting solution was condensed under reduced pressure and the residue neutralized, washed with water, dried and condensed to give an orange oil, (3.77 g) which partly crystallized from dichloromethane with cooling overnight affording orange crystals (1.7 g). Recrystallization from acetone-petroleum gave orange crystals of 4-chloro-(NN-di-2-hydroxyethyl)-3-nitroaniline (1.3 g), m.p. 106.5-108.5°. Final purification of a sample by t.l.c. (ethyl acetate) and recrystallization gave orange crystals of the diol, m.p. 107.5-108°. (Found: C, 45.92; H, 4.94; N, 10.79. $C_{10}H_{13}ClN_2O_4$ requires C, 46.08; H, 5.03; N, 10.75%). τ (acetone-d_6): 2.68 (1H, d, J_o= 9, aromatic), 2.83 (1H, d, J_m= 3, aromatic), 3.05 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 5.9-6.6 (2H, br, exchanged, OH), 6.40 (8H, t, NCH_2CH_2O).

The filtrate and the mother liquor from the crystallization were combined and chromatographed (preparative t.l.c. - dichloromethane) to give in order of increasing R_f: more diol (411 mg), total yield of 56%; an orange-red oil (0.97 g) of a molecular 1:1 complex of 4-chloro-(N-2-hydroxyethyl)-3-nitroaniline and 4-chloro-(N-2-hydroxyethyl-N-2-acetoxyethyl)-3-nitroaniline, $\nu_{max.}$ (film): 3492 (OH), 3395 (NH), 2947/2895 (CH_2), 1725 (CO), 1612, 1535 (NO_2), 1500, 1395 (sh.), 1355 (NO_2), 1290 (sh.), 1255, 1190 (w), 1140, 1050, 960 (w), 860 (sh.), 820, 755, 738. τ: 2.60-3.40 (6H, m, aromatic), 5.5-7.6 (3H, br, CH, NH), 5.6-6.8 (12H, 4 triplets, NCH_2CH_2O), 7.97 (3H, s, C-CH_3). m/e: 304 (P + 2, 33%P, $C_{12}H_{15}Cl^{37}N_2O_5$), 302 (P, $C_{12}H_{15}Cl^{35}N_2O_5$), 271 ($C_{11}H_{12}ClN_2O_4$), 229 ($C_9H_{10}ClN_2O_3$), 216 (P', 26.4%B, $C_8H_9ClN_2O_3$), 187 (B + 2, 33.7±1.4% of B, $C_7H_6Cl^{37}N_2O_2$), 185 (B, 100%, $C_7H_6Cl^{35}N_2O_2$), 139 (42.9%B, C_7H_6ClN), 104 (15.7%B, Cl isotope pattern not apparent,

C_7H_6N), 91, 90, 89, 87, 75 (10%B, C_6H_3), 43, 31; and an orange-red liquid of (N-2-acetoxyethyl)-4-chloro-3-nitroaniline (77.2 mg, 2.6%) (Found: C, 46.56; H, 4.22; N, 10.73. $C_{10}H_{11}ClN_2O_4$ requires C, 46.44; H, 4.29; N, 10.83%). $\nu_{max.}$ (film): 3375 (NH), 2934/2867 (CH_2), 1732 (C=O), 1612, 1530 (NO_2), 1375-1340 (NO_2), 1252, 1140, 1050, 910, 820, 740. τ: 2.67 (1H, d, J_o= 9, aromatic), 2.88 (1H, d, J_m= 3, aromatic), 3.22 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 5.67 (2H, t, C-CH_2-O), 5.90 (1H, br, exchanged, NH), 6.57 (2H, t, N-CH_2-C), 7.90 (3H, s, C-CH_3).

(b) In dioxan at 100° (474)

4-Chloro-3-nitroaniline (1 g), dioxan (3 ml) and freshly redistilled liquid ethylene oxide (2 ml) were sealed inside a Carius tube which was then heated at 100° for 16 h. The tube was then opened, and the contents condensed and chromatographed (preparative t.l.c.-dichloromethane) to give, in order of decreasing R_f: orange-yellow crystals of starting material (444 mg, 44%), m.p. and mixed m.p. 101-103°, τ identical to that of an authentic sample; a red oil which crystallized from chloroform and was recrystallized from dichloromethane-petroleum to orange-yellow needles of 4-chloro-(N-2-hydroxyethyl)-3-nitroaniline (483 mg, 38%), m.p. 73.5° (Found: C, 44.29; H, 4.19; N, 12.76; Cl, 16.69. $C_8H_9ClN_2O_3$ requires C, 44.36; H, 4.19; N, 12.93; Cl, 16.37%). $\nu_{max.}$: 3410 (br, OH), 3302 (NH), 1610, 1530/1342 (NO_2), 1285, 1260 (w), 1220 (w), 1165, 1140, 1072, 1060 (sh.), 970, 887, 837, 821. τ: 2.72 (1H, d, J_o= 8.5, aromatic), 2.90 (1H, d, J_m= 2.5, aromatic), 3.25 (1H, dd, $J_{o,m}$= 8.5, 2.5, aromatic), 5.30 (1H, br, exchanged, NH), 6.13 (2H, t, C-CH_2-O), 6.70 (2H, t, N-CH_2-C), 7.13 (1H, br, exchanged, OH); and a red oil which crystallized from chloroform to orange crystals of the diol (257.1 mg, 17%), m.p. 105-108°, τ identical to that of an authentic sample.

(NN-Di-2-chloroethyl)-4-chloro-3-nitroaniline (436, 565, 567)

4-Chloro-(NN-di-2-chloroethyl)-3-nitroaniline (5.754 g) was dissolved in refluxing dry benzene (25 ml) and phosphoryl chloride (10 ml) in benzene (5 ml) was added dropwise. Reflux conditions were maintained for 4½ h and the reaction mixture was then neutralized, extracted with dichloromethane, washed with water, dried and condensed to give a yellow solid. Partial solution and filtration gave a golden-

yellow solid, of high R_f (CH_2Cl_2), m.p. 141-143°. Recrystallization from hot methanol and slow cooling gave yellow needles of the title compound (3.986 g, 61%), m.p. 143.5-144° (Found: C, 40.26; H, 3.75; N, 9.56; Cl, 36.01. $C_{10}H_{11}Cl_3N_2O_2$ requires C, 40.36; H, 3.73; N, 9.41; Cl, 35.74%). $\nu_{max.}$: 1616, 1538 (s, NO_2), 1498, 1355 (s, NO_2), 1295, 1266, 1230, 1210, 1155, 982 (w), 897, 852, 820, 795, 755, 730. τ(acetone-d_6): 2.58 (1H, d, J_o= 9, aromatic), 2.77 (1H, d, J_m= 3, aromatic), 3.06 (1H, dd, $J_{o,m}$= 9, 3, aromatic), 6.20 (8H, t, NCH_2CH_2Cl).

(N^{1}N^1-Di-2-chloroethyl)-4-chloro-1,3-phenylenediamine (633, 651, 669)

(NN-Di-2-chloroethyl)-4-chloro-3-nitroaniline (1 g) was suspended in methanol (60 ml) and 10% palladium-charcoal (100 mg) was added. The mixture was hydrogenated at room temperature and atmospheric pressure, approximately the theoretical amount of hydrogen (455 ml) being absorbed. It was then quickly filtered into 50% w/w hydrogen chloride in methanol (30 ml) and the clear solution was condensed under reduced pressure, and washed successively with methanol, ether, and dichloromethane. A very viscous yellowish oil was obtained which on trituration with ether gave a sticky solid. It was dissolved in a minimum amount of methanol and this solution was triturated with ether, with cooling, giving white crystals, m.p. 177-190°. Recrystallization from methanol-ether gave white crystals of the title compound hydrochloride forming needles at 158° and m.p. 174-178°. (Found: C, 44.84; H, 5.69; N, 10.12. $C_{10}H_{13}Cl_3N_2$ requires C, 44.88; H, 4.90; N, 10.47% - the hydrochloride apparently decomposed fairly rapidly to the free amine). $\nu_{max.}$: 2640 (NH_3^+), 1638, 1615, 1600 (δNH_3^+), 1565, 1510 (s), 1408, 1390, 1367, 1340, 1318, 1300 (δNH_3^+), 1270, 1218, 1185 (C-N), 1172 (C-N), 1080 (Ar-Cl), 1030, 1005, 950, 935 (sh.), 868 (br, δNH), 792 (C-Cl), 767, 715, 687. τ(methanol-d_4): 2.69 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 3.00-3.37 (2H, m, aromatic), 5.03 (3H, br, exchanged, NH_3^+), 6.20 (8H, s, NCH_2CH_2Cl).

2,3-Dichloronitrobenzene

It was obtained from Aldrich Chemical Co. Ltd, m.p. 61-62°. $\nu_{max.}$: 3072 (w, C-H), 1580 (ring carbons), 1528 (s, NO_2), 1442 (sh., ring carbons), 1435 (s), 1367 (s, NO_2), 1282, 1219, 1167, 1108 (Ar-Cl), 1066, 987 (w), 898 (C-N), 809 (s), 775 (s, C-Cl), 752 (δC-H), 735 (s, C-Cl), 710 (δC-H). τ: 2.03-2.77 (3H, m, aromatic).

3-Chloro-2-(di-2-hydroxyethylamino)nitrobenzene (555)

2,3-Dichloronitrobenzene (5 g) was melted in a conical flask, diethanolamine (10 ml) was added quickly and the solution was stirred for 5 h in an oil bath at 115°. On cooling, the mixture was poured into distilled water (70 ml) and stirred. The products were extracted into dichloromethane, washed (H_2O), dried and condensed to give a dark-red oil (6.21 g). Preparative t.l.c. (CH_2Cl_2) gave in order of increasing R_f: an orange-red oil of the title compound (3.35 g, 49%) crystallizing on standing to tan-yellow crystals m.p. 69-70° (Found: C, 46.15; H, 4.87; N, 10.80; Cl, 13.79. $C_{10}H_{13}ClN_2O_4$ requires C, 46.08; H, 5.03; N, 10.75; Cl, 13.60%). R_f: low. $\nu_{max.}$(film): 3350 (br, OH), 2950/2900 (CH_2), 1530 (NO_2), 1380, 1360 (sh., NO_2), 1080 (Ar-Cl), 1060, 820, 775 (C-Cl), 750 (δC-H). τ: 2.20-2.93 (3H, m, aromatic), 5.97-9.10 (2H, v br, exchanged, OH), 6.35 (4H, t, C-CH_2-O), 6.70 (4H, t, N-CH_2-C); a dark orange-red oil of 3-chloro-2-(2-hydroxyethylamino)nitrobenzene (209 mg, 4%) (lit.,[474] b.p. 155-157° at 2mmHg). $\nu_{max.}$(film): 3380 (br, OH), 3350 (NH), 2950/2890 (CH_2), 1602, 1578, 1530, 1500 (NO_2), 1460, 1345 (NO_2), 1265, 1110 (sh.), 1090 (Ar-Cl), 1070, 915, 882, 800, 748 (C-Cl), 720 (δC-H). τ: 2.10 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 2.52 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 3.18 (1H, br, NH), 3.25 (1H, t, J= 9, aromatic), 6.05-6.62 (4H, m, NCH_2CH_2O), 7.7 (1H, br, OH); an orange-brown solid (159 mg), m.p. greater than 314°, soluble in methanol, only slightly soluble in acetone; a dark purple solid (18.6 mg), slightly soluble in acetone, dichloromethane or methanol; a yellow oil of 2,3-dichloroaniline (147.3 mg, 3.5%) (lit.,[555] m.p. 24°), soluble in ether, $\nu_{max.}$($CHCl_3$): 3470/3380 (NH_2), 1615, 1590, 1540, 1470, 1450, 1110(Ar-Cl), 1020, 910, 700. τ: 2.58-3.52 (3H, m, aromatic), 5.82 (2H, br, NH_2); and scarlet red crystals of starting material (26%) m.p. and mixed m.p. 55-58°. $\nu_{max.}$ and τ identical to those of an authentic sample.

3-Chloro-2-(di-2-chloroethylamino)nitrobenzene (577)

3-Chloro-2-(di-2-hydroxyethylamino)nitrobenzene (1.528 g) was dissolved in dry refluxing benzene (6 ml) and phosphoryl chloride (3 ml) in benzene (4 ml) was added, resulting temporarily in the formation of a precipitate which redissolved within 5 min. Refluxing was continued for 4 h, then the reaction mixture was neutralized,

extracted into dichloromethane, washed, dried and condensed to give an oil (1.6514 g). Preparative t.l.c. (CH_2Cl_2) gave two main products: an orange oil of the title compound (902 mg, 52%) (Found: C, 40.96: H, 3.67; N, 9.14; Cl, 35.67. $C_{10}H_{11}Cl_3N_2O_2$ requires C, 40.36; H, 3.73; N, 9.41; Cl, 35.74%). R_f: high. $\nu_{max.}$(film): 2965/2870 (CH_2), 1530 (NO_2), 1470, 1455, 1375, 1360 (NO_2), 1125 (Ar-Cl), 817, 770 (C-Cl), 740 (δC-H). τ: 2.17-2.88 (3H, m, aromatic), 6.47 (8H, s, NCH_2CH_2Cl); and a yellow oil crystallizing to yellow crystals of N-(2-chloro-6-nitrophenyl)morpholine (255 mg, 18%), m.p. 93-95° (Found: C, 49.52; H, 4.73; N, 11.47; Cl, 15.32. $C_{10}H_{11}ClN_2O_3$ requires C, 49.50; H, 4.57; N, 11.54; Cl, 14.61%). R_f: just lower than that of title compound. Separation of these two products by t.l.c. was very difficult due to their close R_f values.

3-Chloro-2-(di-2-chloroethylamino)aniline (665)

3-Chloro-2-(di-2-chloroethylamino)nitrobenzene (491 mg) was dissolved in dry methanol (10 ml) and hydrogenated at room temperature and atmospheric pressure over 10% palladium-charcoal (60 mg) for 1 h, when the theoretical amount of hydrogen (225 ml) had been absorbed. The catalyst was removed by filtration, collecting the filtrate in a flask containing 50% w/w hydrogen chloride in methanol (10 ml). The filtrate was then condensed under reduced pressure to give an oil which rapidly turned very dark and was therefore immediately used for the next stage.

2'-Chlorophenyl N-[3-chloro-2-(di-2-chloroethylamino)]phenylcarbamate (666)

The above crude aminophenyl chloro mustard hydrochloride was suspended in dry benzene (10 ml) and pyridine (1 ml) was added. 2-Chlorophenyl chloroformate (275 mg) was added resulting in a white precipitate being formed. The mixture was stirred and refluxed overnight. The mixture was then washed with 10% dilute hydrochloric acid, when the red colouration was transferred to the aqueous layer. The light yellow benzene layer was neutralized, washed with water, dried (anhydrous sodium carbonate) and condensed to give an oil. Preparative t.l.c. (dichloromethane) gave a high R_f band again affording an oil (420 mg). Further purification by t.l.c. gave a

very light yellow oil crystallizing from ether-petroleum to white crystals of the title compound (200 mg) m.p. 103-105° (Found: C, 48.31; H, 3.93; N, 6.70; Cl, 33.80. $C_{17}H_{16}Cl_4N_2O_2$ requires C, 48.37; H, 3.82; N, 6.64; Cl, 33.59%). $\nu_{max.}$ (film): 3316 (s, NH), 3070 (C-H), 2966/2856 (CH_2), 1795 (sh.), 1756 (N-CO-O), 1596, 1580, 1516, 1480, 1456, 1380, 1316, 1276, 1240, 1210, 1140, 1076, 1047, 1016, 958, 922, 860, 804, 770 (sh., C-Cl), 756 (C-Cl), 700, 680. τ : 0.70 (1H, br, NH), 1.83 (1H, dd, $J_{o,m}$= 8, 3, aromatic), 2.20-3.20 (6H, m aromatic), 6.10 (8H, s, NCH_2CH_2Cl).

2,4-Dichloronitrobenzene

It was obtained from Aldrich Chemical Co. Ltd in 97% purity, m.p. 29-32°. $\nu_{max.}$: 3100 (w, C-H), 1585, 1575 (sh.), 1535 (NO_2), 1365 (sh.), 1350 (NO_2), 1300, 1260 (w), 1152, 1120 (Ar-Cl), 1063, 880 (vs, C-N), 842 (δC-H), 808 (δC-H), 762 (C-Cl), 740 (w), 687 (w). τ: 2.06 (1H, d, J_o= 9, aromatic), 2.37 (1H, d, J_m= 2.5, aromatic), 2.56 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic).

4-Chloro-2-(di-2-hydroxyethylamino)nitrobenzene (505, 526)

2,4-Dichloronitrobenzene (5 g) was reacted with diethanolamine (10 ml) at 108° for 2 h and then worked up by the method used for the 2,3-isomer to give a red oil which was chromatographed (preparative t.l.c. - ether) to give, in order of decreasing R_f: yellow-orange crystals (from petroleum) of 4-chloro-2-(2-hydroxyethylamino)-nitrobenzene (120 mg, 2.39%), m.p. 115.5-117° (lit.,[474] m.p. 116°). τ : 1.7 (1H, br, NH), 1.87 (1H, d, J_o= 9, aromatic), 3.10 (1H, d, J_m= 2.5, aromatic), 3.38 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 6.03 (2H, t, $C-CH_2-O$), 6.50 (2H, t, $N-CH_2-C$), 7.38 (1H, br, OH); an orange-red oil of 4-chloro-2-(di-2-hydroxyethylamino)nitrobenzene (5.05 g, 74%) (Found: C, 46.03; H, 5.08; N, 10.68; Cl, 13.24. $C_{10}H_{13}ClN_2O_4$ requires C, 46.08; H, 5.03; N, 10.75; Cl, 13.60%). $\nu_{max.}$ (film): 3350 (br, OH), 2950/2900 (CH_2), 1595, 1580, 1515 (NO_2), 1480, 1345 (NO_2), 1300, 1275, 1220, 1170/1150, 1115 (Ar-Cl), 1075, 1005, 935, 860, 740 (C-Cl), 710 (C-H). τ: 2.38 (1H, d, J_o= 9, aromatic), 2.67 (1H, d, J_m= 2.5, aromatic), 3.03 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 6.35 (4H, m, $C-CH_2-O$), 6.63 (4H, m, $N-CH_2-C$), 6.98 (2H, br, exchanged, OH); and a yellow oil which was rechromatographed (ether) giving

yellow crystals of 2-chloro-4-(di-2-hydroxyethylamino)nitrobenzene (383 mg, 5.6%), m.p. 77-79° (Found: C, 46.21; H, 5.10; N, 10.71; Cl, 13.27. $C_{10}H_{13}ClN_2O_4$ requires C, 46.08; H, 5.03; N, 10.75; Cl, 13.60%). $\nu_{max.}$ (film): 3350 (br, OH), 2950/2900 (CH_2), 1595, 1560 (NO_2), 1510, 1400, 1340 (sh.), 1320 (NO_2), 1275, 1230, 1150, 1070 (Ar-Cl), 1030, 1010, 850, 820, 745 (C-Cl), 710 (δC-H). τ: 1.93 (1H, d, J_o= 9, aromatic), 3.1-3.5 (2H, m, aromatic), 6.05 (4H, t, C-CH_2-O), 6.30 (4H, t, N-CH_2-C), 7.14 (2H, br, exchanged, OH).

The reaction was repeated at 75° for 23 h, then at 105° for 1½ h for complete reaction. The products obtained were the title compound (84%), its isomeric diol (5.23%) and the monosubstituted 4-chloro derivative (1.6%).

4-Chloro-2-(di-2-chloroethylamino)nitrobenzene (542, 569)

4-Chloro-2-(di-2-hydroxyethylamino)nitrobenzene (1.51 g) was chlorinated by the method used for the 3-chloro isomer. The resulting yellow-orange oil was chromatographed (preparative t.l.c.-dichloromethane) to give the title compound as an orange-yellow oil (1.28 g, 74.5%) (Found: C, 40.36; H, 3.70; N, 9.53; Cl, 35.51. $C_{10}H_{11}Cl_3N_2O_2$ requires C, 40.36; H, 3.73; N, 9.41; Cl, 35.74%). R_f: high. $\nu_{max.}$ (film): 3100 (C-H), 2965/2860 (CH_2), 1595 (vs), 1560 (s), 1510 (vs) (NO_2), 1480 (s), 1435, 1370 (sh.), 1340 (NO_2), 1304, 1282, 1252, 1188, 1155/1138, 1110 (Ar-Cl), 1084, 1005, 974, 930, 900, 870, 852, 822, 756 (C-Cl), 712 (δC-H), 670. τ: 2.24 (1H, d, J_o= 9, aromatic), 2.65 (1H, d, J_m= 2, aromatic), 2.91 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 6.44 (8H, s, NCH_2CH_2Cl). A small band of slightly lower R_f gave an orange oil which crystallized to orange crystals of N-(5-chloro-2-nitrophenyl)morpholine (87 mg, 6.2%), m.p. 67.5-69.5° (Found: C, 49.71; H, 4.34; N, 11.39; Cl, 14.84. $C_{10}H_{11}ClN_2O_3$ requires C, 49.50; H, 4.57; N, 11.54; Cl, 14.61%). τ: 1.19 (1H, d, J_o= 8.5, aromatic), 2.88 (1H, s, aromatic), 2.98 (1H, dd, $J_{o,m}$= 8.5, 2, aromatic), 6.14 (4H, t, C-CH_2-O), 6.92 (4H, t, N-CH_2-C).

4-Chloro-2-(di-2-chloroethylamino)aniline (685)

4-Chloro-2-(di-2-chloroethylamino)nitrobenzene (1.0196 g) was hydrogenated by the method used for the 3-chloro isomer. The product obtained was a crude red oil insoluble in benzene or dichloromethane. It was used directly to prevent further decomposition.

2'-Chlorophenyl N-[4-chloro-2-(di-2-chloroethylamino)]phenylcarbamate (686)

The above crude product was reacted with 2-chlorophenyl chloroformate as described for the 3-chloro isomer except that triethylamine was used instead of pyridine. The product, however, was a yellowish oil which turned dark brown in air. Repeated purification by t.l.c. (CH_2Cl_2) gave a pale yellow oil of the title compound, $\nu_{max.}$ (film) 3335 (NH), 2960/2860 (CH_2), 1767/1728 (N-CO-O), 1590, 1518(s), 1482, 1458, 1418 (w), 1310 (sh.), 1262 (s), 1235 (s) , 1180, 1138, 1100 (Ar-Cl), 1070, 1040, 960/945, 840, 780, 765, 750, 680. Within 18 h, the light yellow oil had decomposed to a dark brown oil, microanalysis of which was inconsistent with the structure of the title compound.

2-Chloro-4-(di-2-chloroethylamino)nitrobenzene (591)

2-Chloro-4-(di-2-hydroxyethylamino)nitrobenzene (445.6 mg) was chlorinated by the method used for the 3-chloro-2-(di-2-hydroxyethylamino) isomer. After chromatography (t.l.c. - dichloromethane), the products obtained were: a yellow oil which crystallized to yellow crystals of the title compound (93.4 mg, 18.4%), m.p. 115-116° (Found: C, 41.43; H, 3.89; N, 9.12. $C_{10}H_{11}Cl_3N_2O_2$ requires C, 40.36; H, 3.73; N, 9.41%). R_f: high. τ: 2.17 (1H, d, J_o = 8.5, aromatic), 2.60 (1H, d, J_m = 2, aromatic), 2.84 (1H, dd, $J_{o,m}$ = 8.5, 2, aromatic), 6.40 (8H, s, NCH_2CH_2Cl). Total purification of the title compound by t.l.c. was very difficult due to a minor band with an R_f almost identical to it. This small band, R_f just below that of the title compound, gave yellow crystals, m.p. 108-112° which were rechromatographed and recrystallized, affording yellow crystals of N-(3-chloro-4-nitrophenyl)morpholine (44.1 mg, 10.6%), m.p. 121-121° (Found: C, 49.48; H, 4.70; N, 11.46; Cl, 14.72. $C_{10}H_{11}ClN_2O_3$ requires C, 49.50; H, 4.57; N, 11.54; Cl, 14.61%).

2,5-Dichloronitrobenzene

It was obtained from Koch-Light Laboratories Ltd., m.p. 55°. $\nu_{max.}$: 3068 (C-H), 1593, 1570 (sh.), 1544/1355 (NO_2), 1280, 1258, 1166, 1148, 1110 (Ar-Cl), 1060, 900 (C-N), 847, 840, 785 (C-Cl), 770 (C-Cl), 740 (δC-H), 707, 680.

5-Chloro-2-(2-hydroxyethylamino)nitrobenzene (495)

2,5-Dichloronitrobenzene was reacted with monoethanolamine at 110° for 3 h by a similar procedure to that used for the reaction of the 2,3- isomer with diethanolamine. The product obtained, after purification by column chromatography (ether) and crystallization gave orange-yellow crystals, m.p. 103-106°. Recrystallization from dichloromethane-petroleum gave orange crystals of the title compound, m.p. 107-108.5° (lit.,[474] m.p. 107.5° corr.), $\nu_{max.}$: 3350 (NH), 3260 (br, OH), 1620, 1565 (NO_2), 1510, 1410, 1360 (NO_2), 1310, 1265, 1240, 1165, 1080 (Ar-Cl), 1060, 900, 890, 825, 770 (C-Cl), 733 (δC-H). τ: 1.67 (1H, br, exchanged, NH), 1.77 (1H, d, J_m= 2.5, aromatic), 2.53 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 3.10 (1H, d, J_o= 9, aromatic), 6.00 (2H, t, C-CH_2-O), 6.43 (2H, t, N-CH_2-C), 8.32 (1H, br, exchanged, OH).

5-Chloro-2-(di-2-hydroxyethylamino)nitrobenzene (490)

2,5-Dichloronitrobenzene (5 g) was reacted with diethanolamine (10 ml) at 110° for 3 h, and worked up as for the 2,3-dichloro isomer, to give a deep red oil (5.94 g). Preparative t.l.c. (ether) gave, in order of increasing R_f: a red oil of the title compound (3.624 g, 54%) (Found: C, 45.84; H, 5.03; N, 10.66; Cl, 13.57. $C_{10}H_{13}ClN_2O_4$ requires C, 46.08; H, 5.03; N, 10.75; Cl, 13.60%), $\nu_{max.}$(film): 3350 (br, OH), 2950/2900 (CH_2), 1601, 1520 (NO_2), 1490, 1360 (NO_2), 1280, 1220, 1180, 1120 (Ar-Cl), 1070, 1050, 880, 825, 770 (C-Cl), 740 (δC-H). τ: 2.10-2.77 (3H, m, aromatic), 6.33 (4H, t, C-CH_2-O), 6.67 (4H, t, N-CH_2-C), 7.12 (2H, br s, exchanged, OH); dark purple crystals of 2,2',5,5'-tetrachloroazobenzene (118.5 mg, 3.6%), m.p. 185° (lit.,[554] m.p. 189°); and orange-yellow crystals recrystallized from dichloromethane-petroleum to give orange crystals of 5-chloro-2-(2-hydroxyethylamino)nitrobenzene (85.6 mg, 1.6%), m.p. 107-109°, mixed m.p. with the above prepared sample, m.p. 105-108° (lit.,[474] m.p. 107.5° corr.). $\nu_{max.}$ and τ identical to those of an authentic sample.

5-Chloro-2-(di-2-chloroethylamino)nitrobenzene (573)

5-Chloro-2-(di-2-hydroxyethylamino)nitrobenzene (1.594 g) in benzene (6 ml) was chlorinated according to the method used for the 3-chloro isomer. An orange oil (1.95 g) was obtained which was chromatographed to give a yellowish-orange oil of the title compound (1.587 g, 87%) (Found: C, 40.27; H, 3.76; N, 9.45; Cl, 35.96. $C_{10}H_{11}Cl_3N_2O_2$ requires C, 40.36; H, 3.73; N, 9.41; Cl, 35.74%). R_f: high. $\nu_{max.}$(film): 3095 (C-H), 2965/2860 (CH_2), 1604, 1522 (NO_2), 1485, 1457/1439 (w), 1350 (NO_2), 1310, 1280, 1258, 1192, 1162, 1142, 1120 (Ar-Cl), 890, 830, 770, 750 (C-Cl), 670. τ: 2.30 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 2.52 (1H, d, J_m= 2.5, aromatic), 2.65 (1H, d, J_o= 9, aromatic), 6.45 (8H, s, NCH_2CH_2Cl). A small band with R_f just below that of the title compound, was extracted to give a reddish oil which crystallized on standing to an orange solid of N-(4-chloro-2-nitrophenyl)morpholine (55 mg, 3.7%), m.p. 43-45°. (Found: C, 49.35; H, 4.35; N, 11.75; Cl, 14.58. $C_{10}H_{11}ClN_2O_3$ requires C, 49.50; H, 4.57; N, 11.54; Cl, 14.61%).

5-Chloro-2-(di-2-chloroethylamino)aniline (639)

5-Chloro-2-(di-2-chloroethylamino)nitrobenzene (1.3487 g) was hydrogenated by a similar procedure to that used for the 3-chloro isomer. On filtration the filtrate started to get dark very quickly, but on condensation and trituration with ether, pinkish mauve crystals, soluble in methanol and dichloromethane, insoluble in ether, were obtained. These were recrystallized to brown crystals of the title compound hydrochloride (Found: C, 41.06; H, 4.97; N, 9.52. $C_{10}H_{13}Cl_3N_2$ requires C, 44.89; H, 4.90; N, 10.47%. $C_{10}H_{13}Cl_3N_2$.HCl requires C, 39.50; H, 4.64; N, 9.21%). $\nu_{max.}$($CHCl_3$): 3450/3360 (w) (NH_2), 2975/2850 (CH_2), 2565 (NH_3^+), 1610 (s), 1500 (vs), 1360 (w), 1318, 1250-1220, 1187, 1142, 1112 (Ar-Cl), 1030, 925 (s), 868/842 (C-H), 675. τ: 0.27 (2 to 3H, br, NH_3^+), 2.00-2.97 (3H, m, aromatic), 6.41 (8H, s, NCH_2CH_2Cl). It was used directly for the next stage.

2'-Chlorophenyl N-[5-chloro-2-(di-2-chloroethylamino)]phenylcarbamate (641)

The above crude aminophenyl chloro mustard hydrochloride (1 g) in benzene (25 ml) was treated with 2-chlorophenyl chloroformate (624 mg) and pyridine (700 mg) and worked up by a similar procedure to that used for the 3-chloro isomer, to give a dark brown oil (1.185 g). Preparative t.l.c. (dichloromethane) gave a high R_f band which on extraction gave a yellowish oil. Trituration with petroleum-ether gave, on cooling, white crystals, m.p. 94-97°. Recrystallization from ether-petroleum gave white crystals of the title compound (660 mg), m.p. 98° (Found: C, 48.42; H, 3.89; N, 6.57; Cl, 33.50. $C_{17}H_{16}Cl_4N_2O_2$ requires C, 48.37; H, 3.82; N, 6.64; Cl, 33.59%). $\nu_{max.}$ ($CHCl_3$): 3325 (NH), 3015 (C-H), 2965/2840 (CH_2), 1754 (s)(N-CO-O), 1585, 1520 (s), 1480, 1463 (sh.), 1450, 1428, 1270, 1230, 1200 (s), 1178, 1132, 1068, 1038 (w), 1013, 934, 916, 890, 824, 700-670 (br). τ: 1.11 (1H, br, NH), 1.75 (1H, d, J_m= 2.5, aromatic), 2.41-3.18 (6H, m, aromatic), 6.58 (8H, q, NCH_2CH_2Cl).

3,4-Dichloronitrobenzene

It was obtained from Koch-Light Laboratories Ltd., m.p. 40.5-42° $\nu_{max.}$: 3090 (C-H), 1602, 1573, 1535 (NO_2), 1520 (sh.), 1348 (NO_2), 1292, 1250, 1160 (s), 1142, 1117 (w), 1044 (s), 907/900 (δC-H), 851/840 (C-N, δC-H), 785 (s) (C-Cl), 733 (C-Cl), 692.

3-Chloro-4-(di-2-hydroxyethylamino)nitrobenzene (536)

3,4-Dichloronitrobenzene (5 g) was reacted with diethanolamine (10 ml) at 118° for 24 h as described for the 2,3-dichloro isomer, obtaining a dark brown-black viscous oil which, on dissolving in dichloromethane and standing, gave partial crystallization. The mixture was filtered and the crystals were washed with dichloromethane-petroleum to give orange-yellow crystals (228.6 mg), m.p. 150-154°. The filtrate was chromatographed (preparative t.l.c. - ethyl acetate) to give, in order of decreasing R_f: a further amount of orange crystals (154.7 mg), m.p. 151-154°. Recrystallization from ether gave yellowish-orange crystals of 3,3',4,4'-tetrachloroazobenzene (9.2%), m.p. 152-154° (lit.,[475] m.p. 195.5° corr., [550] m.p. 158°)

(Found: C, 44.62; H, 2.11; N, 8.96; Cl, 44.64. Calc. for $C_{12}H_6Cl_4N_2$: C, 45.04; H, 1.89; N, 8.75; Cl, 44.32%). τ: 2.06 (2H, dd, $J_{o,m}$= 9, 2, aromatic), 2.26 (2H, d, J_m= 2, aromatic), 2.41 (2H, d, J_o= 9, aromatic); on trituration with ether-petroleum, brownish-yellow crystals of 3-chloro-4-(2-hydroxyethylamino)nitrobenzene [(2-chloro-(N-2-hydroxyethyl)-4-nitroaniline] (130 mg, 2.3%), m.p. 115-117° (lit.,[474] m.p. 120°), $\nu_{max.}$ and τ identical to those of the earlier sample (page 152); and a dark orange-brown oil of the title compound (4.15 g, 61%) (Found: C, 45.97; H, 5.22; N, 10.60; Cl, 13.80. $C_{10}H_{13}ClN_2O_4$ requires C, 46.08; H, 5.03; N, 10.75; Cl, 13.60%). $\nu_{max.}$(film): 3340 (s, OH), 2940/2890 (CH_2), 1584 (s), 1504 (NO_2), 1340 (NO_2), 1278, 1223, 1143 (sh.), 1130, 1065 (sh.), 1044, 1005, 900, 858, 835, 775, 755 (C-Cl), 725 (δC-H). τ: 1.68 (1H, d, J_m= 2, aromatic), 1.88 (1H, dd, $J_{o,m}$= 9, 2, aromatic), 2.65 (1H, d, J_o= 9, aromatic), 6.30 (8H, sextuplet, NCH_2CH_2Cl), 7.39 (2H, br s, OH).

3-Chloro-4-(di-2-chloroethylamino)nitrobenzene (585)

3-Chloro-4-(di-2-hydroxyethylamino)nitrobenzene (1.5234 g) in benzene (6 ml) was chlorinated with phosphoryl chloride as above to give the crude product as an oil (1.672 g) which was chromatographed (preparative t.l.c. - dichloromethane) affording a yellowish-orange oil of the title compound (1.256 g, 72%) (Found: C, 40.63; H, 3.66; N, 9.50; Cl, 35.86. $C_{10}H_{11}Cl_3N_2O_2$ requires C, 40.36; H, 3.73; N, 9.41; Cl, 35.74%). $\nu_{max.}$(film): 3095 (C-H), 2965/2860 (CH_2), 1580 (s), 1508 (NO_2), 1485 (sh.), 1458, 1440, 1382, 1337 (NO_2), 1280, 1255, 1190, 1125 (s), 1070, 1040, 1016, 1002, 962, 916 (sh.), 895, 834, 765, 747 (C-Cl), 720 (δC-H), 665. A small band of slightly lower R_f to that of the title compound, was extracted and crystallized to give yellow crystals of N-(2-chloro-4-nitrophenyl)morpholine (60.3 mg, 4.3%), m.p. 125-126° (Found: C, 49.22; H, 4.36; N, 11.63; Cl, 14.53. $C_{10}H_{11}ClN_2O_3$ requires C, 49.50; H, 4.57; N, 11.54; Cl, 14.61%). τ: 1.69 (1H, d, J_m= 2.5, aromatic), 1.84 (1H, dd, $J_{o,m}$= 9, 2.5, aromatic), 2.89 (1H, d, J_o= 9, aromatic), 6.06 (4H, t, C-CH_2-O), 6.76 (4H, t, N-CH_2-C).

3-Chloro-4-(di-2-chloroethylamino)aniline (661)

3-Chloro-4-(di-2-chloroethylamino)nitrobenzene (1.137 g) was hydrogenated using the procedure adopted for the 2,3-dichloronitrobenzene derivative. On filtration, the methanolic acidic solution turned dark blue-black. So it was condensed affording a dark blue-black oil of the crude title compound hydrochloride which was immediately characterized as its carbamate.

2'-Chlorophenyl N-[3-chloro-4-(di-2-chloroethylamino)] phenylcarbamate (662)

The above crude product was refluxed in benzene (25 ml) containing pyridine (1 ml) and 2-chlorophenyl chloroformate (730 mg) was added. The mixture was refluxed for 2 h, then neutralized, washed, dried, condensed and chromatographed (preparative t.l.c. - dichloromethane) to give a yellowish oil crystallizing from dichloromethane - petroleum, on cooling, to white crystals. When filtration was attempted, the crystals started melting at room temperature. Recrystallization from ether-petroleum with cooling afforded white crystals of the title compound in good yield, m.p. 28-32° (Found: C, 48.31; H, 4.16; N, 6.48; Cl, 32.87. $C_{17}H_{16}Cl_4N_2O_2$ requires C, 48.37; H, 3.82; N, 6.64; Cl, 33.59%). Drying this low m.p. solid was extremely difficult particularly as the room temperature was 31°. $\nu_{max.}$ (film): 3300 (NH), 3070 (C-H), 2975/2865 (CH_2), 1750/1720 (sh.)(N-CO-O), 1608, 1583, 1525, 1503, 1480, 1450, 1405/1395, 1310, 1270, 1220 (s), 1165, 1140, 1110 (Ar-Cl), 1065, 1035, 1015, 940, 920, 885, 855, 835, 800, 755 (C-Cl), 735 (δC-H), 686. τ: 2.00-3.00 (8H, m, aromatic & NH), 6.48 (8H, s, NCH_2CH_2Cl).

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