A Thesis entitled

STUDIES ON SOME POTENTIALLY CYTOTOXIC COMPOUNDS IN THE NITROGEN- AND SULPHUR- MUSTARD SERIES

presented by

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ABSTRACT

Part I:

Several new phenylthio-esters designed to be selectively cytotoxic for tumours, have been synthesised, by acylation of p-[di-(2-chloroethyl)amino]thiophenol, or the dibromo analogue, with a variety of α-N-acylamino-acids in the presence of dicyclohexylcarbodi-imide. A few 'Model' compounds analogous to the new mustards were also prepared from thiophenol or from p-(diethylamino)thiophenol, as possible 'enzymic potentiators' for the mustards. In all these preparations, the amino group of the amino-acid moiety was protected with enzymically fissionable groups such as formyl, acetyl and methoxycarbonyl. The extent of hydrolytic fission of the halogen atoms under standard conditions revealed that the deactivation of the mustard, brought about by linkage of the thiol group to the amino-acid residues, is approximately the same as that caused by simple acetylation.

Part II:

2-(Phenylthio)propane-1,3-diol has been synthesised

from 1,3-O-benzylideneglycerol. The alkylating properties of the 1,3-dimethanesulphonate derived from this diol, and of the isomeric 1,2-dimethanesulphonate, were investigated in detail by a study of their reactions with various nucleophiles. With dry acetic acid (a weak nucleophile) the 1,3-compound underwent molecular rearrangement to give only the 1,2-diacetate, whilst with methanol it gave a partly rearranged product; identical products were obtained from the 1,2-dimethanesulphonate, indicating that the reactions proceed through a common sulphonium intermediate. Attack of a powerful nucleophile, such as the benzylmercaptide anion, proceeded normally on both dimethanesulphonates without rearrangement. With a strong base (methoxide ion), each dimethanesulphonate underwent elimination rather than substitution. Unsaturated products were also formed in the reactions with tetraethylammonium acetate.

Nuclear magnetic resonance spectroscopy was particularly useful for the identification of the products and for establishing whether molecular rearrangements involving cyclic sulphonium intermediates had occurred.

This thesis is dedicated to

my

mother, wife and children.

ACKNOWLEDGMENTS

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I also wish to record my thanks to Professor D.H.R. Barton for the opportunity provided to work in his department.

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I wish to thank the staff of the Microanalytical Laboratory of the college, for analyses, Mrs. A.I. Boston for the nuclear magnetic resonance spectrographic information, and the technical staff of the department for their cooperation.

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M.V.A. Baig,
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CONTENTS

Part I

NITROGEN MUSTARDS

CHAPTER I

CANCER AND ITS CHEMOTHERAPY

'The degree to which a disease is open to therapeutic attack is inversely related to the number of remedies that we possess.'

Otto Volker.

CANCER is a covering term under which are grouped a number of diseases which are characterized by the invasive growth of normal healthy tissue by an abnormal tissue, although they may differ in presentation. As a disease it was recognized in antiquity and presents perhaps the most serious challenge offered by nature to science. 'Oncology' or 'research in the field of cancer', requires a close coordination of many independent scientific disciplines viz. clinical medicine, surgery, pathology, radiation physics, genetics, immunology, endocrinology,and biochemistry, with the common object of controlling the neoplastic transformation. The disappointments, so far experienced, in the practical control of this horrible disease, suggest that success depends only on the basis of a deep and multifaceted understanding of the nature of neoplastic cells.

CHEMOTHERAPY is essentially a study of selective inhibition or regression of a parasite species with minimum damage to the host species, by chemical means.

The art of chemotherapy is as old as civilisation, but the science of chemotherapy is the child of today. Ehrlich is regarded as the father of the science of chemotherapy. The quest for a medical, as opposed to surgical, treatment is also very ancient. Perhaps the treatment of cancer began with the local application of Arsenic, Zinc and various other caustics in ancient India, Egypt and Persia. With the progress in surgical skill, the use of caustics in cancer therapy became less popular. Perhaps on the principle of 'setting a thief to catch a thief', tumours themselves have been used for treatment. This sort of experimentation was soon abandoned when found not only useless but possibly dangerous and wholly unjustifiable from a scientific point of view.

During the early part of the 20th century more effective surgery was developed and the value of irradiation in the treatment of cancer was discovered. Since then surgery, radiation therapy and chemotherapy came to be regarded as complementary to each other. Special interest in chemotherapeutic investigations in

cancer was aroused when Von Wasserman and his associates reported in 1911 the inhibitory effect of subcutaneously or intravenously administered selenium and eosin on certain animal tumours.

The problem of selective toxicity of a chemotherapeutic agent towards a cancerous species is particularly difficult to achieve because of the marked similarity between the cancerous species and its normal ancestor. Any selective cytotoxicity arrived at, is frequently overcome by the ability of the tumour to develop drug resistance. Consequently it is desirable to make a wide range of anti-cancer drugs so that if drug resistance is noted with one, the treatment may be continued with another.

Although Warburg[1] concluded in a biochemical investigation that all the known chemical constituents of normal tissues were also present in cancerous tissues, certain quantitative chemical differences between host and parasite are our real hope to be taken into account in designing chemotherapeutic agents. Danielli[2] has pointed that 'the larger the number of cell variables concerned in determining a drug action, the more selective will that drug be.' There are a number of biological variables which are concerned in

determining the specificity of a drug, and these must be exploited successfully to achieve the highest possible degree of selectivity. The modern tendency of workers in the field of cancer therapy has been to elaborate a relatively simple chemical molecule of known anti-cancer activity, with the object of increasing the specificity, whilst leaving the active centre intact (or reproducible *in vivo*).

As it is not possible to present here in detail the vast field of the chemotherapy of cancer, the reader is directed to the excellent reviews by Stock[3] and Farber *et al.*[4]; the publications[5,6] of the American Association for the Advancement of Science; and Dyer's[7] tabulated compilation of data on clinical and experimental investigations.

CHAPTER 2

CHEMOTHERAPEUTIC AGENTS

The majority of the compounds, used for the chemotherapeutic treatment of cancer, are classified into two main groups, viz. (A) the 'antimetabolites and, (B) the 'biological alkylating agents'.

A. Antimetabolites

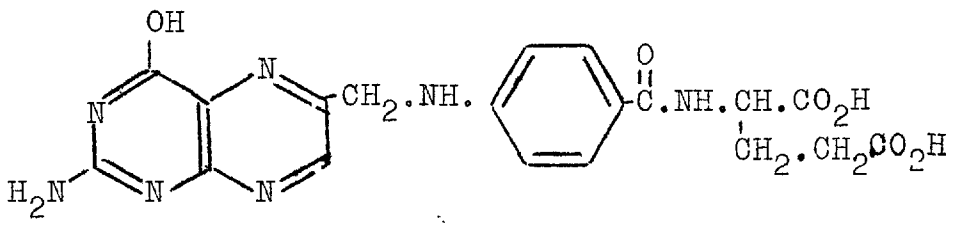
Antimetabolites may be further classified into two sub-groups. The first group consists of antagonists to folic acid, to riboflavin or pyridoxine. The second group comprises antagonists to purines, pyrimidines, amino-acids etc.

Antimetabolites are largely derivatives of the parent metabolites. Their inhibitory effect is specific for certain reactions and probably depends upon their ability to block certain metabolic processes for which the parent compounds are essential, and is reversed by supplying a large excess of the metabolite.

1. Folic Acid Antagonists or 'Antifolics'

The folic acid antagonists prevent or obstruct the

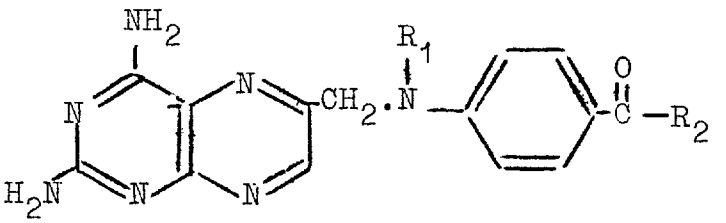
transformation of folic acid to folinic acid which acts as a co-factor in nucleic acid synthesis. Leuchtenberger and his colleagues[8] reported the significant tumour-growth inhibitory effect of folic acid. Pteroylglutamic acid or folic acid is represented by (I).



I

Pteridine p-aminobenzoic acid glutamic acid

The following 'aminopterins' are powerful inhibitors of the enzyme dihydrofolic reductase. It is this group of compounds that is most widely used in cancer chemotherapy.



II

4-Aminoptercylglutamic acid ('Aminopterin'). [R_1 = H; R_2 = glutamic acid]

4-Amino-N^{10}-methyl-pteroylglutamic acid ('A-methopterin' or 'Methotrexate' MTX). [R_1 = Me; R_2 = glutamic acid]

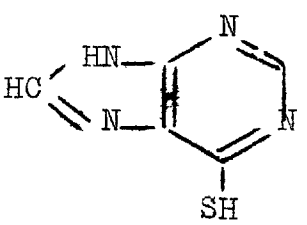
4-Aminopteroyl aspartic acid ('Amino-an-fol'). [R_1 = H; R_2 = aspartic acid].

Aminopterin was reported to induce a high frequency of remissions in acute leukemia, especially in children. It was soon largely replaced by the comparatively less toxic MTX and amino-an-fol, which showed greater activity.

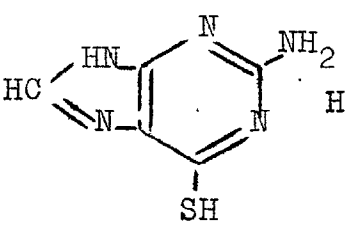
2. Purine and Pyrimidine Antagonists

The division of a cell depends upon the synthesis of DNA ('deoxyribose nucleic acid'). In this process purines and pyrimidines are required as precursors. Hence if the cell is fed with the antagonists of purines and pyrimidines, they will interfere with the synthesis of nucleic acid, and thus mitosis of the cell may be hindered or stopped.

6-Mercaptopurine (III) was found to inhibit growth and sometimes to cause permanent regression of sarcoma 180. The activity of thioguanine (IV) is similar to that of (III). The gastrointestinal side effects seriously limited the usefulness of 2,6-diaminopurine (V) and it is now only of historic interest.



III



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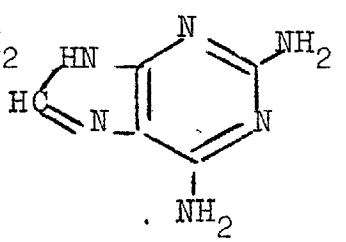
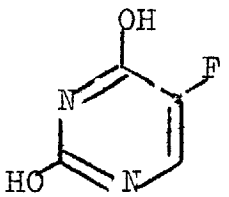
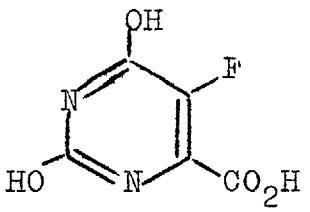


V

Heidelberger et al.[9] have synthesised a number of 5-fluoropyrimidines and their 2-thio derivatives. 5-Fluorouracil (VI) and 5-fluoro-orotic acid (VII) exert considerable anti-tumour activity against transplanted tumours in animals because of the obstruction in the synthesis of DNA.



VI



VII

3. Amino-Acid Antagonists

It is said[10] that the syntheses of proteins and ribonucleic acid go simultaneously during the growth, and, generally, inhibitors of ribonucleic acid synthesis produce simultaneous inhibition of protein synthesis. Various results suggested that somewhere in the synthetic chain the compounds of amino-acids with nucleotides act as common precursors for both protein and nucleic acid syntheses. Hence amino-acid antagonists merit investigation as possible chemotherapeutic agents. It is also believed that α-methyl amino-acids are strongly concentrated intracellularly. Thus Connors and Ross[11] were prompted to prepare α-methylated methionine,

ethionine, cysteine etc. in an attempt to produce antagonists for the naturally occurring amino-acids but with no useful tumour inhibiting properties.

4. Carbamates

Ethyl-(VIII) and isopropyl-(IX) phenylcarbamates[12] and simple urethane[3] (X) produced inhibition in the growth of the Walker rat carcinoma 256.

$Ph.NH.CO.OEt$ $Ph.NH.CO.OPr^{i}$ $NH_2.CO.OEt$

VIII IX X

It was observed[13] that patients suffering from myeloid leukemia showed undoubted response to simple urethane in that a dramatic fall took place in the white cell-count, but in cases of lymphatic leukemia the response was less effective. The effect of urethane and deep x-ray therapy are strikingly similar both in the differential count and the duration of the time required for the response.

For detailed information on the subject, the reader is recommended to the works of Farber[4] and Delmonte[14].

B. The Biological Alkylating Agents

These compounds, which can function as alkylating agents under physiological conditions of temperature and pH, have recently been reviewed by Ross[15]. This group includes methanesulphonyl esters of simple diols and of sugars, polyepoxides, polyethyleneimines and the nitrogen mustards.

1. Sulphonic Acid Esters

Extensive studies on the dimethanesulphonates of dialkanolamines and simple aliphatic diols by Timmis[16] resulted in the development of 'Myleran' (1, 4-dimethanesulphonyloxybutane) (XI), which was found[17] to be a powerful inhibitor of the transplanted carcinoma in the rat and a useful drug for the treatment of myeloid leukemia.

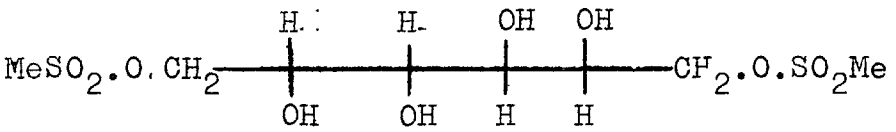
$$MeSO_2.O.(CH_2)_4.O.SO_2Me \qquad XI$$

2-Chloroethylmethanesulphonate[18] (XII) and ethyl-methanesulphonate[19] (XIII) showed effective inhibition on the growth of the transplanted Walker rat carcinoma 256.

$$Me.SO_2.O.CH_2.CH_2Cl \qquad XII$$

$$Me.SO_2.O.Et \qquad XIII$$

This work was extended to synthesise Myleran-like derivatives, and the first compound of the series, the 'mannitol myleran' (1,6-di-O-methane-sulphonyl-D-mannitol) (XIV) was shown[20] to possess powerful tumour-inhibiting properties.



XIV

Further work on polyol dimethane-sulphonates revealed that both the methanesulphonyloxy groups are required to be present at the end of the chain[21].

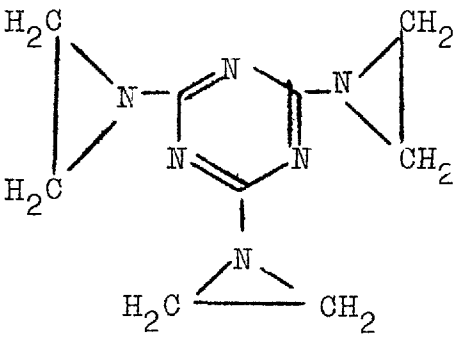
On the basis of structural similarity to myleran, the synthesis and screening of certain bifunctional aliphatic nitrososulphonamides[22] of the type XV, where n = 3, 4 and 5, were reported.

$$Me.N(NO).O_2S.(CH_2)_n.SO_2(NO)N.Me \qquad XV$$

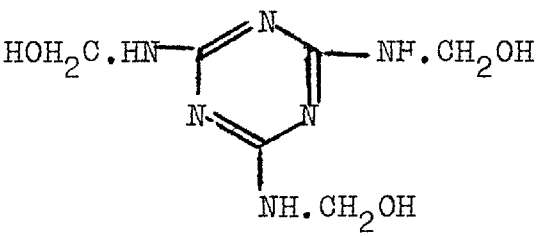
2. Polyethyleneimines

The two factors which evoked the investigation of a number of polyethyleneimino-compounds were:
(i) the suggestion that the mode of action of nitrogen mustards might involve an ethyleneimine-type ion as an intermediate, and, (ii) the necessity for at least two possible active centres for anti-tumour activity.

The most extensively studied of the polyethyleneimines is the so-called triethylenemelamine (TEM) otherwise 2, 4, 6-tris-ethyleneimino-1, 3, 5-triazine[23,24] (XVI), a cross linking agent which was developed in the German synthetic fibre industry. Although quite toxic, it had found clinical application. It was found very useful in inhibiting sarcoma 180 and Hodgkin's disease.



XVI

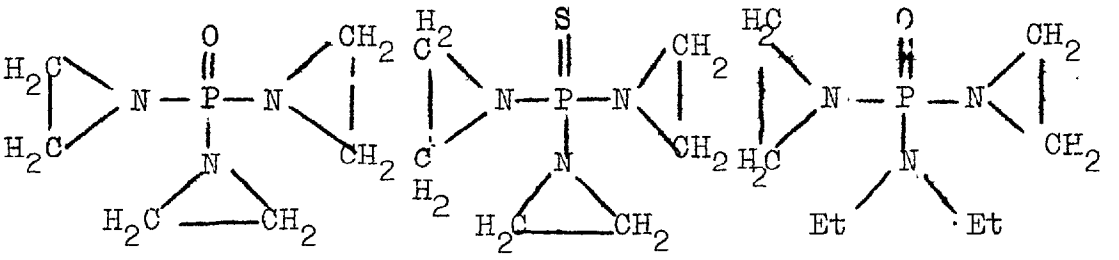


XVII

Trimethylolmelamine (XVII) and several other polymethylolamides were also found[25] active as tumour inhibitors.

Extension of these studies to the ethylenephosphoramides has resulted in the preparation of a number of compounds[26] of chemotherapeutic importance, e.g. N, N', N''-triethylenephosphoramide (XVIII); N, N', N''-triethylenethiophosphoramide (XIX) and N, N-diethyl-N',N'' -diethylene-

phosphoramide (XX).

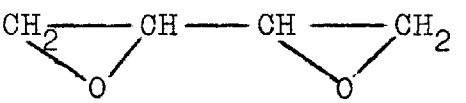


XVIII TEPA XIX Thio-TEPA XX DEPA

The phosphoramides were synthesised by the American Cynamid Research Laboratories and were found to be active against sarcoma 180 in mice. Thio-TEPA has been reported the most active compound in preventing metastases of rat mammary adenocarcinoma.

3. Bis-Epoxides

Hendry et al.[27] found that the bis-epoxides, such as butadiene dioxide (XXI), were inhibitory for the Walker rat carcinoma 256, but only at toxic levels.



XXI

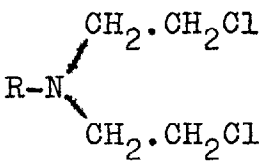
In addition, the compounds were found to be carcinogenic, therefore it was concluded that they would be unlikely to have any therapeutic application.

4. Nitrogen Mustards

During the Second World War, the mustards HN2 (XXII) and HN3 (XXIII) were primarily designed as highly toxic vesicants suitable for tactical use in chemical warfare. Their hydrochlorides were used to study their physiological action and clinical use in the treatment of neoplastic disease. Quite stimulating results were reported by

$Me.N(CH_2.CH_2Cl)_2$ ... XXII $N(CH_2.CH_2Cl)_3$ ... XXIII

Gilman and Philips[28] and Rhoads[29], which gave great impetus to the search for effective cancer chemotherapeutics in general and nitrogen mustards in particular. In the beginning HN2 was used almost exclusively for extensive clinical trial, but its therapeutic application has been limited[30]. Although many encouraging but imperfect results were published from time to time, the so-called 'nitrogen mustards' as active inhibitors of cell division, are still in the process of extensive study as possible anti-cancer agents. Their fundamental chemical structure is (XXIV). The bis(β-chloroethyl) group is apparently essential for the toxicological characteristics of these agents. Although many of them have proved valuable in the treatment of certain neoplastic diseases, the limiting factor in their



XXIV

chemotherapeutic use has been their injurious effect on certain healthy tissues, and thus their initial promise as curative agents has not been fulfilled so far.

An account will now be given of the attempts which have been made to make these compounds more efficient in their action.

Some Approaches to Drug Design

'If no use is made of the labours of past ages, the world must remain always in the infancy of knowledge.'

Cicero.

In the therapy of cancer, the aim is to arrest the cell multiplication responsible for malignant growth. Injury to the mitotic mechanism is one way by which cell multiplication can be stopped. Screening of a great number of compounds and exploitation of the differences between normal and neoplastic cells will be a rational approach in modifying the structure of the drug as an approach to the problem of selective action on a tumour. The problem is acute in the case of nitrogen

mustards as they attack all proliferating tissue indiscriminately. The biological variables[31], which must be exploited, as far as possible, to attain drugs of highest possible degree of therapeutic efficiency, include (a) enzymic constitution, (b) permeability properties, (c) facilitated diffusion and (d) adaptation and resistance.

The following are the principal methods for obtaining selective activity.

1. Enzymic Activation

Liberation of cytotoxic compounds from non-toxic compounds by the action of an enzyme *in vivo*, is referred to as 'enzymic activation'. This method has been quite widely exploited in an attempt to increase the selectivity of the nitrogen mustards. To accomplish this a nitrogen mustard is at first 'deactivated' so that little or no reaction occurs in its passage through normal cells, and then on arrival at the tumour site the free active nitrogen mustard must be regenerated so as to attack the neoplastic cells. Hebborn and Danielli[32] during their studies on *p*-(*NN*-di-2-chloroethyl)-aminophenol (XXV) and *p*-(*NN*-di-2-chloroethyl)-phenylenediamine (XXVI) and their acylation products, found that when a tumour

contains an enzyme capable of splitting off the acyl group, selectivity is increased. There is a complete correlation between the presence of an activating enzyme in the tumour and the inhibitory action of the drug.

<u>p</u>-HO.C_6H_4.N(CH_2.CH_2Cl)$_2$ XXV

<u>p</u>-RHN.C_6H_4.N(CH_2.CH_2Cl)$_2$

where, R = H XXVI; R = .COEt XXVIII

R = Ac XXVII; R = Bz XXIX

Let us consider the nitrogen mustard (XXVI). This parent amine is known to be a substance of high toxicity and powerful vesicant action[33]. The introduction of electron-withdrawing acyl groups causes a considerable fall in the toxicity as well as in the alkylating power, but the <u>N</u>-acyl compound (XXVII) and the <u>N</u>-propionyl derivative (XXVIII) retain significant effect on the tumour, whilst the <u>N</u>-benzoyl derivative (XXIX) is inactive. The tumour was found to possess an enzyme which can deacylate an acetamido- but not a benzoylamido-group, liberating the parent amine mustard (XXVI) from (XXVII) but not from the more resistant benzoate (XXIX). Thus it can be seen that should a tumour possess enzymes at levels higher than in the normal tissue, then they

can be used to liberate an active nitrogen mustard from its inactive derivative.

A large number of compounds have been prepared to show selective action by enzymic hydrolysis, reduction or oxidation. In tumour growth inhibition tests, the O-acetate and O-benzoate derivatives of p-(NN-di-2-chloroethyl)-aminophenol (XXV) were found to be more active than the phenol itself although the chlorine atoms in them were shown to be comparatively less reactive. This may be due to the liberation of the phenol within cells into which the free phenol could not so easily diffuse.

Ross and Wilson[34] turned their attention to prepare some NN-di-2-chloroalkyl derivatives of aliphatic and aromatic nitrogen mustard carboxy-amides of the type (XXX), which were expected to liberate tumour growth inhibitory di-2-chloroalkylamines by the hydrolysis (enzymic action) *in vivo* of the amide linkage. But this hope could not be fulfilled due to the rearrangement of the carboxyamides to the esters 2-acyloxy-ethyl-2'-chloroethylammonium chlorides (XXXI).

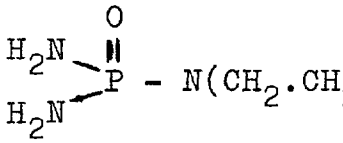
$$\underset{\text{XXX}}{R.CO.N(CH_2.CH_2Cl)_2} \xrightarrow[\text{Acetone/HCl}]{H_2O} \underset{\text{XXXI}}{R.CO_2.CH_2.CH_2.NH(HCl).CH_2.CH_2Cl}$$

However, the succinic acid derivative (XXXII) and the phthalic acid derivative (XXXIII) showed activity of a low order.

$HO_2C.CH_2.CH_2.CO.N(CH_2.CH_2Cl)_2$ XXXII

<u>o</u>-$HO_2C.C_6H_4.CO.N(CH_2.CH_2Cl)_2$ XXXIII

As the chemical activity and the biological potency of the nitrogen mustard is presumed to depend on the basicity of the nitrogen atom, it is expected that <u>N</u>-phosphorylation might give a product significantly less toxic than the parent mustard. <u>NN</u>-Di-(2-chloroethyl) phosphorotriamidate (XXXIV) was prepared[35] and was found to have only a fraction of the toxicity of the parent bis(β-chloroethyl)-amine. Phosphamidated mustard would be expected to yield the parent mustard in cells of tumours if the enzyme, phosphamidase, were present. Ross <u>et al.</u>[36] prepared the diethyl (XXXV; R = Et) and the diphenyl phosphoramidate (XXXV: R = Ph), related to the amine (XXVI). It was found that only the diethyl derivative was an active tumour-growth inhibitor.



XXXIV

<u>p</u>-$(RO)_2PO.NH.C_6H_4.N(CH_2.CH_2Cl)_2$ XXXV

Nitromin[15] (XXXVI), an oxidation product of HN2 has caused complete regression of the Yoshida sarcoma, presumably due to its reduction *in vivo* to the parent amine HN2 by a reductase present at a higher level in the sarcoma.

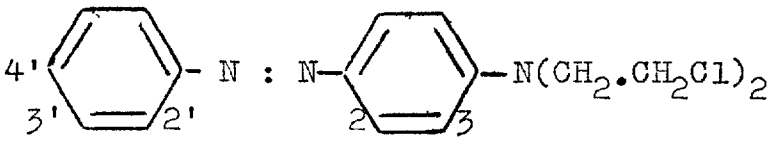
$CH_3.N(\rightarrow O).(CH_2.CH_2Cl)_2$ XXXVI

The moderate anti-tumour activity of p-(NN-di-2-chloroethylamino)-benzaldehyde XXXVII, in spite of unreactive chlorine atoms, is said to be due to its oxidation[37] by enzymic activation into the amino-benzoic acid derivative (XXXVIII), which exists in the more reactive anionic form at physiological pH.

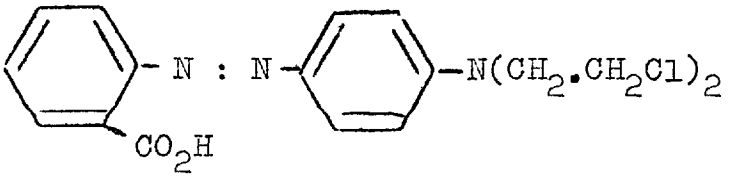
p-$OHC.C_6H_4.N(CH_2.CH_2Cl)_2$ XXXVII

p-$HOOC.C_6H_4.N(CH_2.CH_2Cl)_2$ XXXVIII

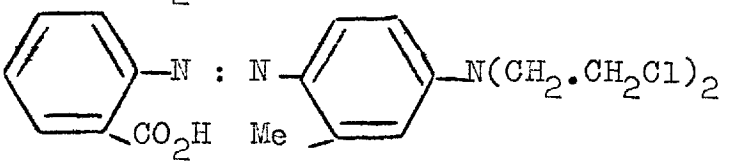
Since the azo compound (XXXIX) and its 2'-carboxy derivative (XL) were known to inhibit the growth of the transplanted Walker rat carcinoma, Ross and Warwick[38] synthesised a large number of substituted 4-[di-(2-chloro-ethyl)amino] azobenzenes. Among them 2'-carboxy-2-methyl-derivative (XLI) had the most promising biological activity, being 10 fold[39] that of the parent azo compound (XXXIX).



XXXIX



XL



XLI

The azo compound probably owed its activity to a reduction *in vivo* into the amine, e.g. (XXXIX) is reduced to the parent amine (XXVI), the active entity. The compound (XLI) thus ranks as one of the most active nitrogen mustards.

In view of the importance of thiol groups in biological systems, the thiol mustards p-(NN-di-2-chloroethylamino)thiophenol (XLII), its bromo analogue (XLIII) and thier derivatives were synthesised[40].

M.SH XLII M'.SH XLIII

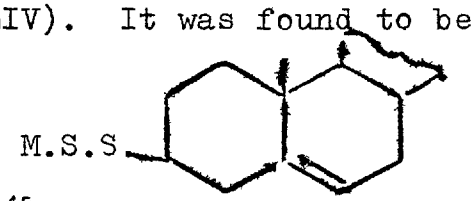
where M = p-C_6H_4.N(CH_2.CH_2Cl)$_2$ and

M' = p-C_6H_4.N(CH_2.CH_2Br)$_2$

The thiophenol mustard M.SH was shown[41,42] to have only a moderate activity against experimental tumours whilst its acyl (M.SAc, M.SBz) and carbamic acid

($M.S.CONH_2$) derivatives were quite inactive. However, M'.SH and M'.SCN both caused a high proportion of complete regressions and were non-toxic.

Since a disulphide is subject to reductive fission *in vivo*, some mixed disulphides[40], derived from the thiol mustard M.SH or M'.SH with other thiols were synthesised, e.g. α-cholesteryl *p*-(*NN*-di-2'-chloroethylamino)phenyl-disulphide (XLIV). It was found to be relatively inactive.



XLIV

Compounds[43] in which the nitrogen mustard unit was linked to a molecule which was toxic by itself (e.g. 2,4-dinitrophenol), were quite interesting, because the product, which might as such show diminished toxicity, on fission *in vivo* would liberate two toxic substances and thus was expected to show greater activity e.g.

M.S.S.(2,4-dinitrophenyl) XLV

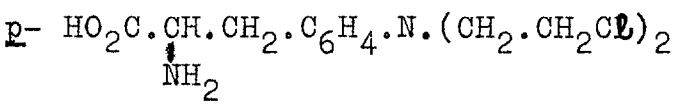
and M'.S.S.(2,4-dinitrophenyl) XLVI

2. Facilitated Diffusion or Active Transport

It is known[31] that molecules of physiological importance, such as those of natural amino acids or sugars are able to pass more selectively than other

molecules through cell membranes. The approach by exploitation of this variable remains very limited as not much is known about its mechanism.

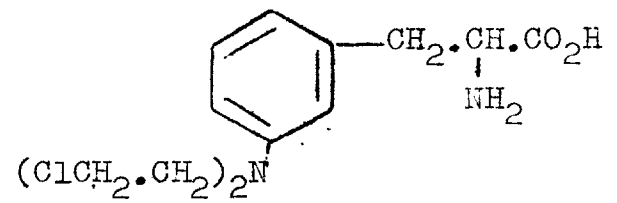
Compounds of this type have been prepared, the most outstanding being DL-p-di-(2-chloroethylamino) phenylalanine (named 'Sarcolysine') (XLVII).



XLVII

It was synthesised by Bergel and Stock[44] and was independently studied by Larionov et al.[45], who found it to be particularly active against sarcomas.

'Melphalan', p-(NN-di-2-chloroethyl)amino-L-phenylalanine, produced intense inhibition[44,47] of growth of the Walker rat carcinoma (1 mg/kg. producing complete inhibition of the tumours). It is more active than its racemic form sarcolysine. The m-isomer[48] (XLVIII) of sarcolysine has been synthesised both in California and Russia.

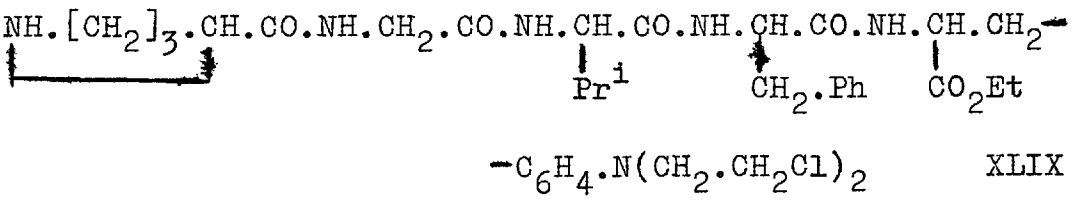


XLVIII

It was more than 4 times as effective as sarcolysine against adenocarcinoma and only twice as toxic.

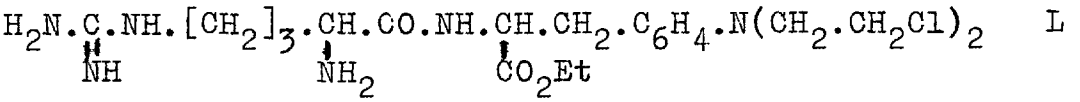
As melphalan also powerfully affects the blood-forming organs and has a high general toxicity, Bergel and Stock[49] have prepared derivatives, which fall into two main groups: (1) simple derivatives including amides and esters, and (2) di-, tri- and tetra-peptides. It was found that the general toxicity, haemotoxicity and anti-tumour activity (with special reference to Walker rat carcinoma 256) were exhibited to the highest degree in those derivatives which possessed a free primary amine group.

A series of N-acetyl-L- and -D-aminoacyl derivatives of melphalan ethyl ester was synthesised, and it was noticed[50] that large differences in anti-tumoural activity exist between the L-L and D-L stereoisomeric pairs; the L-L pair being more active than the D-L pair by a factor of up to 100. Johnson and Stock[51] extended this work to synthesise a pentapeptide of the arbitrary sequence prolylglycylvalylphenylalanylmelphalan (XLIX) (all the asymmetric centres having the L-configuration). This compound markedly inhibited the growth of the Walker rat carcinoma 256 at a single dose of 5 mg./kg., approximately one tenth of the toxic dose.



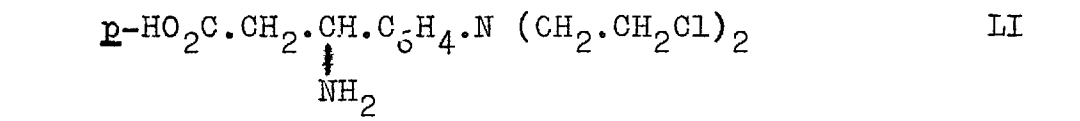
XLIX

They also prepared[51] another derivative, L-arginylmelphalan (L). This peptide completely inhibited the Walker tumour at a single dose of 2 mg./kg., approximately one twelfth of the toxic dose.



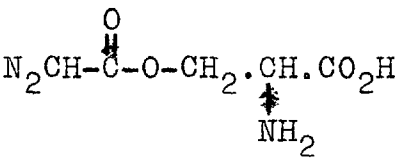
L

The synthesis of the β-amino analogue of sarcolysine, namely, p-(NN-di-2-chloroethyl)aminophenyl-DL-β-alanine (LI), has been reported[52] both in England and Russia. It was found to inhibit completely the Walker rat carcinoma 256 at a dose of 20 mg./kg.

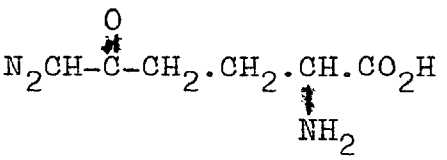


LI

Interest in diazo derivatives of amino-acids has been stimulated by the discoveries that 'azaserine',[53] O-diazoacetyl-L-serine (LII) and 'DON'[54] 6-diazo-5-oxo-L-norleucine (LIII) showed potent antitumour activity against sarcoma 180. It is not clear yet whether azaserine will be useful against human cancer. Two such compounds, namely, 3-(p-diazoacetylphenyl)propionic acid

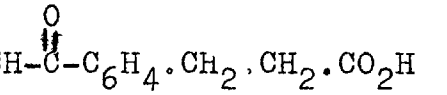


LII

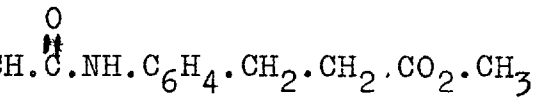


LIII

(LIV), and methyl 3-(p-diazoacetamidophenyl)-propionate (LV) were synthesised[55], and were evaluated against various tumours. The only positive activity observed was a 37% life extension by the former on mice bearing Ehrlich ascites.

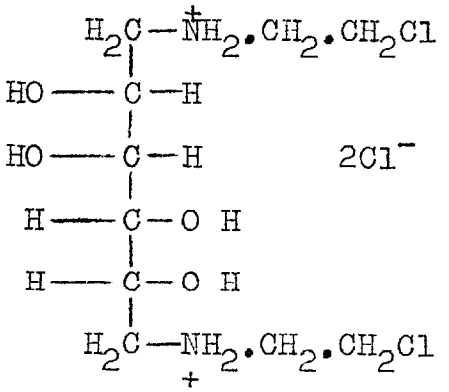


LIV

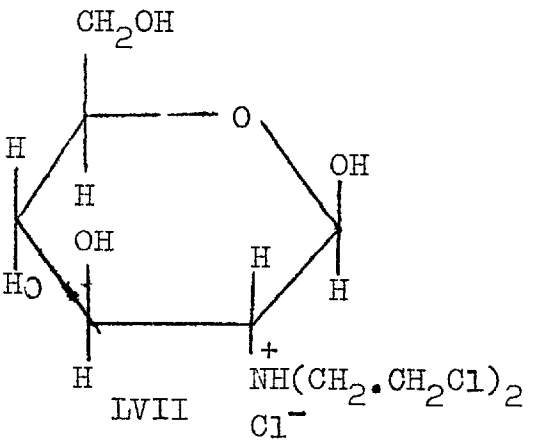


LV

Vargha et al.[56] prepared 1, 6-di-(2-chloroethylamino) -1, 6-dideoxy-D-mannitol dihydrochloride ('BCM') (LVI) which has shown strong cytoactive and tumour-inhibiting activity. Also was prepared[57] NN-di(2-chloroethyl)-D-glucosamine hydrochloride (LVII) but it gave disappointing results being almost as toxic as nitrogen mustard HN2.

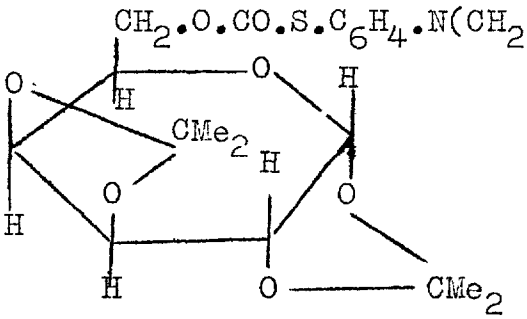


LVI



LVII

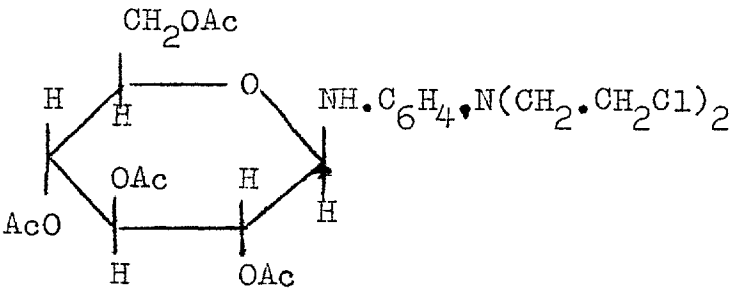
Creighton[41] synthesised isopropylidene derivatives of D-galactose of the type (LVIII) with the idea of removing the isopropylidene groups to give the free sugar, which should be activated by the active transport mechanism.



where X = Br or Cl

LVIII

p-(NN-di-2-chloroethylamino)phenyl 2', 3', 4', 6' - tetra-O-acetyl-β-D-glucosylamine (LIX) did not show much activity and was highly toxic[58].



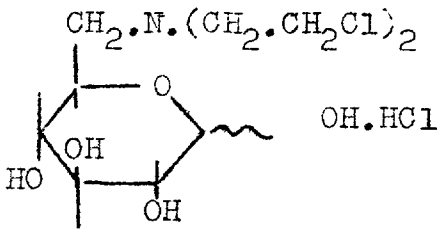
LIX

Ross *et al.*[59] prepared a number of derivatives (LX) of *NN*-di-2-chloroethylaniline containing carboxyl group. Compound (LX; n = 1) shows high activity, whilst *p*-(*NN*-di-2-chloroethylamino)phenyl butyric acid 'Chlorambucil' (LX; n = 3) was found[60] to be a powerful inhibitor of the transplanted Walker rat tumour 256. This alkylating agent is also most effective in Hodgkin's disease and allied lymphomas.

p-$(ClCH_2.CH_2)_2.N.C_6H_4.[CH_2]_n.CO_2H$ LX

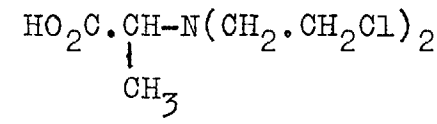
where n = 0 to 4.

Reist *et al.*[61] instituted a programme to synthesise a variety of nitrogen mustards of amino sugars, but only one (LXI) of them namely 6-[bis(2-chloroethyl)-amino]-6-deoxy-D-glucose hydrochloride, derived from 6-amino-6-deoxy-D-glucose, showed selective action by an active transport mechanism.



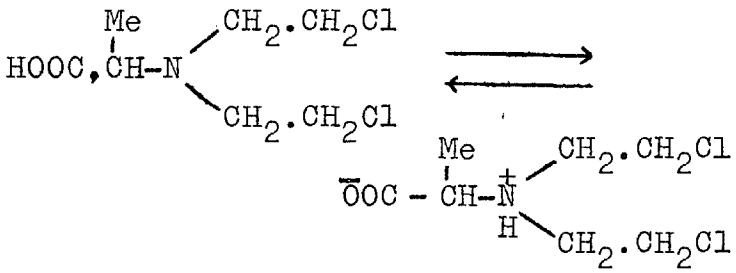
LXI

Izumi[46] reported that *N*-bis(β-chloroethyl)alanine (LXII) is predominantly effective against a wide range of neoplastic diseases. This compound has shown the

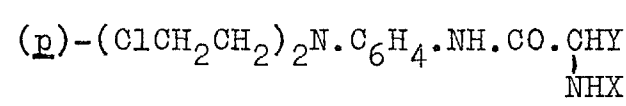


LXII

most surpassing chemotherapeutic index ($\frac{LD_{50}}{MED} = \frac{30}{0.05} = 600$). Its most predominant anti-cancer efficiency was presumed partly due to the formation of an amphoteric ion in the animal body.



It was thought that if the release of the active nitrogen mustard is made dependent upon the successive action of two or more enzymes, the selectivity of the drug should be increased. Based upon this principle a great number of α-(acylamino)acyl derivatives (LXIII) were prepared[62,63] by the condensation of the amino mustard (XXVI) with the appropriate α-N-acylamino acid. The amino-acids used were glycine, DL-methionine, DL-ethionine



LXIII

etc., the α-N-acyl groups 'X' were formyl, acetyl, fluoroacetyl, dichloroacetyl, or methoxycarbonyl. The methoxycarbon

-yl

group is of special interest because of the presence of the -NH.CO.OMe (urethane) grouping in the product, the fission of which at the tumour site should be capable of potentiation by prior administration of a simple urethane (see the next section). It was presumed that on administration of such derivatives (LXIII), the acyl group 'X' would undergo enzymic removal first, producing the intermediate (LXIII; X = H) which would then be attacked by another enzyme (aminopeptidase), when the active nitrogen mustard (XXVI) would be released due to the fission of the amide linkage.

3. Adaptation and Resistance

The phenomena of drug resistance and dependence are among the most obscure in experimental therapeutics and perhaps the most troublesome in actual practice. For two reasons at least, namely, the toxicity of the drug and the resistance developed to them, no agent has been found as yet to be completely effective in the treatment of cancer.

Carbamates[12] are known to possess cytotoxic properties, but tumours which are initially sensitive to such compounds generally soon become resistant to them. Danielli et al.[42] suggested that this phenomenon is due

to the formation of an 'adaptive enzyme', which can destroy the urethane by fission of the ester or amide linkage, and, if this is so, the formation of an adaptive enzyme may then be used to activate a detoxicated carbamate mustard. In favour of his hypothesis[31] that where a tumour becomes resistant to a urethane, it will be hypersensitive to a 'urethane nitrogen mustard', Danielli presented evidence[64] that administration of isopropyl phenylurethane (IX) alone induced only a small

$$Ph.NH.CO.OPr^{i} \qquad IX$$

decrease in the rate of tumour growth, and dosage with (LXIV) alone caused growth inhibition but no regression. However, if (IX) was administered first in a small

$$p\text{-} \quad (ClCH_2.CH_2)_2\ N.C_6H_4.NH.CO.OPr^{i} \qquad LXIV$$

dosage for several days and then discontinued before treatment with the mustard urethane (LXIV) for 10 days, complete and permanent regression of the Walker carcinoma was recorded. Thus compound (IX) has proved to be a highly effective potentiator This principle has, however, been extended to other types of nitrogen mustards. In the following table five compounds and their potentiators are given.

Nitrogen Mustard	Potentiator	%CR with mustard alone	% CR with potentiator followed by mustard
M'.NH.CO.OPr^i	Ph.NH.CO.OPr^i	0	75
M'.S.CO.OPr^i	Ph.S.CO.OPr^i	10	50
M.NH.COCH$_3$	R'.NH.CO.CH$_3$	20	>75
M.NH.CO.NH.CH$_2$.CO$_2$H	Ph.NH.CO.NH.CH$_2$.CO$_2$H	*	*
M.S.CO.NH.CH$_2$CO$_2$H	Ph.S.CO.NH.CH$_2$.CO$_2$H	*	*

M = .C$_6$H$_4$.N(CH$_2$.CH$_2$Cl)$_2$; M' = .C$_6$H$_4$.N(CH$_2$.CH$_2$Br)$_2$

R' = Et$_2$N.Ph ; CR = complete regression

* Potentiation improves performance, but optimal conditions for use not yet known.

With all the above five substances, there was a striking improvement in performance when the appropriate potentiator was used.

4. Miscellaneous Variables

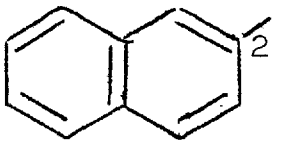
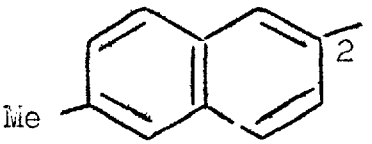
If the 'rate of hydrolysis' (as measured by the extent of reaction after 30 minutes in boiling 50% aqueous

acetone) of a nitrogen mustard is 20% or more[65], it is usually effective as a tumour-growth inhibitor, while a compound with a lower rate of hydrolysis shows little or no activity. Introduction of bromine for β-chloro atoms in a nitrogen mustard, increases the rate of hydrolysis. Benn[40] synthesised p-(NN-di-2-bromoethylamino)thiophenol (XLIII) and found that its rate of hydrolysis was 70% while the value for the chloro analogue was just 15%. The bromo mustard was also much more biologically active. Ross[65] studied the effect of variation in the halogen group on the rate of hydrolysis. There is an increase in the reaction rate on passing from chlorine to bromine, but in all examples of halogenoethyl compounds there is a surprising decrease when the bromine is replaced by an iodine atom. (See table on page 35).

The rate of hydrolysis also increases with the substitution of electron donating groups, e.g. methoxy or methyl on a benzene ring, while it decreases with electron attracting groups, e.g. nitro and carboxyl.

An increase in the length of the reactive side chain of the nitrogen mustard has the expected effect of reducing the rate of hydrolysis. Thus the chlorine atoms in NN-di-(3-chloro-n-propyl)- (LXV) and NN-di-(6-chloro-n-hexyl)-

Type of Compound $R.N:R'_2$

R	R'	rate of hydrolysis
 2-naphthyl	$.CH_2.CH_2Cl$	15%
	$.CH_2.CH_2Br$	80%
	$.CH_2.CH_2.I$	64%
 6-methyl-2-naphthyl	$.CH_2.CH_2Cl$	25%
	$.CH_2.CH_2Br$	85%
	$.CH_2.CH_2I$	74%

aniline (LXVI)

$$Ph.N(CH_2.CH_2.CH_2Cl)_2 \qquad \text{LXV}$$

$$Ph.N(CH_2.CH_2.CH_2.CH_2.CH_2.CH_2Cl)_2 \qquad \text{LXVI}$$

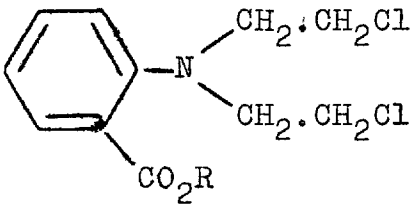
are not appreciably hydrolysed under the standard conditions[33], whilst (LXVII) is hydrolysed to the extent of 20%. Introduction of a methyl group such as in (LXVIII) increased the hydrolysis to 90%.

$$Ph.N(CH_2.CH_2Cl)_2 \qquad \text{LXVII}$$

$$Ph.N(CH_2.\underset{\underset{Me}{|}}{CH}Cl)_2 \qquad \text{LXVIII}$$

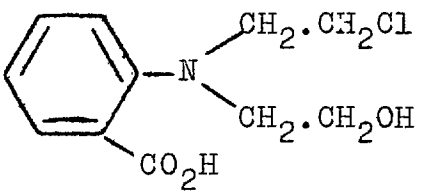
Compounds with only one halogenoethylamino group are generally much less biologically active, e.g.

β-(chloroethyl)ethylmethylamine, β-(chloroethyl)diethylamine and β-(chloroethyl)dimethylamine are 5 to 100 times less potent than bis(β-chloroethyl)methylamine (HN2). In any closely related series of monofunctional and polyfunctional derivatives, those that are more damaging generally possess reactive moieties at least in duplicate[66]. NN-Di-2-chloroethyl-o-aminobenzoic acid (LXIX; R = H) and its methyl ester (LXIX; R = Me) were synthesised[67] and were found to be active growth inhibitors, but monofunctional chloroethylamines (LXX)

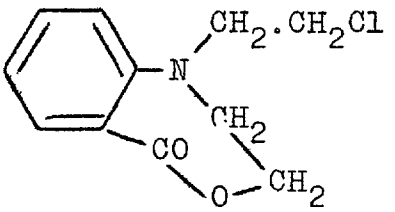


LXIX

and (LXXI), in spite of structural similarity with (LXIX) both proved inactive. This again emphasises the dependence of maximum cytotoxic activity upon the presence in the molecule of at least two chloroalkylamino groups. However, increasing the number of alkylating



LXX



LXXI

groups beyond two does not seem to cause any corresponding increase in effectiveness. Thus HN3 (XXIII) was no more effective than HN2 (XXII). In the non-aromatic series of chloroethylamines, the two halogencethylamino groups need not be situated on the same nitrogen atom, e.g. NN'-di-(2-chloroethyl)piperazine[33] is an effective compound.

Generally a drug which is soluble at physiological pH shows more activity than one which is insoluble; however, when it is required to be released slowly, injection in an oil becomes preferable.

In short, Bergel[68] was of the opinion that if we know (1) all about the mechanism of carcinogenesis, (2) every detail of cell and tissue composition, (3) every shade of difference between normal and abnormal material, and (4) the exact nature of interaction between a drug and the cellular constituents, then we could sit down at a desk and devise the most perfect remedies for all forms of malignancy.

CHAPTER 3

PROTECTION OF α-AMINO GROUPS AND THE FORMATION OF PEPTIDE LINKAGES

Although the synthesis of a peptide requires only the establishment of amide (peptide) linkages by condensation of the respective component amino-acids, this cannot be achieved directly with free amino-acids. The non-reacting functional groups are protected to avoid unwanted side reactions. It is essential that the protecting group must be capable of easy removal by reactions which do not lead to rupture of the amide bond after it has been established.

Since the α-N-acylamino acids (i.e. the N-protected amino acids) were used by the writer in the synthetic work that will be described in the forthcoming chapters of this thesis, it will not be out of place to give a brief account of a few methods which are generally employed for the protection of α-amino groups, and also of some coupling methods, well-known in peptide synthesis.

1. Benzyloxycarbonyl ('Carbobenzoxy') and related derivatives

The introduction, in 1932, by Bergmann and Zervas[69] of benzyloxycarbonyl chloride, as a reagent for the protection of α-amino groups in peptide synthesis, opened a new era in this field and enabled the preparation of a wide variety of peptides.

N-Benzyloxycarbonyl derivatives (LXXII) are prepared under Schotten-Baumann conditions by the action of benzyl chloroformate[69,70] (obtained by the action of phosgene on benzyl alcohol)[71], on amino-acids.

$$R.CH(NH_2).CO_2H + Cl.CO.O.CH_2Ph \rightarrow R.CH(NH.CO.O.CH_2Ph).CO_2H \qquad \text{LXXII}$$

The benzyloxycarbonyl group can be removed by catalytic hydrogenolysis[69], or by acid catalysed fission with hydrogen bromide[72,73] in acetic acid, or by using sodium in liquid ammonia. Birkofer *et al.*[74] have recommended the use of triethylsilane for this purpose.

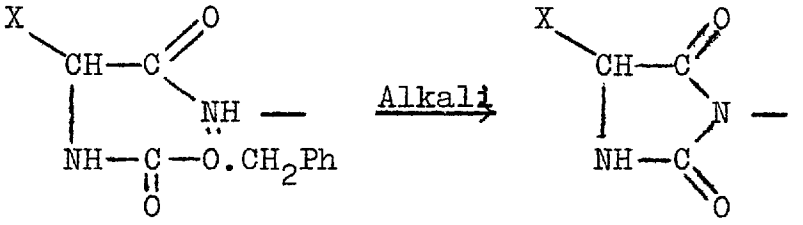
$$R.CH(NH.CO.O.CH_2Ph).COOH + Et_3SiH \xrightarrow[Et_3N]{PdCl_2} H_2 + R.CH(NH.CO.O.CH_2Ph).CO.O.SiEt_3$$

$$R.CH(NH.CO.O.CH_2Ph).CO.O.SiEt_3 \rightarrow CO_2 + PhCH_3 + R.CH(NH.SiEt_3).CO.O.SiEt_3$$

$$R.CH(NH.SiEt_3).CO.O.SiEt_3 \xrightarrow{2MeOH} R.CH(NH_2).CO.OH + 2Et_3SiOMe$$

Carpenter and Gish[75,76] investigated the application of p-nitrobenzyl chloroformate in peptide synthesis and reported the preparation of p-nitrobenzyloxycarbonyl derivatives of several amino-acids. These derivatives were characterized by the ease with which they were crystallized.

Although the wide applicability of the carbobenzoxy method is now well established and the advantages of using this protecting group are well known, it has certain disadvantages, and side reactions are sometimes encountered. The group is not very stable to alkali and it may cyclize to the substituted imidazolidine derivative.



2. Formyl Derivatives

Although the use of the formyl group as an amino protective function in the synthesis of peptides was known to a number of earlier workers[77,78,79,80], it did not gain wide acceptance until it was thoroughly

studied by Sheehan and Yang[81] in 1958.

Amino-acids and peptides are best formylated by treatment with formic acid and acetic anhydride[78], the formylating agent probably being the mixed anhydride:

$$H.CO.O.CO.CH_3 + H_2N.CHR.CO_2H \longrightarrow$$

$$H.CO.NH.CHR.CO_2H + CH_3.CO_2H.$$

This potential peptide protective function offers many valuable advantages.

(i) It can be introduced readily without racemization of the parent amino acids.

(ii) With the introduction of the carbodi-imide method of peptide bond formation, it becomes feasible to employ the N-formyl grouping in conjunction with the carbodi-imide procedure, when the optical integrity is maintained.

(iii) This group can be removed smoothly and selectively by treatment of the formyl peptide with a slight excess of 0.5 N HCl in methanol. In the examples studied, peptide bond fission does not occur[80].

(iv) The formyl group is very resistant to the alkaline conditions employed for the removal of ester groups by saponification.

Its main disadvantages are:

(i) Racemization of N-formylamino acids did occur when the mixed carbonic-anhydride procedure or azide method of coupling was used.

(ii) Substantial decomposition of many N-formylamino acids by interaction with such reagents as thionyl chloride and phosphorus pentachloride was recorded.

COUPLING METHODS

Although a variety of coupling methods pertaining to peptide chemistry have been described in the literature, only a few of them which gave fruitful results will be reviewed here.

1. Acid Azides

This method was first introduced by Curtius, and is still one of the best and most popular. The following are its characteristic features.

(i) It is a multi-stage process.

$$X_2N.CHR.CO_2H \xrightarrow{EtOH/HCl} X_2N.CHR.CO_2Et \xrightarrow{N_2H_4}$$

$$X_2N.CHR.CO.NH.NH_2 \xrightarrow{HNO_2} X_2N.CHR.CO.N_3$$

$$\xrightarrow{H_2N.CHR'.CO_2Et} X_2N.CHR.CO.NH.CHR'.CO_2Et.$$

(ii) It is necessary to carry out this process quite rapidly, in the cold, because of the somewhat unstable

nature of the azide, which decomposes to form the isocyanate and finally gives ureido-compounds as by-products.

$$X_2N.CHR.CON_3 \xrightarrow{-N_2} X_2N.CHR.NCO$$

$$\xrightarrow{H_2N.CHR'.CO_2Et} X_2N.CHR.NH.CO.NH.CHR'.CO_2Et.$$

(iii) This is the only coupling method which has never been known to cause racemization of either of the two amino-acid residues involved in the coupling.

2. Mixed Anhydrides Derived from Carboxylic Acids

The development of this method of formation of the amide bond is the result of the pioneering work of Wieland and his collaborators[82]. They, in 1950, prepared mixed anhydrides of N-protected amino-acids and benzoic acid, by treatment of the former with benzoyl chloride and a tertiary amine in an inert solvent and showed that they reacted with amino-acid esters to form protected dipeptide esters.

$$X_2N.CHR'.CO_2H + Cl.COPh \longrightarrow X_2N.CHR'.CO.O.COPh$$

$$\xrightarrow{H_2N.CHR''.CO_2Et} X_2N.CHR'.CO.NH.CHR''.CO_2Et + Ph.CO_2H$$

Shortly it was found[83] that mixed anhydrides derived

from ethyl chloroformate also could be used to form the peptide.

$$X_2N.CHR.CO_2H \xrightarrow{Cl.CO_2Et} X_2N.CHR.CO.O.CO.OEt$$

$$\xrightarrow{H_2N.CHR'.CO_2Et} X_2N.CHR.CO.NH.CHR'.CO_2Et + CO_2 + EtOH.$$

The superiority of isobutyl chloroformate over ethyl chloroformate has been reported by Vaughan and Osato[84].

This 'mixed carbonic anhydride' procedure has proved very popular and successful. One of the disadvantages is that racemization is quite prevalent. Many examples can be found in the literature[85,86] and a review is given by Neuberger[87]. Convincing evidence was advanced that azlactone formation caused the racemization, although the azlactones were not isolated from the reaction mixtures[86]. Racemization increases with the time and temperature used for the anhydride forming step. Solvent also plays an important part[88]. When chloroform was used, complete racemization occurred, but with dioxan or tetrahydrofuran there was practically none.

Another disadvantage is the instability of the anhydride, which results in disproportionation. It is

favoured by long reaction times during the anhydride forming step.

$$2Y.NH.CHR.CO.O.CO.OR' \longrightarrow (Y.NH.CHR.CO)_2O + (R'O)_2CO + CO_2$$

The loss of carbon dioxide from the mixed anhydride sometimes brings about the formation of an ester.

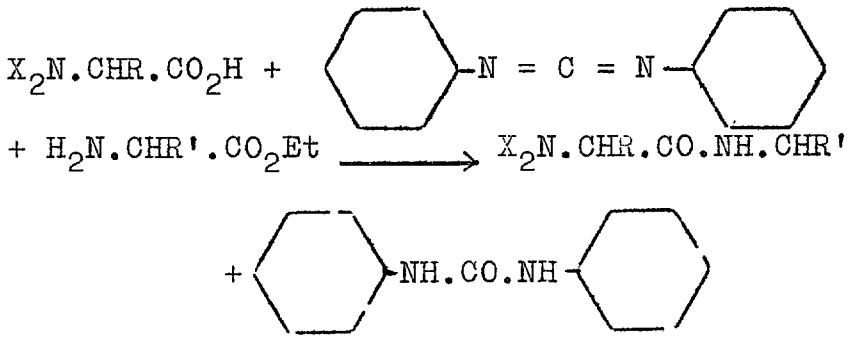
$$Y.NH.CHR.CO.O.CO.OR' \longrightarrow Y.NH.CHR.CO.OR' + CO_2$$

These and other spurious side reactions can be prevented by the right selection of alkyl chloroformate, the time required for the formation of the mixed anhydride, the nature of the solvent, and the temperature.

3. Carbodi-imides

NN'-Dicyclohexylcarbodi-imide (hereafter abbreviated to DCC) (LXXIII) was introduced by Sheehan and Hess[89] in 1955 as a direct coupling or condensing agent in peptide synthesis. Its ready availability, simplicity of use under mild conditions with frequent good yields of peptides, and the apparent lack of racemization in the formation of optically active peptide derivatives has resulted in its widespread popularity. In this method, the two components, one containing a free carboxyl function and the other a free amino group, couple directly and rapidly, on treatment

with DCC in an inert solvent like methylene chloride, tetrahydrofuran or acetonitrile.

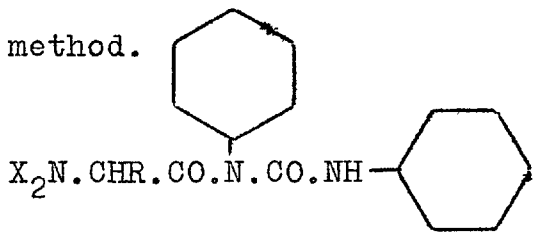


$X_2N.CHR.CO_2H$ + $-N = C = N-$ LXXIII

+ $H_2N.CHR'.CO_2Et$ ⟶ $X_2N.CHR.CO.NH.CHR'.CO_2Et$ LXXIV

+ $-NH.CO.NH-$ LXXV

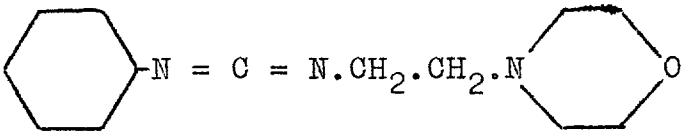
Khorana[90] used dioxan, tetrahydrofuran, chloroform or ether as solvents. Anderson and Callahan[91] have reported that this method is not entirely free from the danger of racemization, which actually depends upon the temperature and the nature of the solvent. The reaction is not sensitive to moisture and indeed it may be carried out in aqueous medium. The co-product NN'-dicyclohexylurea (LXXV) has a very low solubility in most organic or aqueous solvents, and can usually be separated without any difficulty. The possible formation of the N-acylurea (LXXVI) may be regarded as one of the disadvantages of this method.



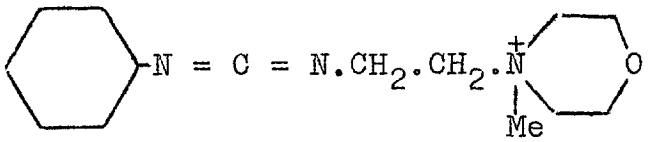
$X_2N.CHR.CO.N.CO.NH-$ LXXVI

In certain instances e.g. the synthesis of high molecular weight peptides, the co-product (LXXV) and the

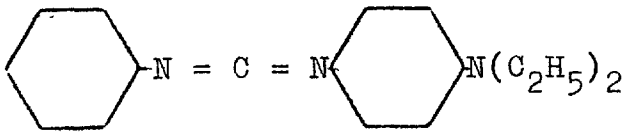
peptide derivative (LXXIV) may have similar solubility properties, thus rendering separation of the products difficult. Sheehan and Hlavka[92] therefore prepared several new acid-soluble and water-soluble carbodi-imides, bearing tertiary or quaternary amine substituents. The most promising for general peptide bond synthesis are 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodi-imide (LXXVII), the corresponding metho-p-toluenesulphonate (LXXVIII) and 1-cyclohexyl-3-(4-diethylaminocyclohexyl)carbodi-imide (LXXIX).



LXXVII

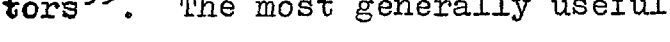


LXXVIII



LXXIX

Besides these, some other new acid- and water-soluble carbodi-imides were described by Sheehan and his collaborators[93]. The most generally useful compounds are 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (LXXX) and the corresponding hydrochloride salt.



LXXX

From these carbodi-imides, peptides have been synthesised in high yields and in very pure form.

CHAPTER 4

SYNTHESES OF SOME POTENTIALLY CYTOTOXIC S-α-(ACYLAMINO)ACYL-p-[Di-(2-HALOGENOETHYL)-AMINO]THIOPHENOLS

The synthetic studies undertaken by the present writer are a continuation of the research programme that was begun in 1954 in this Department, with the objective of synthesising drugs which were expected to show selective action on tumours either by enzymic attack at the site or by selective transport within the body.

A great majority of the aromatic 'nitrogen mustards', reported so far are derivatives of NN-di-2-chloroethyl-p-phenylenediamine (XXVI) or p-(NN-di-2-chloroethylamino)-phenol (XXV).

$$p\text{-}(ClCH_2.CH_2)_2N.C_6H_4.NH_2 \qquad \text{XXVI}$$

$$p\text{-}(ClCH_2.CH_2)_2N.C_6H_4.OH \qquad \text{XXV}$$

Amino-acid derivatives of the above 'nitrogen mustards' were described by Bergel and Stock[94] in 1959, but those prepared by Johnson[95] were claimed to be of a novel type. Johnson synthesised a large number of N'-[α-(acylamino)acyl]-NN-di-2'-chloroethyl-p-phenylenediamines

(LXIII; x = acyl) by acylation of the amino-group in the amine mustard (XXVI), with a variety of N-acyl-α-amino-acids.

$$\underline{p}\text{-}(ClCH_2.CH_2)_2N.C_6H_4.NH.CO.\underset{\underset{NHX}{|}}{CHY} \qquad \text{LXIII}$$

Various principles which motivated the synthesis of such compounds are:

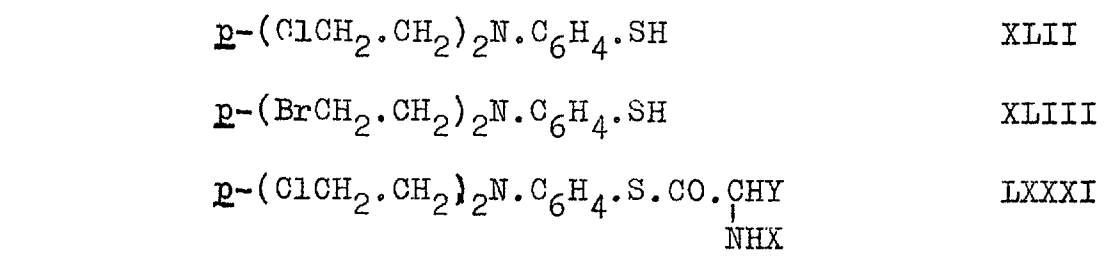
(i) Acylation of the free amino group in the parent amine (XXVI) deactivates the 'nitrogen mustard' resulting in a fall of general toxicity.

(ii) The principle of facilitated diffusion applies to amino-acids, which are actively transported through cell membranes.

(iii) The release of the active nitrogen mustard (XXVI) from such derivatives (LXIII) requires the successive action of two enzymes. The acyl group 'X' undergoes enzymic removal first, and then the enzyme 'amino-peptidase' is able to split the peptide linkage between the amino-acid and nitrogen mustard and release the free alkylating agent (XXVI).

Because of the importance of thiol groups in biological systems, a study has now been made of the synthesis of some derivatives (LXXXI) from p-(NN-di-2-chloroethylamino)thiophenol (XLII) and also of analogues

from the corresponding dibromo compound (XLIII). Such products are of additional interest because they would be 'phenylthioesters', a class of compound ('active esters') from which the parent thiol is usually very readily liberated under mild conditions by virtue of the ease with which the acyl group is transferred to other acceptors.



XLII

XLIII

LXXXI

As has been seen (Chapter 3), it is necessary to protect one of the functional groups of an amino-acid in order to form a peptide bond with the other. Similar considerations apply if a thiolester is to be formed. In the present series the N-acetyl, N-formyl and N-methoxycarbonyl groups have been used. The condensation with the thiol group of the appropriate nitrogen mustard (either the chloro-mustard XLII or the bromo-mustard XLIII) was carried out at room temperature in the presence of NN'-dicyclohexylcarbodi-imide in various solvents. Sufficient time (14 to 18 hours) was allowed for the completion of the reaction. The mixed anhydride

method of preparation was tried in a few cases, but was generally not so good. The products obtained by the DCC method were not only far cleaner but were also formed in good yield. All the reactions were performed in an atmosphere of nitrogen to minimise the oxidation of the thiol into its disulphide. The presence of the disulphide rendered the purification of the products very troublesome. Their chromatcgraphic purification on alumina or on silica was not effective, possibly because of the sensitivity of the thiol ester group. Fractional crystallisation was the only effective method for the removal of the contaminants. In almost all the reactions, the condensation product was obtained initially as an oil, but fourteen 'thiolesters' were eventually obtained in pure crystalline form when a suitable solvent system was found.

Derivatives of Glycine

Glycine is known to be the precursor of a wide variety of nitrogenous compounds of great physiological importance and is believed to be converted into serine, glutathione, proteins, porphyrins, uric acid and nucleic acid purines during certain metabolic pathways, the

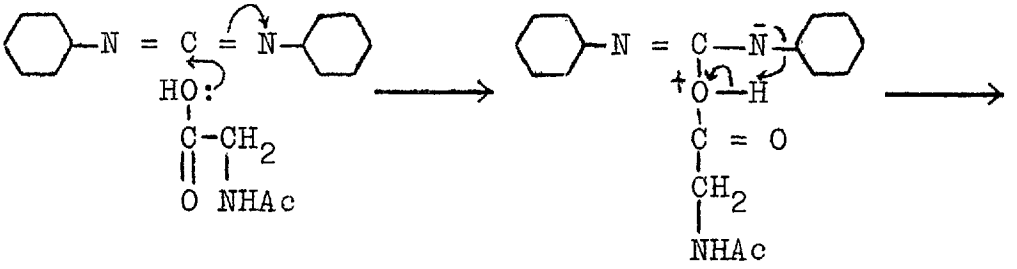
most important being the one in which glycine is converted into serine by an active form of folic acid. Because nitrogen mustard derivatives of it may be actively transported across cell membranes, a number of glycine derivatives have been prepared. The use of various N-substituents on the α-amino groups was to confer more specificity to the drugs, as explained above.

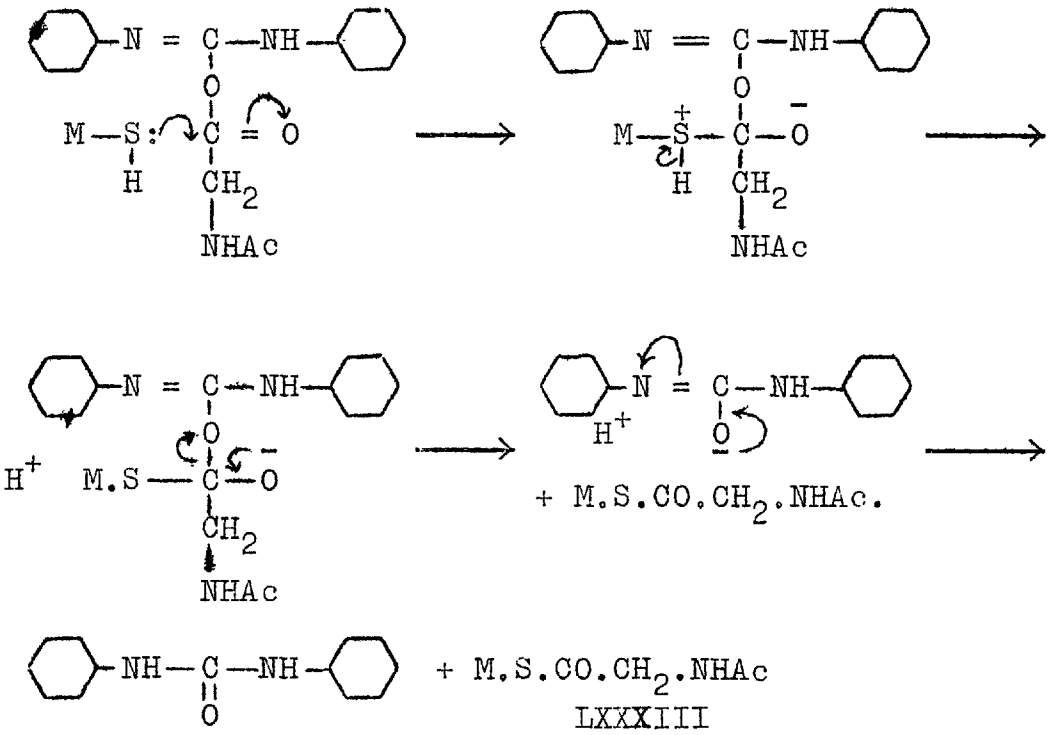
N-Acetylglycine (LXXXII) was prepared by the

$$Ac.NH.CH_2.CO_2H \qquad \text{LXXXII}$$

method of Herbst and Shemin[96]. It was then condensed with the thiol chloro-mustard, suspended in dichloromethane, using NN'-dicyclohexylcarbodi-imide as condensing agent. The product, p-(NN-di-2-chloroethylamino)phenyl (acetamido)thiolacetate (LXXXIII; I.C. 203) was obtained in 80% yield.

$$p\text{-}(ClCH_2.CH_2)_2N.C_6H_4.S.CO.CH_2.NHAc \qquad \text{LXXXIII}$$



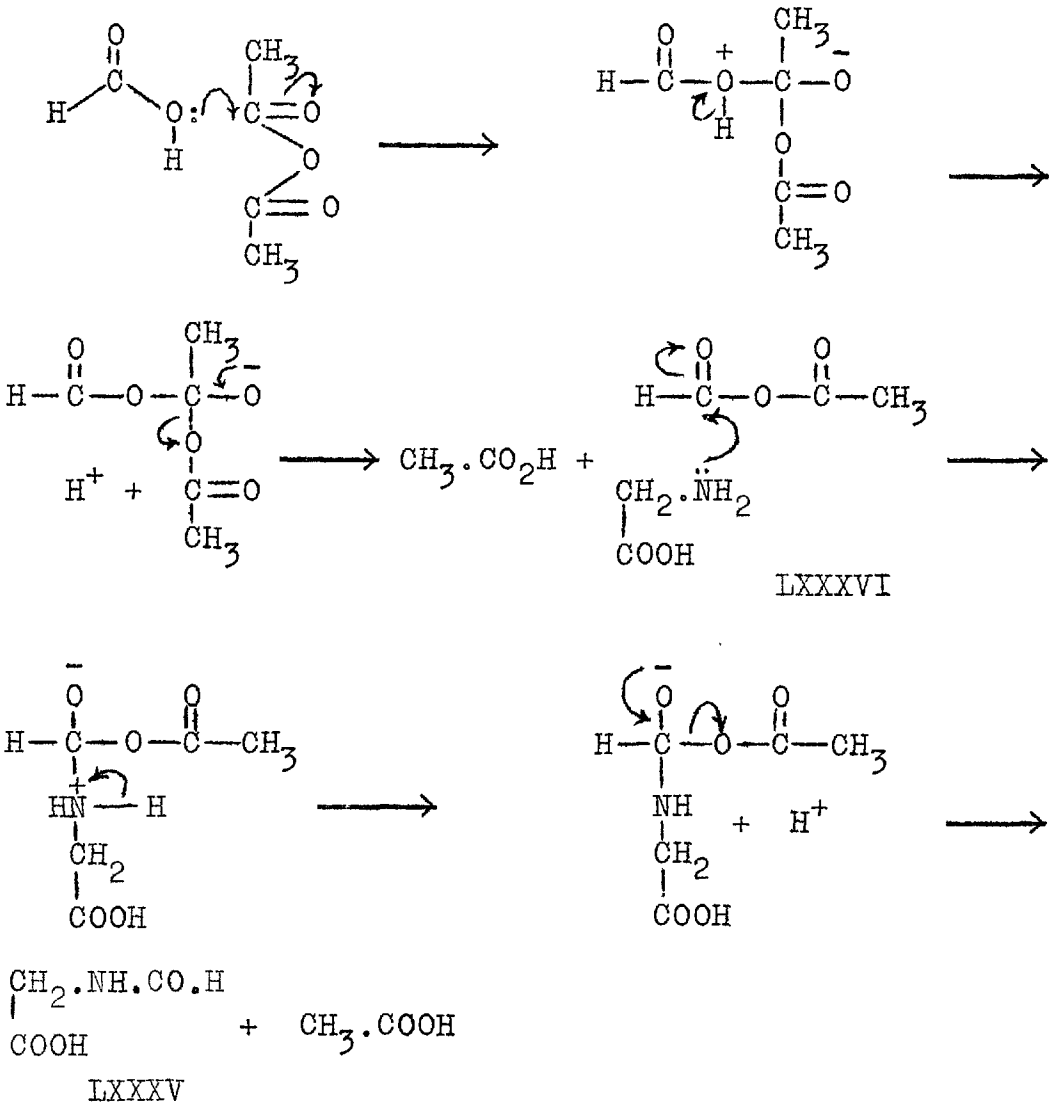


LXXXIII

The condensation of N-acetylglycine with the bromo-mustard under similar conditions gave p-(NN-di-2-bromoethylamino)phenyl (acetamido)thioi-acetate (LXXXIV; I.C. 202). The yield was 38%.

p-$(BrCH_2.CH_2)_2N.C_6H_4.S.CO.CH_2.NHAc$ LXXXIV

N-Formylglycine (LXXXV) was prepared by the method of Sheehan and Yang[81] from 88% formic acid and acetic anhydride, the formylating agent probably being the mixed anhydride (LXXXVI).



LXXXVI

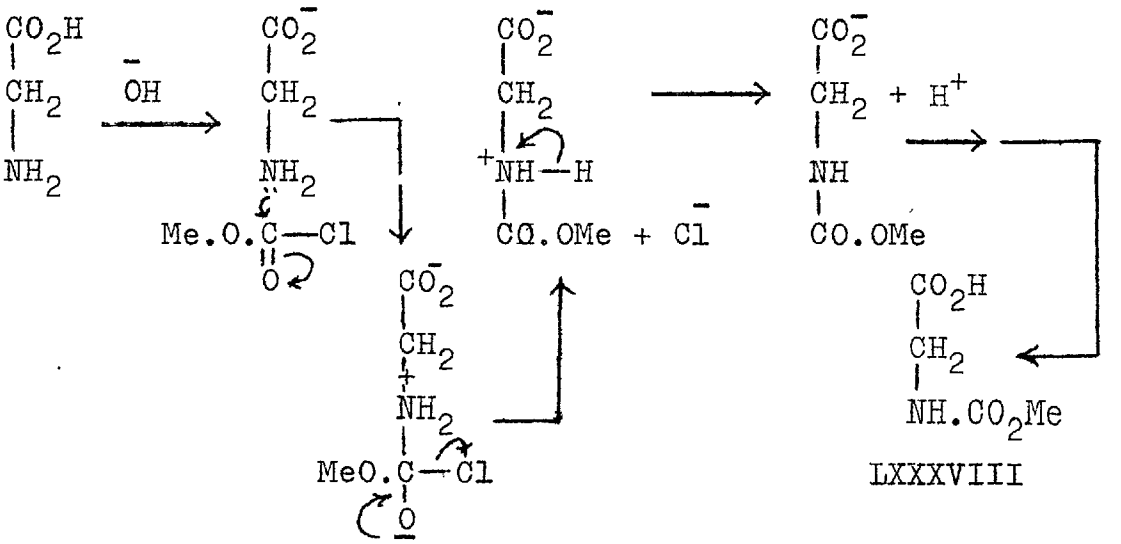
LXXXV

The N-formylated derivative was then used for coupling with the chloro-mustard using DCC as a coupling reagent. The oily product crystallised to give p-(NN-di-2-chloroethylamino)phenyl (formamido)thiolacetate (LXXXVII; I.C. 204) in 80% yield.

p-$(ClCH_2.CH_2)_2N.C_6H_4.S.CO.CH_2.NH.CHO$ LXXXVII

The condensation of N-formylglycine with the bromo-mustard was attempted by the DCC method as well as the mixed anhydride method, but in both cases the product was an oil which could not be induced to crystallise. An attempt was made, using lithium bromide in boiling isobutyl methyl ketone, to convert the dichloro derivative (LXXXVII) into its dibromo analogue, but this also gave an oily product. However, the infrared spectrum of the product in each case showed absorption bands at 3450 $cm.^{-1}$ (:NH), and 1690 $cm.^{-1}$ (>CO, -CONH).

N-Methoxycarbonylglycine (LXXXVIII) was prepared[97] using normal Schotten-Baumann conditions, by the action of methyl chloroformate on glycine in sodium carbonate solution



LXXXVIII

The suspension of N-methoxycarbonylglycine in dichloromethane when treated with the chloro-mustard in the presence of DCC, produced p-(NN-di-2-chloroethylamino)phenyl (methoxycarbonylamino)thiolacetate (LXXXIX; I.C. 205) in 35% yield. Its dibromo analogue could not be obtained in crystalline form by a similar method, but the infrared spectrum, recorded with the oily product, showed absorption bands at 3350 $cm.^{-1}$ (:NH) and 1720 - 1680 $cm.^{-1}$ ($\rangle C = O$; -CONH).

p-$(ClCH_2.CH_2)_2N.C_6H_4.S.CO.CH_2.NH.CO_2Me$ LXXXIX

Derivatives of DL-phenylalanine

N-Acetyl-DL-phenylalanine (XC) was prepared in 92.5% yield essentially by the method described in the literature[98]. It was then condensed with the chloro-

$$Ph.CH_2.\underset{\underset{NHAc}{|}}{CH}.CO_2H$$ XC

mustard using DCC as the condensing reagent; because of the insolubility of N-acetyl-DL-phenylalanine in dichloromethane, a mixture of NN-dimethylformamide and dichloromethane (1 : 1) was used. Crystalline p-(NN-di-2-chloroethylamino)phenyl 2-acetamido-3-(phenyl)thiolpropionate (XCI; I.C. 207) was attained in 32% yield.

Condensation of N-acetyl-DL-phenylalanine with the

p-$(ClCH_2.CH_2)_2N.C_6H_4.S.CO.\underset{\underset{NHAc}{|}}{CH}.CH_2.Ph$ XCI

bromo-mustard under similar conditions did not give a crystalline product.

N-Formyl-DL-phenylalanine (XCII) was prepared by the Vigneaud-Meyer method[99] as well as by that described by Overby and Ingersoll;[100] the yield in the former case was better. Its condensation with the chloro-mustard thiol gave no crystalline dichloroethyl

$Ph.CH_2.\underset{\underset{NH.CHO}{|}}{CH}.CO_2H$ XCII

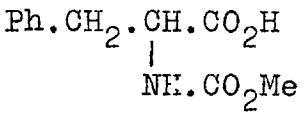
analogue of (XCIII), although its formation was indicated by the infrared spectrum. However, p-(NN-di-2-bromoethylamino)phenyl 2-formamido-3-(phenyl)thiolpropionate (XCIII; I.C.210) was obtained by the condensation of N-formyl-DL-phenylalanine with the bromo-mustard thiol by the DCC method. This reaction was carried out in a mixture of NN-dimethyl

p-$(BrCH_2.CH_2)_2N.C_6H_4.S.CO.\underset{\underset{NH.CHO}{|}}{CH}.CH_2.Ph$ XCIII

formamide and dichloromethane (1 : 2). The crude residue did not crystallise immediately, but when the impurities

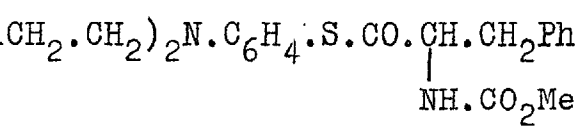
(especially the disulphide) were removed by passing a solution in dichloro-methane through a layer of decolourising charcoal, the product could be crystallised, although in poor yield (27%).

N-Methoxycarbonyl-DL-phenylalanine (XCIV) was



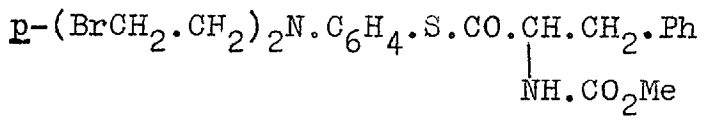
XCIV

prepared by the method described by Petri and Staverman[101]. At first it was obtained as a viscous colourless oil as reported by these workers, but on leaving at room temperature for about four months, it crystallised. Chibnall and Spahr[102] also reported in 1958 its eventual solidification on storing at room temperature. Even in the viscous oily form, it was apparently pure and gave clean condensation products. The condensation with the chloro mustard thiol was carried out in dichloromethane, whereas a mixture of NN-dimethylformamide and dichloromethane in equal proportions was used when the reaction was done with the bromo mustard thiol. In both cases DCC was the coupling reagent. The products were p-(NN-di-2-chloroethylamino)phenyl 2-methoxycarbonylamino-3-(phenyl)thiolpropionate (XCV; I.C. 208), and



XCV

p-(NN-di-2-bromoethylamino)phenyl 2-methoxycarbonylamino-3-(phenyl)thiolpropionate (XCVI; I.C. 209) respectively. Their respective yields were 55% and 92%.

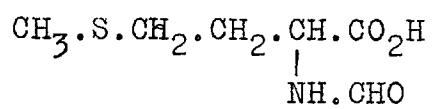


XCVI

Derivatives of DL-Methionine

It is known that methionine is an 'essential' amino-acid. Not only it is indispensible but both the D- and the L- form can be utilized by the rat. Thus it would seem reasonable that if a nitrogen-mustard attached to DL-methionine, was given to a tumour-bearing rat which had been fed on a diet deficient in DL-methionine, then the chances of the drug being utilized and of reaching the required sites should be greater.

N-Formyl-DL-methionine (XCVII) was prepared from DL-methionine by the method of Sheehan and Yang[81]. It was then linked with the chloro mustard thiol by the DCC



XCVII

method. p-(NN-di-2-Chloroethylamino)phenyl 2-formamido-4-(methylthio)thiolbutyrate (XCVIII; I.C. 206), was thus obtained in crystalline form. The yield was poor (35%).

$$p\text{-}(ClCH_2.CH_2)_2N.C_6H_4.S.CO.\underset{\displaystyle NH.CHO}{\underset{|}{CH}}.CH_2.CH_2.S.CH_3 \qquad \text{XCVIII}$$

However, p-(NN-di-2-bromoethylamino)phenyl 2-formamido-4-(methylthio)thiolbutyrate, the condensation product of the N-formyl-DL-methionine and the bromo mustard thiol was not obtained in crystalline form, though the oily product showed an infrared spectrum consistent with the presence of the required product.

MODEL COMPOUNDS

On page 32 it has been explained that isopropyl phenylurethane (IX) has been used as a potentiator for the mustard urethane (LXIV). A few model compounds have therefore been prepared from thiophenol and from

$Ph.NH.CO.OPr^i$ (IX) $\qquad$ $p\text{-}(ClCH_2.CH_2)_2N.C_6H_4.NH.CO.OPr^i$ (LXIV)

p-(diethylamino)thiophenol by the present writer, as possible potentiators for the thiol mustards.

A. From Thiophenol

The condensation of N-acetylglycine with thiophenol in dichloromethane was carried out in the presence of NN'-dicyclchexylcarbodi-imide and gave S-(N-acetylglycyl)

thiophenol (XCIX; X56) in 57% yield.

$$Ph.S.CO.CH_2.NHAc \qquad XCIX$$

The product of the reaction between N-formylglycine and thiophenol in dichloromethane was S-(N-formylglycyl) thiophenol(C; X55). The yield was 78% when NN'-dicyclohexyl-carbodi-imide was used as the condensing agent.

$$Ph.S.CO.CH_2.NH.CHO \qquad C$$

S-(N-Methoxycarbonylglycyl)thiophenol (CI; X57) was

$$Ph.S.CO.CH_2.NH.CO_2Me \qquad CI$$

obtained in 74% yield by condensation between N-methoxycarbonylglycine and thiophenol under similar conditions.

B. From p-(NN-Diethylamino)thiophenol

NN-Diethyl-p-thiocyanatoaniline (CII), which was

$$p\text{-}Et_2N.C_6H_4.SCN \qquad CII$$

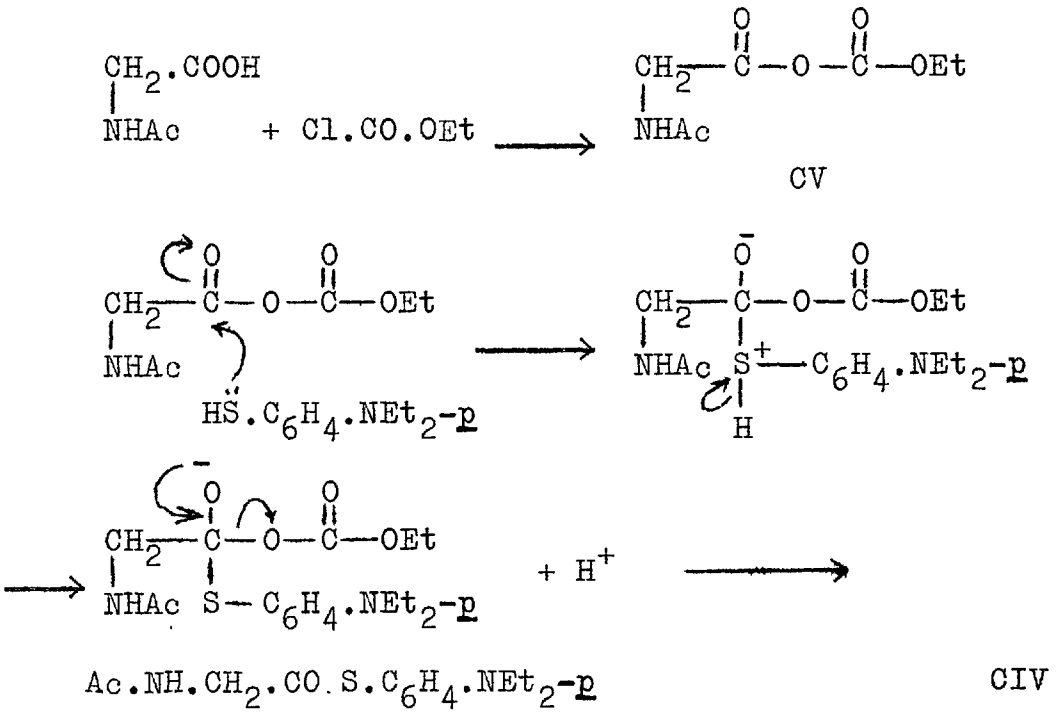
prepared from NN-diethylaniline by the method described by Brewster and Schroeder[103] was converted into p-(NN-diethylamino)thiophenol (CIII) by two methods. The

$$p\text{-}Et_2N.C_6H_4.SH \qquad CIII$$

lithium aluminium hydride method described by Benn et al.[40] gave the reduction product (CIII) in better yield (83%)

than the Zinc dust-HCl method described by Montgomery et al.[104]

p-(N-Acetylglycylthio)-NN-diethylaniline (CIV; X59) was then prepared from (CIII) by two methods. In one method N-acetylglycine was converted into the mixed anhydride (CV) by reaction with ethyl chloroformate and a linkage was then established with the thiophenol (CIII) in NN-dimethylformamide to give the desired compound (CIV).



CV

CIV

The second method for the preparation of p-(N-acetylglycylthio)-NN-diethylaniline (CIV) was based upon the use of DCC as a condensing reagent between N-acetylglycine and p-(NN-diethylamino)thiophenol. A far cleaner

product was obtained by this method.

In a separate experiment N-formylglycine was dissolved in NN-dimethylformamide-dichloromethane. (1 : 1) before it was condensed with the p-(NN-diethylamino) thiophenol in the presence of DCC to give p-(N-formyl-glycylthio)-NN-diethylaniline (CVI; X58).

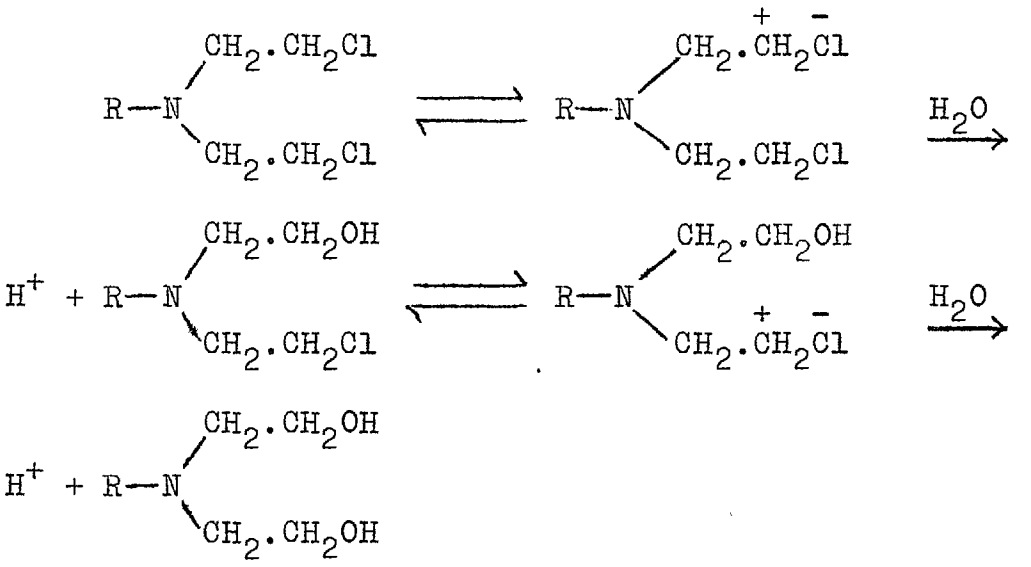
p-$Et_2N.C_6H_4.S.CO.CH_2.NH.CHO$ CVI

Although both the mixed anhydride and the DCC procedures were attempted for the coupling of N-methoxycarbonylglycine and p-(NN-diethylamino)thiophenol, neither process gave p-(N-methoxycarbonylglycylthio)-NN-diethylaniline in crystalline form. However, its presence in the crude oily product was indicated by the characteristic absorption bands in the infrared spectrum.

QUANTITATIVE HYDROLYSIS

Ross[105] prescribed a standard procedure for the determination of the extent of hydrolysis of the derivatives of nitrogen mustards.

The same standard conditions were employed and the extent of hydrolysis on three of the thiol mustard derivatives was determined.



0.5 m.mole of the chloro mustard derivative (0.25 m.mole in case of bromo mustard derivative) was dissolved in AnalaR acetone; water was added and the solution was raised as rapidly as possible to its boiling point (66°) under reflux for 30 minutes. The solution was then cooled quickly in ice and the liberated hydrogen ion determined by the use of standard $N/_{10}$ potassium hydroxide employing phenolphthalein as indicator.

M = p-C_6H_4.N(CH_2.CH_2Cl)$_2$

M' = p-C_6H_4.N(CH_2.CH_2Br)$_2$

I.C.NO	Compound	wt. taken (g.)	vol. of acetone (ml.)	vol. of water (ml.)	H^+%
206	M.S.CO.CH(NH.CHO).CH_2.CH_2.S.CH_3	0.2045	25	25	4
203	M.S.CO.CH_2.NH.$COCH_3$	0.1730	25	25	4
202	M'.S.CO.CH_2.NH.$COCH_3$	0.1096	12.5	12.5	28

Benn et al.[40] reported a value of 3% for the S-acetyl derivative, M.SAc, of p-(NN-di-2-chloroethylamino)thiophenol while a value of 26% was reported by Creighton et al.[106] for the S-acetyl derivative, M'.SAc, of the bromo-analogue. The respective values[40] for the free thiols themselves were reported to be 15 and 70%. The closeness of the present values to those of the acetyl compounds clearly shows that the deactivation brought about by linkage of the thiol group to the amino-acid residues is about the same as that caused by simple acetylation.

BIOLOGICAL RESULTS

The following data are the results of the biological tests on a selection of the compounds carried out in the Department of Biochemical

Pharmacology of the State University of New York at Buffalo, under the direction of Professor J.F. Danielli, F.R.S.

Code No.	Formula of the Drug	LD_{50} mouse	LD_{50} rat	ED_{90}	TI $\frac{LD_{50}}{ED_{90}}$
I.C.202	M'.S.CO.CH_2.NHAc	200	150	90	1.7
I.C.203	M.S.CO.CH_2.NHAc	>1000	90	>90	<1.0
I.C.206	M.S.CO.CH(NH.CHO)(CH_2.CH_2.S.Me)	1000	ca.400	200	2
I.C.208	M.S.CO.CH(NH.$COOCH_3$)(CH_2.Ph)	>1000	>800	>800	1
I.C.209	M'.S.CO.CH(NH.$COOCH_3$)(CH_2.Ph)	480	>400	50	>8.0
*I.C.211	Ph.S.CH($CH_2O.SO_2CH_3$)$_2$	12.5	12	>12	<1.0
*I.C.212	Ph.S.CH_2.CH(O.SO_2CH_3)(CH_2.O.SO_2CH_3)	38	35	>35	<1.0

*These compounds will be described in Part II of the thesis.

From the study of the above data, I.C.209 appears to be considerably better than I.C.202 or 206.

A brief account of the method[107] employed for obtaining the above data is given below.

Male Holtzman rats, weight 160 to 180 g., were fed a controlled diet and housed in special cages at 74-76°F. Walker carcinosarcoma 256 was implanted subcutaneously; on the next day a single intraperitoneal injection of various dose levels was administered, and carcinostatic effects of the drugs were assessed. When the rats were killed, the tumours were dissected out and weighed. The therapeutic index was obtained from the ratio LD_{50}/ED_{90}, where ED_{90} is the dose corresponding to a T/C ratio of 0.1. T/C is the ratio of the mean weight of treated tumours to the mean weight of control tumours.

CHAPTER 5

EXPERIMENTAL

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

All concentrations of solutions were performed under reduced pressure.

Infrared spectra were run on a Unicam S.P.200 spectrometer, and ultra violet spectra on a Unicam S.P.800 instrument. Nuclear magnetic resonance (N.M.R.) spectra were measured by Mrs. A.I. Boston using a Varian A.60 spectrometer.

Melting points were determined on a Kofler block and are uncorrected.

In the following text p-(NN-di-2-chloroethylamino) thiophenol will be designated as nitrogen mustard A, and p-(NN-di-2-bromoethylamino)thiophenol as nitrogen mustard B, and their formulae will be abbreviated to MSH and M'SH respectively.

The nitrogen mustards A and B that were used in the present investigations were kindly provided by the Wellcome Foundation. They contained 12.4% and 8.2% of

thiol-S respectively (estimated volumetrically); calculated, 12.81% and 9.50%.

NN-Diethyl-p-thiocyanatoaniline. (5,7)

It was prepared from NN-diethylaniline by the method[103] described for the NN-dimethyl analogue, and had b.p. 140-145°/0.5 mm., and $n_D^{22.5}$ 1.5932. (lit.[108] b.p. 138 /1 mm.). $\nu_{max.}$ (liquid film), 2200 (-SCN), 1365 (=N-) cm^{-1}.

p-(NN-Diethylamino)thiophenol. (30,32; 36,50,58,71)

It was prepared by two methods.

(a) Zinc dust- HCl method.[104]

NN-Diethyl-p-thiocyanatoaniline was reduced by zinc dust and aqueous hydrochloric acid (1 : 1) essentially as described by Edna M. Montgomery et al.[104] to give p-(NN-diethylamino)thiophenol as a colourless oil, b.p. 85-90°/0.05 mm., $n_D^{16.5}$ 1.6010. (lit.[40] b.p. 97-98°/0.06 mm., n_D^{20} 1.5942). (Found: thiol-S, 17.61. Calc. for $C_{10}H_{15}NS$: thiol-S, 17.71%).

(b) Lithium aluminium hydride method.

NN-Diethyl-p-thiocyanatoaniline was reduced with lithium aluminium hydride by the method described by Benn et al.[40] to give p-(NN-diethylamino)thiophenol as a colourless oil in 83% yield, b.p. 90°/0.03 mm., and 106-108°/0.4 mm. $\nu_{max.}$ in chloroform, 2550 $cm.^{-1}$ (-SH). (Found: thiol-S, 17.70. Calc. for $C_{10}H_{15}NS$: thiol-S, 17.70%).

DERIVATIVES OF GLYCINE

N-Acetylglycine. (9)

It was prepared from glycine and 95% acetic anhydride by the method described by Herbst and Shemin[96]. It had m.p. 208-209°. (lit. 207-208°).

S-(N-Acetylglycyl)thiophenol. (92)

N-Acetylglycine (0.585 g.) was suspended in dichloromethane (20 ml.) to which was added a solution of thiophenol (0.55 g.) in dichloromethane (15 ml.). Then a solution of NN'-dicyclohexylcarbodi-imide (1.1 g.) in dichloromethane (5 ml.) was added to the mixture which was then shaken for 16 hours. The precipitated

urea was filtered off and the clear filtrate treated with acetic acid (ca. 1 ml.) and left for 3 hours at room temperature. It was then washed with water (3 x 15 ml.), dried ($MgSO_4$) and concentrated to give S-(N-acetylglycyl)thiophenol (0.6 g., 57%) as a white solid which was recrystallized from benzene-petroleum (b.p. 40-60°) and had m.p. 99-99.5°, $\nu_{max.}$ in chloroform, 3490 (>NH), 1690-1680 (doublet; -SAc and -CONH) $cm.^{-1}$ (Found: C, 57.50; H, 5.41; S, 15.26. $C_{10}H_{11}NO_2S$ requires C, 57.39; H, 5.30; S, 15.32%).

p-(N-Acetylglycylthio)-NN-diethylaniline. (39,41,54,67)

It was prepared by two methods.

(a) Ethyl chloroformate (0.99 ml.) was added to an ice cold solution of N-acetylglycine (1.17 g.) in dry NN-dimethylformamide (7.5 ml.) and triethylamine (2.86 ml.) and the mixture was left for 20 minutes in an ice bath. A cooled solution of p-(NN-diethylamino) thiophenol (1.81 g.) in NN-dimethylformamide (15 ml.) was added to this mixed anhydride solution, which was then left for 4 hours at room temperature and then for 4 days at ca.2°before the precipitated triethylamine

hydrochloride was filtered off. The filtrate was concentrated to give an oil which was taken up in chloroform and washed with water (3 x 25 ml.), dried ($MgSO_4$) and concentrated to give a viscous pale yellow oil which crystallized on trituration with ether. This p-(N-acetylglycylthio)-NN-diethylaniline (1.68 g., 61%) was recrystallized from chloroform-petroleum (b.p. 40-60°) and had m.p. 99-100°. $\nu_{max.}$ (nujol), 3325 (>NH), 1690 (>CO) and 1680 (-CONH) $cm.^{-1}$ (Found: C, 60.39; H, 6.98; S, 11.70. $C_{14}H_{20}N_2O_2S$ requires C, 59.96; H, 7.19; S, 11.43%).

(b) N-Acetylglycine (0.585 g.) was suspended in dichloromethane (20 ml.) to which was added a dichloromethane solution (15 ml.) of NN-diethyl-p-aminothiophenol (0.905 g.), followed by a solution of NN'-dicyclohexylcarbodi-imide (1.1g.) in dichloromethane (5 ml.). The mixture was shaken for 16 hours; filtered and the filtrate was treated with acetic acid (0.5 ml.). After 2 hours it was washed with water (3 x 15 ml.), dried (Na_2SO_4) and concentrated to give p-(N-acetylglycylthio)-NN-diethylaniline, which was recrystallized from chloroform-petroleum (b.p.40-60°) (0.55 g., 42%)

and had m.p. 100-101°. $\nu_{max.}$ (nujol), 3325 (>NH), 1690 (>CO) and 1680 (-CONH) $cm.^{-1}$ (Found: C, 59.66; H, 7.24; S, 11.76. $C_{14}H_{20}N_2O_2S$ requires C, 59.96; H, 7.19; S, 11.43%).

p-(NN-Di-2-chloroethylamino)phenyl (acetamido)thiolacetate. (110)

To a suspension of N-acetylglycine (0.585 g.) in dichloromethane (20 ml.) was added a dichloromethane solution (15 ml.) of the nitrogen mustard A (1.25 g.) followed by a solution of NN'-dicyclohexylcarbodi-imide (1.24 g.) in dichloromethane (5 ml.). After shaking for 16 hours, it was worked up as described above to give p-(NN-di-2-chloroethylamino)phenyl (acetamido)thiolacetate (1.44 g., 80%) which was recrystallized from benzene-petroleum (b.p. 40-60°) and had m.p. 119-120°. $\nu_{max.}$ (nujol), 3325 (>NH), 1700 (>CO) and 1645 (-CONH) $cm.^{-1}$ (Found: C, 48.56; H, 5.07; Cl, 20.26. $C_{14}H_{18}Cl_2N_2O_2S$ requires C, 48.14; H, 5.19; Cl, 20.30%).

p-(NN-Di-2-bromoethylamino)phenyl (acetamido)thiolacetate (124,136)

N-Acetylglycine (1.17 g.) was suspended in dichloromethane (40 ml.). To this suspension was added a solution of nitrogen mustard B (3.39 g.) in dichloromethane (30 ml.), and then a solution of NN'-dicyclohexylcarbodi-imide (2.5 g.) in dichloromethane (10 ml.). Shaking for 16 hours and then working up as usual gave a yellow oil which crystallized as p-(NN-di-2-bromoethylamino)phenyl (acetamido)thiolacetate on trituration with ether. It was recrystallized from benzene-petroleum (b.p. 40-60°) and had m.p. 125-127° (1.66 g., 38%). $\nu_{max.}$ (nujol), 3300 (>NH), 1685 (>CO) and 1645 (-CONH) $cm.^{-1}$ (Found: C, 38.90; H, 4.18; Br, 36.46. $C_{14}H_{18}Br_2N_2O_2S$ requires C, 38.37; H, 4.14; Br, 36.48%).

N-Formylglycine. (14)

This was prepared by the action of 88% formic acid and acetic anhydride on glycine following the general procedure described by Sheehan and Yang[81]. It had m.p. 154° (lit. m.p. 160°)

S-(N-Formylglycyl)thiophenol. (96)

A solution of thiophenol (0.55 g.) in dichloromethane (15 ml.) was added to a suspension of N-formylglycine (0.515 g.) in dichloromethane (20 ml.), followed by NN'-dicyclohexylcarbodi-imide (1.3 g.) in dichloromethane (5 ml.). The mixture was shaken for 16 hours and then worked up as usual to give S-(N-formylglycyl)-thiophenol (0.76 g., 78%). It was recrystallized from chloroform-petroleum (b.p. 40-60°) and had m.p. 105-106°. $\nu_{max.}$ in chloroform, 3450 (>NH), 1700-1688 (>CO; -CONH) $cm.^{-1}$ (Found: C, 55.29; H, 4.79; S, 16.62. $C_9H_9NO_2S$ requires C, 55.36; H, 4.65; S, 16.42%).

p-(N-Formylglycylthio)-NN-diethylaniline. (45,60,74,81,89)

N-Formylglycine (0.515 g.) was dissolved in NN-dimethylformamide-dichloromethane (20 ml., 1 : 1) to which was added a solution (10 ml.) of p-(NN-diethylamino) thiophenol (0.905 g.), using the same solvents in the same proportions. To this mixture was added NN'-dicyclohexylcarbodi-imide (1.3 g.) and the solution shaken for $16\frac{1}{2}$ hours at 18°. The precipitate was filtered off, and the filtrate treated with acetic acid

(0.5 ml.) and left for 2 hours at 18°. The precipitate, formed again, was filtered off, and the filtrate concentrated to give an oil which was dissolved in chloroform, washed quickly with water (3 x 10 ml.), dried ($MgSO_4$) and concentrated to give an oil which crystallized out as p-(N-formylglycylthio)-NN-diethyl-aniline (0.83 g., 63%) on trituration with petroleum (b.p. 40-60°). It was recrystallized from chloroform-petroleum and had m.p. 108°. $\nu_{max.}$ in chloroform, 3475 (>NH), 1690 (>CO, -CHO) $cm.^{-1}$ (Found: C, 58.87; H, 6.91; S, 11.71. $C_{13}H_{18}N_2O_2S$ requires C, 58.62; H, 6.81; S, 12.04%).

p-(NN-Di-2-chloroethylamino)phenyl (formamido)thiolacetate. (114,153)

N-Formylglycine (1.03 g.) was suspended in dichloromethane (40 ml.) to which was added a solution of nitrogen mustard A (2.5 g.) in dichloromethane (30 ml.) followed by a solution (10 ml.) of NN'-dicyclohexyl-carbodi-imide (2.65 g.) in dichloromethane. The mixture was shaken for 16 hours and then worked up as usual to give a light yellow oil which crystallized out

as p-(NN-di-2-chloroethylamino)phenyl (formamido)thiolacetate (2.7 g., 80%) on trituration with benzene-petroleum (b.p. 40-60°), and had m.p. 92-94°. $\nu_{max.}$ in chloroform, 3320 (>NH), 1700 (>CO, -CHO), 1660 (-CONH) $cm.^{-1}$ (Found: C, 46.97; H, 5.02; Cl, 21.13. $C_{13}H_{16}Cl_2N_2O_2S$ requires C, 46.57; H, 4.81; Cl, 21.15%).

Attempted Preparation of p-(NN-di-2-bromoethylamino) phenyl (formamido)thiolacetate. (128,131,140,155,156)
(a) N-Formylglycine (0.515 g.) was dissolved in NN-dimethylformamide-dichloromethane (20 ml., 1 : 1) to which was added a solution (10 ml.) of the nitrogen mustard B (1.695 g.) followed by a solution (5 ml.) of NN'-dicyclohexylcarbodi-imide (1.3 g.) using the same solvents in the same proportions. The mixture was shaken for 14 hours at 22° and then worked up to give a viscous yellow oil which could not be induced to crystallize. $\nu_{max.}$ in chloroform, 3450 (>NH), 1690 (>CO, -CONH) $cm.^{-1}$ (Found: Br, 40.36. $C_{13}H_{16}Br_2N_2O_2S$ requires Br, 37.68%).

(b) p-(NN-Di-2-chloroethylamino)phenyl (formamido)-thiolacetate (0.19g.), prepared by the method described

before, was dissolved in isobutyl methyl ketone (6 ml.). To this solution was added lithium bromide (0.675 g.) and the mixture refluxed for $1\frac{3}{4}$ hours. It was then diluted with chloroform (25 ml.); washed quickly with ice water (3 x 25 ml.); dried ($MgSO_4$) and concentrated to give a yellow oil which could not be induced to crystallize. $\nu_{max.}$ in chloroform, 3450 (>NH), 1690 (>CO; -CONH) $cm.^{-1}$

(c) N-Formylglycine (0.515 g.) was dissolved in dry NN-dimethylformamide (5 ml.) and triethylamine (1.43 ml.). The solution was cooled to 4° and ethyl chloroformate (0.5 ml.) was added. After 20 minutes, a cooled solution (7.5 ml.) of nitrogen mustard B (1.695 g.) in NN-dimethylformamide was added to the mixture, which was stirred for one hour and then left at ca. 3° for 24 hours. The precipitated triethylamine hydrochloride was filtered off and concentrated to give an oil which was taken up in chloroform (25 ml.), washed quickly with ice water (4 x 20 ml.), dried ($MgSO_4$) and concentrated to give a pale yellow oil. $\nu_{max.}$ in chloroform, 3450 (>NH), 1685 with

shoulder at 1670 (>CO, -CONH) $cm.^{-1}$

N-Methoxycarbonylglycine. (17)

It was prepared by the action of methyl chloroformate on a mixture of glycine and aqueous sodium carbonate, according to the method described by Leuchs.[97] It was recrystallized from ether-petroleum (b.p. 60-80°) and had m.p. 94-95° (lit. 95-96°). $\nu_{max.}$ in chloroform 3450 (>NH), 2950-2500 (a group of bands, -COOH), 1755-1690 (-COOH, -CONH, -COOMe) $cm.^{-1}$

S-(N-Methoxycarbonylglycyl)thiophenol. (100)

To a suspension of N-methoxycarbonylglycine (0.665 g.) in dichloromethane (20 ml.) was added a solution (15 ml.) of thiophenol (0.55 g.) in dichloromethane followed by

(continued on page 80)

a solution of NN'-dicyclohexylcarbodi-imide (1.3 g.) in dichloromethane (5 ml.). The mixture was shaken for 16 hours and then treated as described earlier to give S-(N-methoxycarbonylglycyl)thiophenol (0.83 g., 74%). It was recrystallized from benzene-petroleum (b.p. 40-60°) and had m.p. 78-79°. $\nu_{max.}$ in chloroform, 3500 (>NH), 1730-1710 (>CO, -COOMe) $cm.^{-1}$ (Found:C, 53.14; H, 4.92; S, 14.44. $C_{10}H_{11}NO_3S$ requires C, 53.32; H, 4.92; S, 14.23%).

Attempted preparation of p-(N-methoxycarbonylglycylthio)-NN-diethylaniline. (61,78,85)

(a) To a cooled solution of N-methoxycarbonylglycine (0.665 g.) in dry NN-dimethylformamide (5 ml.) and triethylamine (1.43 ml.), was added ethyl chloroformate (0.5 ml.). After 20 minutes a cold solution (7.5.ml.) of p-(NN-diethylamino)thiophenol (0.91 g.) in NN-dimethylformamide was added to the mixture which was kept stirred at ca. 0° for 4 hours and then stored in the refrigerator for 48 hours. On treating the mixture by the usual method a yellow oil was obtained which could not be induced to crystallize. $\nu_{max.}$ in chloroform, 3490 (>NH), 1750-1710, and 1670 (>CO, -CONH,

-COOMe) $cm.^{-1}$

(b) N-Methoxycarbonylglycine (0.665 g.) was dissolved in NN-dimethylformamide-dichloromethane (15 ml., 1 : 1). To this solution was added a dichloromethane solution (20 ml.) of p-(NN-diethylamino)thiophenol (0.897 g.) followed by a solution of NN'-dicyclohexylcarbodi-imide (1.1 g.) in dichloromethane (5 ml.). The mixture was shaken for $16\frac{1}{2}$ hours at the end of which it was worked up as usual to give a pale yellow oil, which could not be crystallized. $\nu_{max.}$ (liquid film), 3390 (>NH), 1730-1650 (>CO, -CONH, -COOMe) $cm.^{-1}$

p-(NN-Di-2-chloroethylamino)phenyl (methoxycarbonylamino) thiolacetate. (118)

To a suspension of N-methoxycarbonylglycine (0.665 g.) in dichloromethane (20 ml.), was added a dichloromethane solution (15 ml.) of nitrogen mustard A (1.25 g.), followed by NN'-dicyclohexylcarbodi-imide (1.3 g.) in dichloromethane (5. ml.). Shaking of the mixture for 18 hours and then working it up as described previously afforded a viscous pale yellow oil, which, on trituration with ether gave crystalline p-(NN-di-2-chloroethylamino)-

phenyl (methoxycarbonylamino)thiolacetate. It was recrystallized from benzene-petroleum (b.p. 40-60°) (0.5 g., 35%) and had m.p. 92-93°. $\nu_{max.}$ (nujol mull), 3375 (>NH), 1710 and 1698 (>CO, -CONH, -COOMe) $cm.^{-1}$ (Found: C, 46.15; H, 5.05; Cl, 19.66. $C_{14}H_{18}Cl_2N_2O_3S$ requires C, 46.03; H, 4.97; Cl, 19.42%).

Attempted preparation of p-(NN-di-2-bromoethylamino)-phenyl (methoxycarbonylamino)thiolacetate. (133)

The condensation of N-methoxycarbonylglycine (0.665 g.), with the nitrogen mustard B (1.695 g.) in the presence of NN'-dicyclohexylcarbodi-imide (1.3 g.), using dichloromethane as solvent according to the method described before, gave a viscous yellow oil, which could not be induced to crystallize. $\nu_{max.}$ (liquid film), 3350 (>NH) 1720-1680 (>CO, -CONH, -COOMe) $cm.^{-1}$

DERIVATIVES OF DL-PHENYLALANINE

N-Acetyl-DL-phenylalanine. (158)

It was prepared in 92.5% yield by the action

of acetic anhydride on a suspension of DL-phenylalanine in 25% acetic acid, essentially by the procedure described in the literature.[98] It was recrystallized from hot water and had m.p. 147-148° (lit. m.p. 148°). $\nu_{max.}$ (nujol mull), 3375 (>NH), 2700-2300 (a group of bands, -COOH), 1700 and 1620 (>CO, -CONH)$cm.^{-1}$

p-(NN-Di-2-chloroethylamino)phenyl 2-acetylamino-3-(phenyl)thiolpropionate. (160)

A solution of N-acetyl-DL-phenylalanine (1.04 g.) was prepared in dry NN-dimethylformamide-dichloromethane (15 ml., 1 : 1), to which was added a solution (10 ml.) of the nitrogen mustard A (1.25 g.) followed by a solution (5 ml.) of NN'-dicyclohexylcarbodi-imide (1.3 g.) using the same solvents in the same proportions. The mixture, after having been shaken at 20° for 16 hours, was worked up as described earlier to give a yellow oil, which, on treatment with acetone-petroleum (b.p. 40-60°) crystallized out as p-(NN-di-2-chloroethylamino)phenyl 2-acetamido-3-(phenyl)thiolpropionate (0.682 g., 32%), It was recrystallized from benzene-petroleum (b.p. 40-60°) and had m.p.133.5-134.0°. $\nu_{max.}$ (nujol mull), 3325 (>NH), 1700 and 1650 (>CO, -CONH) $cm.^{-1}$ (Found: C, 57.63; H, 5.44;

Cl, 16.14. $C_{21}H_{24}Cl_2N_2O_2S$ requires C, 57.40; H, 5.51; Cl, 16.14%).

Attempted preparation of p-(NN-di-2-bromoethylamino)phenyl 2-acetamido-3-(phenyl)thiolpropionate. (164)

The preparation was attempted by the method described above for the chloro analogue. The resulting yellow oil could not be induced to crystallize. $\nu_{max.}$ in chloroform, 3450 (>NH), 1820 and 1685 (>CO, -Ac, -CONH) $cm.^{-1}$

N-Formyl-DL-phenylalnine. (166,168)

(a) It was prepared from DL-phenylalanine, 90% formic acid and acetic anhydride according to the method described by du Vigneaud and Meyer[99] in 87% yield and had m.p. 171.5-172.5° (lit.[99] 91%; m.p. 168-169°). $\nu_{max.}$ (nujol mull), 3275 (>NH), 2800-2400 (a group of bands, -COOH), 1740 (-CHO), 1620 (-CONH) $cm.^{-1}$

(b) N-Formyl-DL-phenylalanine was also prepared by the action of 90% formic acid on DL-phenylalanine at reflux temperature according to the method described by Overby and Ingersoll.[100] It was recrystallized from hot water

and had m.p. 170-171° (lit.[100] m.p. 165-166°).

Attempted preparation of p-(NN-di-2-chloroethylamino)phenyl 2-formamido-3-(phenyl)thiolpropionate. (170)

To a solution (15 ml.) of N-formyl-DL-phenylalanine (0.965 g.) in dry NN-dimethylformamide-dichloromethane (1 : 1), was added a solution (10 ml.) of the nitrogen mustard A (1.25 g.), followed by a solution (5 ml.) of NN'-dicyclohexylcarbodi-imide (1.4 g.) using the same solvents taken in the same proportions. The mixture was shaken for 16 hours and then treated as described previously to give a yellow oil which could not be crystallized. $\nu_{max.}$ in chloroform, 3450 (>NH), 1700-1680 (>CO, -CONH) $cm.^{-1}$

p-(NN-Di-2-bromoethylamino)phenyl 2-formamido-3-(phenyl)-thiolpropionate. (173)

N-Formyl-DL-phenylalanine (0.965 g.) was dissolved in dry NN-dimethylformamide-dichloromethane (18 ml., 1 : 2). To this solution was added a solution (18 ml.) of the nitrogen mustard B (1.695 g.) and then a solution (4.5 ml.) of NN'-dicyclohexylcarbodi-imide (1.3 g.) using the same solvents in the same proportions. The

mixture was shaken for 16 hours and treated in the usual way to produce an oil which at first could not be induced to crystallize, but when a solution in dichloromethane was passed through a layer of decolorising charcoal and the filtrate treated with petroleum (b.p. 40-60°), cooled with acetone-CO_2, and the sides of the container scratched with a glass rod, p-(NN-di-2-bromoethylamino)phenyl 2-formamido-3-(phenyl)-thiolpropionate (0.69 g., 27%) crystallized out. It was recrystallized from benzene-petroleum (b.p.40-60°) and had m.p. 147-149°. $\nu_{max.}$ in chloroform, 3450 (>NH) 1690 (>CO) cm^{-1} (Found: C, 46.70; H, 4.31; S, 6.23. $C_{20}H_{22}Br_2N_2O_2S$ requires C, 46.62; H, 4.21; S, 6.34%).

N-Methoxycarbonyl-DL-phenylalanine. (176)

A mixture of DL-phenylalanine (10 g.), N sodium hydroxide (60 ml.), methyl chloroformate (6 ml.) and aqueous sodium carbonate (3.2 g.) was treated in the same way as described by Petri and Staverman.[101] The product, a viscous colourless oil (11.5 g., 85 %) (lit. 77.5%), was left at room temperature when after 3 months 20 days it crystallized out as a white mass and had m.p.

75–76°. Chibnall and Spahr[102] also reported that the oil solidified on standing at room temperature. $\nu_{max.}$ in chloroform, 3450 (>NH), 2800-2400 (a group of bands, -COOH), 1740-1690 (>CO, -CONH, -COOMe) $cm.^{-1}$

p-(NN-Di-2-chlorcethylamino)phenyl 2-methoxycarbonylamino-3-(phenyl)thiolpropionate. (179)

To a solution of N-methoxycarbonyl-DL-phenylalanine (1.115 g.) in dichloromethane (10 ml.), was added a solution (10 ml.) of the nitrogen mustard A (1.25 g.) and then a solution (5 ml.) of NN'-dicyclohexylcarbodi-imide (1.3 g.) using dichloromethane as a solvent in both cases. After shaking the mixture for 16 hours, it was treated in the usual manner to give a glass, which crystallized out with dichloromethane-petroleum (b.p. 30-40°) to give p-(NN-di-2-chloroethylamino)phenyl 2-methoxycarbonylamino-3-(phenyl)thiolpropionate (1.27 g., 56%). It was recrystallized from dichloromethane-petroleum (b.p. 30-40°) and had m.p. 132-133°. $\nu_{max.}$ in chloroform, 3450 (>NH), 1720-1700 (a doublet, >CO, -CONH, -COOMe) $cm.^{-1}$ (Found: C, 55.38; H,5.76; Cl, 15.92. $C_{21}H_{24}Cl_2N_2O_3S$ requires C, 55.38; H, 5.31; Cl, 15.57%).

p-(NN-Di-2-bromoethylamino)phenyl 2-methoxycarbonylamino-3-(phenyl)thiolpropionate. (182)

N-Methoxycarbonyl-DL-phenylalanine (1.115 g.) was dissolved in dry NN-dimethylformamide-dichloromethane (10 ml., 1:1) to which was added a solution (15 ml.) of the nitrogen mustard B (1.695 g.) followed by a solution (5 ml.) of NN'-dicyclohexylcarbodi-imide (1.3 g.) using the same solvents in the same proportions. The mixture was stirred for 16 hours and the usual procedure was followed for its work-up to give a pale yellow oil which crystallized when treated with dichloromethane-petroleum (b.p. 30-40°) to form p-(NN-di-2-bromoethylamino)-phenyl 2-methoxycarbonylamino-3-(phenyl)thiolpropionate (2.5 g., 92%) and had m.p. 136-137°. (Found: C, 46.36; H, 4.69. $C_{21}H_{24}Br_2N_2O_3S$ requires C, 46.33; H, 4.44%).

DERIVATIVES OF DL-METHIONINE

N-Formyl-DL-methionine. (239)

It was prepared from DL-methionine (7.45 g.), 88% formic acid (125 ml.) and acetic anhydride (42.5 ml.) at ca.60° according to the method of Sheehan and Yang[81] in 75% yield (after two crystallizations) and had m.p.

103-104°. (lit.103-104°).

p-(NN-Di-2-chloroethylamino)phenyl 2-formamido-4-(methylthio)thiolbutyrate. (144,150,242)

N-Formyl-DL-methionine (0.885 g.) was dissolved in dry NN-dimethylformamide-dichloromethane (15 ml., 1 : 1) to which was added a solution (10 ml.) of the nitrogen mustard A (1.25 g.) followed by a solution (5 ml.) of NN'-dicyclohexylcarbodi-imide (1.28 g.) using the same solvents in the same proportions. The mixture was shaken at 22° for 14 hours. The usual treatment of the mixture produced a yellow oil which crystallized out when treated with acetone-petroleum (b.p.40-60°) to give p-(NN-di-2-chloroethylamino)phenyl 2-formamido-4-(methylthio)thiolbutyrate (0.7 g., 35%). It was recrystallized from benzene-petroleum (b.p.40-60°) and had m.p. 96-97.5°. $\nu_{max.}$ (nujol mull), 3340 (>NH), 1690 (>CO), 1660 (-CONH) $cm.^{-1}$ (Found: C, 46.91; H, 5.30; Cl, 17.57. $C_{16}H_{22}Cl_2N_2O_2S_2$ requires C, 46.94; H, 5.42; Cl, 17.32%).

Attempted preparation of p-(NN-di-2-bromoethylamino)phenyl 2-formamido-4-(methylthio)thiolbutyrate. (148,152)

To a solution of N-formyl-DL-methionine (0.885 g.) in dry NN-dimethylformamide-dichloromethane (15 ml., 1 : 1), was added a solution (15 ml.) of the nitrogen mustard B (1.695 g.), followed by a solution (5 ml.) of NN'-dicyclohexylcarbodi-imide (1.3 g.) using the same solvents in the same proportions. The mixture was shaken for 14 hours and treated as above to give a yellow oil which could not be crystallized. $\nu_{max.}$ in chloroform, 3450 (>NH), 1775 and 1690 (>CO, -CONH) $cm.^{-1}$

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Part II

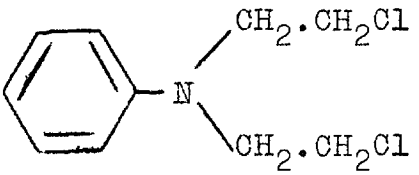
SULPHUR MUSTARDS

CHAPTER I

INTRODUCTORY REVIEW

SULPHONIUM INTERMEDIATES AND MOLECULAR REARRANGEMENTS

In part I, the nitrogen mustards and their derivatives, which were designed to exhibit cytotoxic activity, have been described. It has been seen that in the so-called nitrogen mustards of the type (I), the reactivity can be varied by structural modifications in the benzene ring, thus affecting the chemical reactivity of the halogen atoms and the biological activity of the compound. The structural requirements for activity in

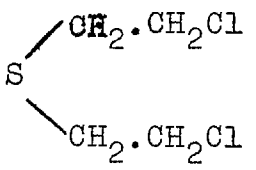


I

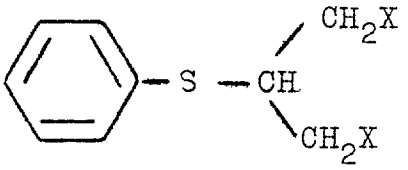
the nitrogen mustards suggested that two alkylating groupings, capable of reacting with functional centres of biological systems, were required for the compounds to be effective as cytotoxic agents. The vesicant War Gas, ββ'-dichlorodiethyl sulphide (Mustard Gas) (II),

also is cytotoxic and although prior to 1939 it was not regarded as particularly reactive under physiological conditions, work done since that time has shown that it is capable of direct reaction with many functional groups.

As sulphur is divalent in the mustard gas (II), it cannot carry an additional phenyl substituent, and hence its structure cannot be modified as is possible with the nitrogen-mustard (I). But because the reactivity of the nitrogen- and sulphur- mustards is associated with the fact that the halogen atoms are in the β-position to a nitrogen or sulphur atom, a structure (III) would allow the usual variables to be made in the benzene ring.



II

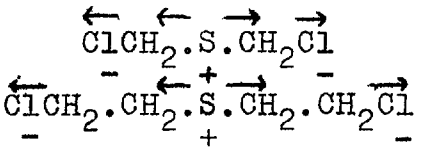


III

Since structure (III) might undergo rearrangement under the influence of certain nucleophiles, it will be relevant first to consider the formation of sulphonium ions and their subsequent molecular rearrangements.

The remarkable chemical reactivity of ββ'-dichloro-

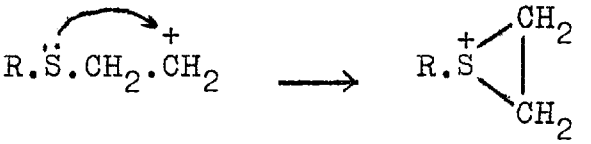
diethyl sulphide must be attributed to the influence of the sulphur atom upon the two chlorine atoms. A high degree of reactivity is generally shown by the chlorine atoms in compounds of the general formula $R.S.CH_2.CH_2Cl$. The chlorine in the isomeric αα'-dichlorodiethyl sulphide is also in a reactive condition. γγ'-Dichlorodipropyl sulphide is decidedly less reactive than ββ'-dichlorodiethyl sulphide and ββ'-dichlorodipropyl sulphide.[1] The high reactivity of chlorine in the α- and β- positions was originally attributed to a general polar effect of the sulphur atom, which by repelling electrons increased the negative charge on the chlorine atoms, the effect being greater in the α- than in the β- position owing to the smaller distance of chlorine from sulphur in the former case.[2]



This simple type of inductive effect is no longer considered adequate to explain the high reactivity of such compounds.

As a result of their studies on the hydrolysis of mustard gas, Price and Wakefield[3] postulated a

cyclic ethylenesulphonium salt (IV) as an intermediate.

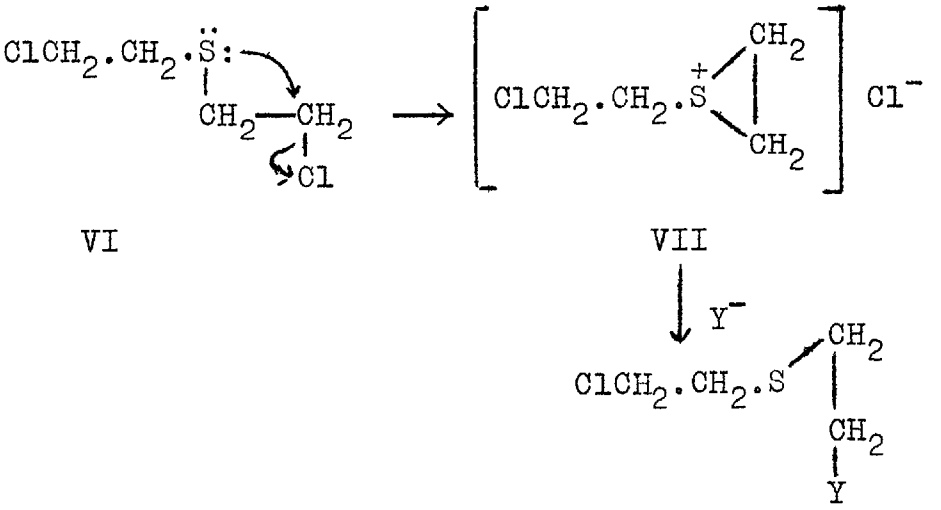


V IV

It appeared[4] that this intermediate must be the result of the stabilization of the carbonium ion (V) initially formed. Thus it was considered that the formation of the ion precedes cyclization. On the basis of this primary ionization to yield a carbonium ion, it is certainly possible to interpret the reactions of mustard gas, but more recent work has led to a modified mechanism.

The ability of certain sulphur moieties to participate as neighbouring groups has been ably demonstrated by Bartlett and Swain[5] in a kinetic study of the hydrolysis of mustard gas and 'mustard chlorohydrin'. It was recognized that the ethylenesulphonium ion (IV) offered an attractive explanation of the first order displacement reactions of mustard gas (II), but these authors attributed the rapid solvolysis of mustard gas to the fact that the neighbouring sulphur accelerates the ionization of the carbon-chlorine bond by direct participation as an internal displacing

reagent, as shown in (VI). The three membered ring sulphonium intermediate (VII) is highly susceptible[6]

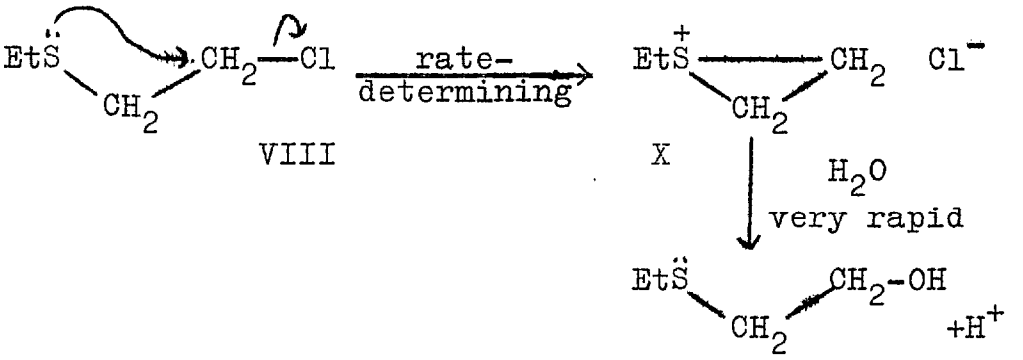


VI VII

to nucleophilic attack. Reaction occurs rapidly with any available nucleophilic species Y^-. The reagent Y^- may attack[7] the sulphonium ion at either of the ring carbon atoms, and if an aqueous medium is used, water[6] can act as nucleophilic agent.

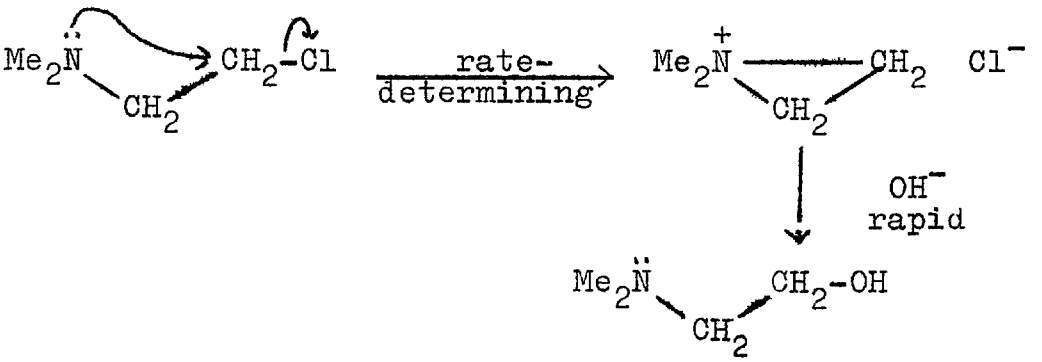
This concept of an intermediate cyclic sulphonium ion has been applied in many other cases. The extremely rapid hydrolysis of $ClCH_2.CH_2.SEt$ (VIII) in contrast to $ClCH_2.CH_2.OEt$ (IX) under the same conditions is thought to be due to the rate-determining formation of a cyclic sulphonium salt (X), which being highly strained undergoes extremely ready and rapid

hydrolysis.[8]



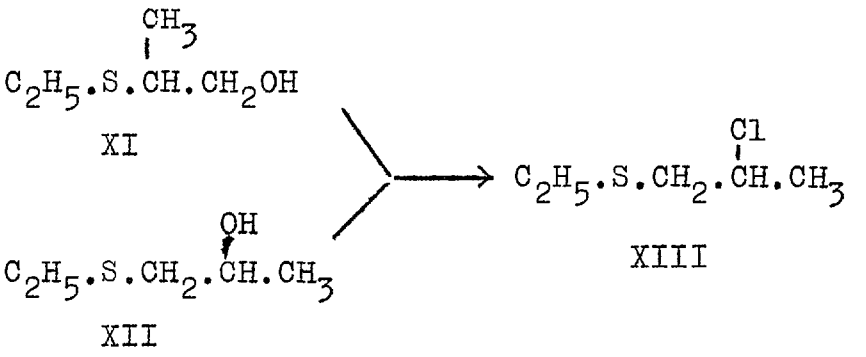
VIII X

The oxygen in the ether (IX) being more electronegative, does not part with its unshared electrons so readily as sulphur, hence no cyclic salt is formed and the chlorine undergoes hydrolysis by a normal displacement reaction. The hydrolysis of similar compounds containing nitrogen proceeds less rapidly than that of the sulphur compounds, reflecting the greater stability of the cyclic nitrogen-, as compared with the cyclic sulphur- intermediates.

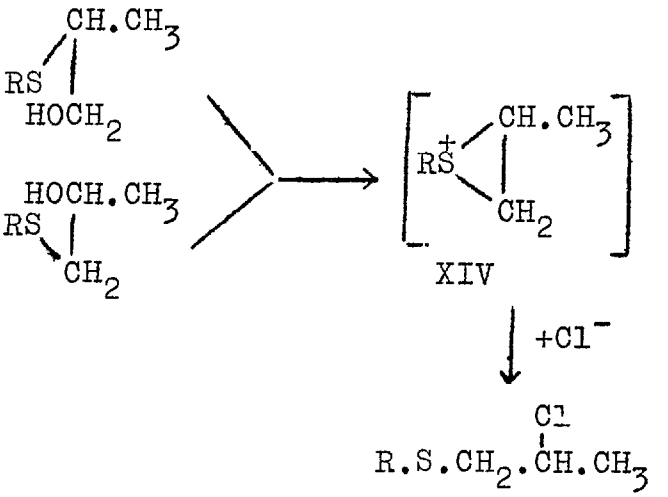


The reaction[9] of either 2-ethylthio-1-propanol (XI) or 1-ethylthio-2-propanol (XII) with hydrochloric acid

or thionyl chloride yields the same product, 2-chloro-n-propyl ethyl sulphide (XIII). The rearrangement of the isopropyl to the n-propyl structure is readily explained by the suggestion that nucleophilic replacement



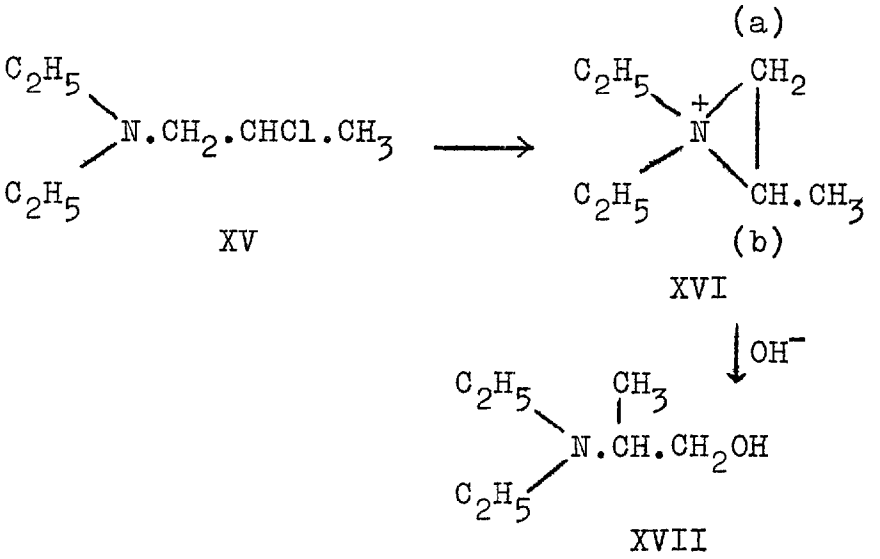
reactions in β-thioalkyl radicals may occur through a cyclic sulphonium intermediate (XIV). The formation



of (XIII) requires attack by a chloride ion at the secondary carbon atom in preference to the primary.

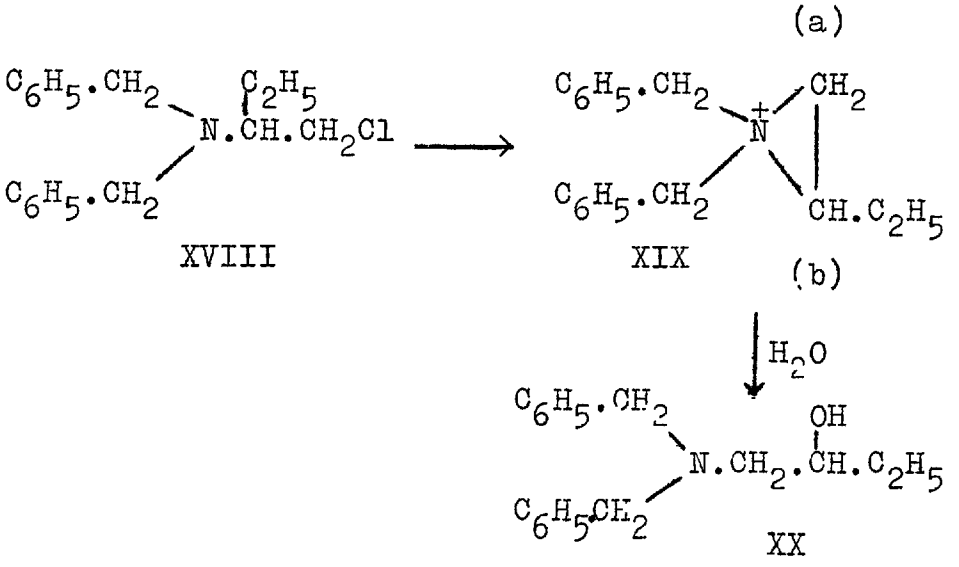
These results can be compared with those obtained

on a comparable nitrogen-mustard.[10] Basic hydrolysis of 1-diethylamino-2-chloropropane (XV) yields 2-diethylamino-1-propanol (XVII), and the neutral hydrolysis of



XV XVI XVII

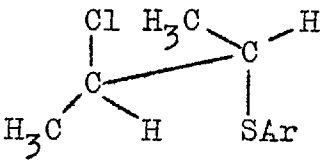
2-dibenzylamino-1-chlorobutane (XVIII) yields 1-dibenzylamino-2-butanol (XX). Both reactions undoubtedly proceed[11]



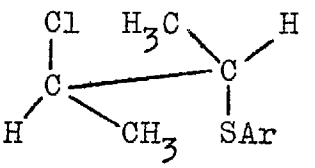
XVIII XIX XX

through the same analogous intermediate (XVI or XIX) which reacts with hydroxide ion at (a) but with water at (b).

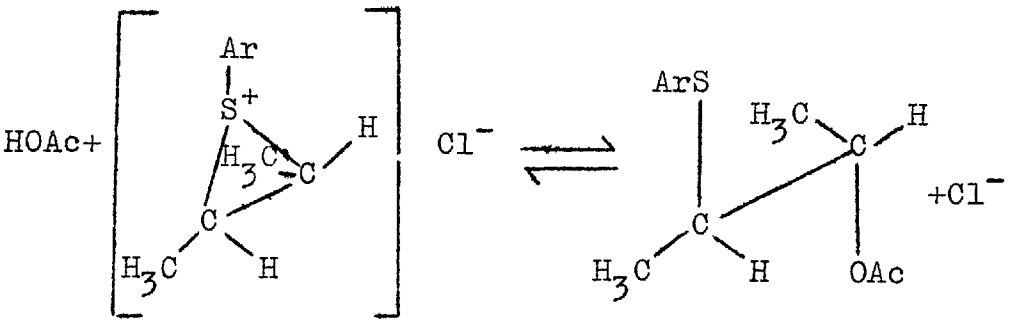
More recently, Havlik and Kharasch[12] studied the acetolysis of diastereoisomeric compounds of the type (XXI) and (XXII) and concluded that this must involve a cyclic sulphonium ion (XXIII) as an intermediate so as to account for the formation of identical racemic mixtures from each stereoisomer.



and

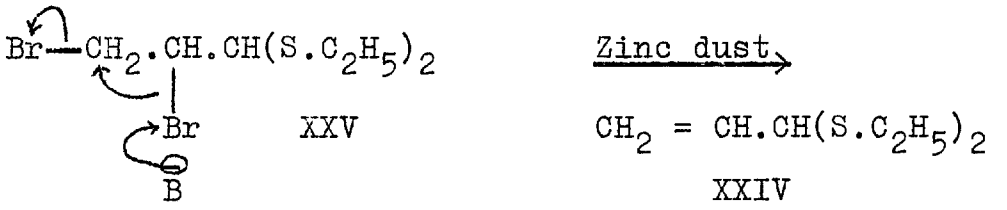


XXI XXII



XXIII

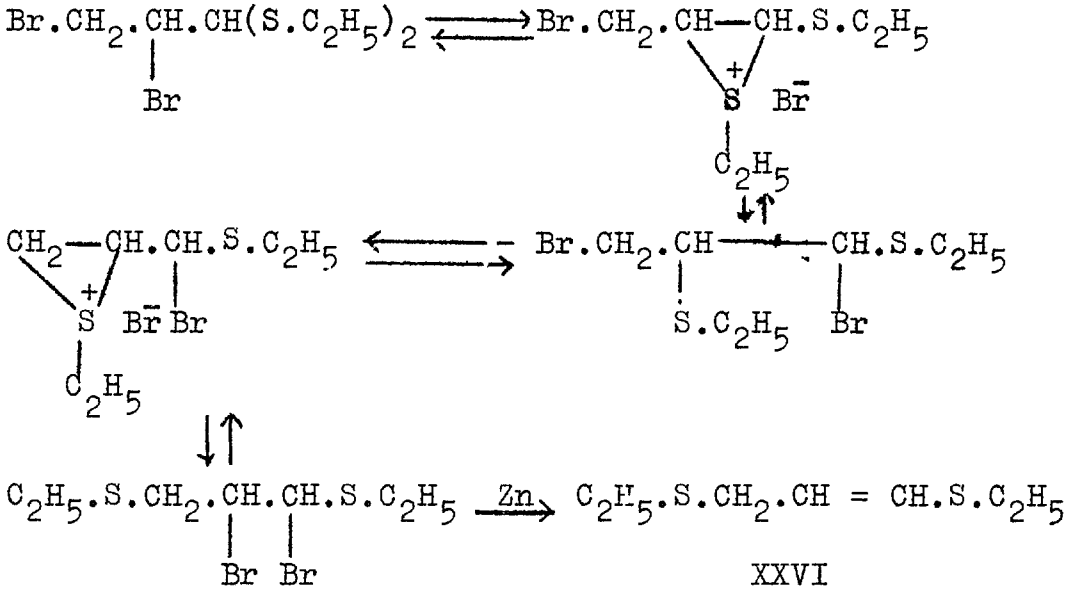
Rothstein[13] claimed to have prepared αα-bis(ethylthio)-prop-2-ene (XXIV) from βγ-dibromo-αα-bis(ethylthio)propane (XXV). A re-examination of the structure (XXIV) revealed[14]



that the product was actually 1,3-bis(ethylmercapto)-prop-1-ene (XXVI). The following mechanism was

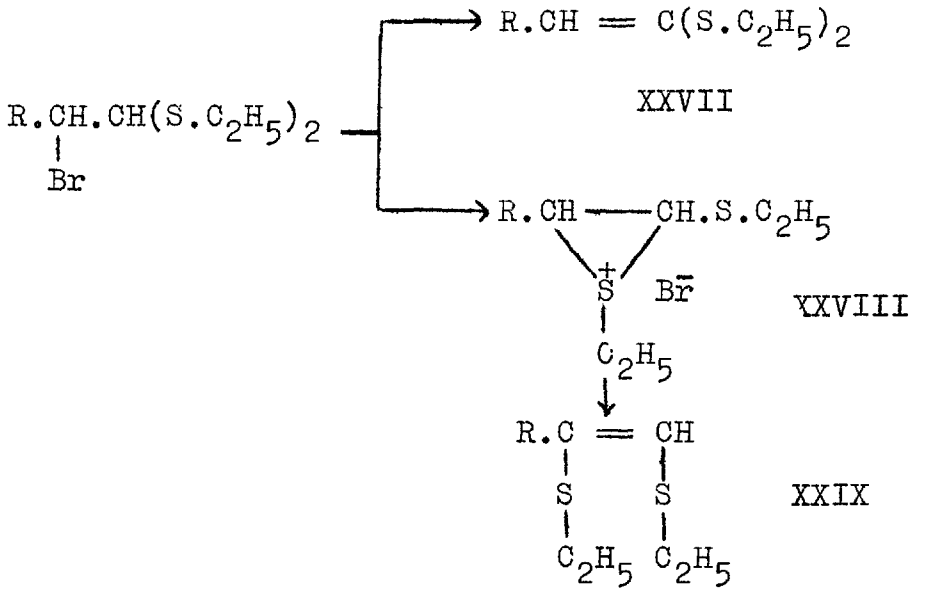
$$C_2H_5.S.CH_2.CH = CH.S.C_2H_5 \qquad \text{XXVI}$$

proposed[14] to account for the migration of one ethylmercapto group, which involves the formation of an ethylenesulphonium intermediate.



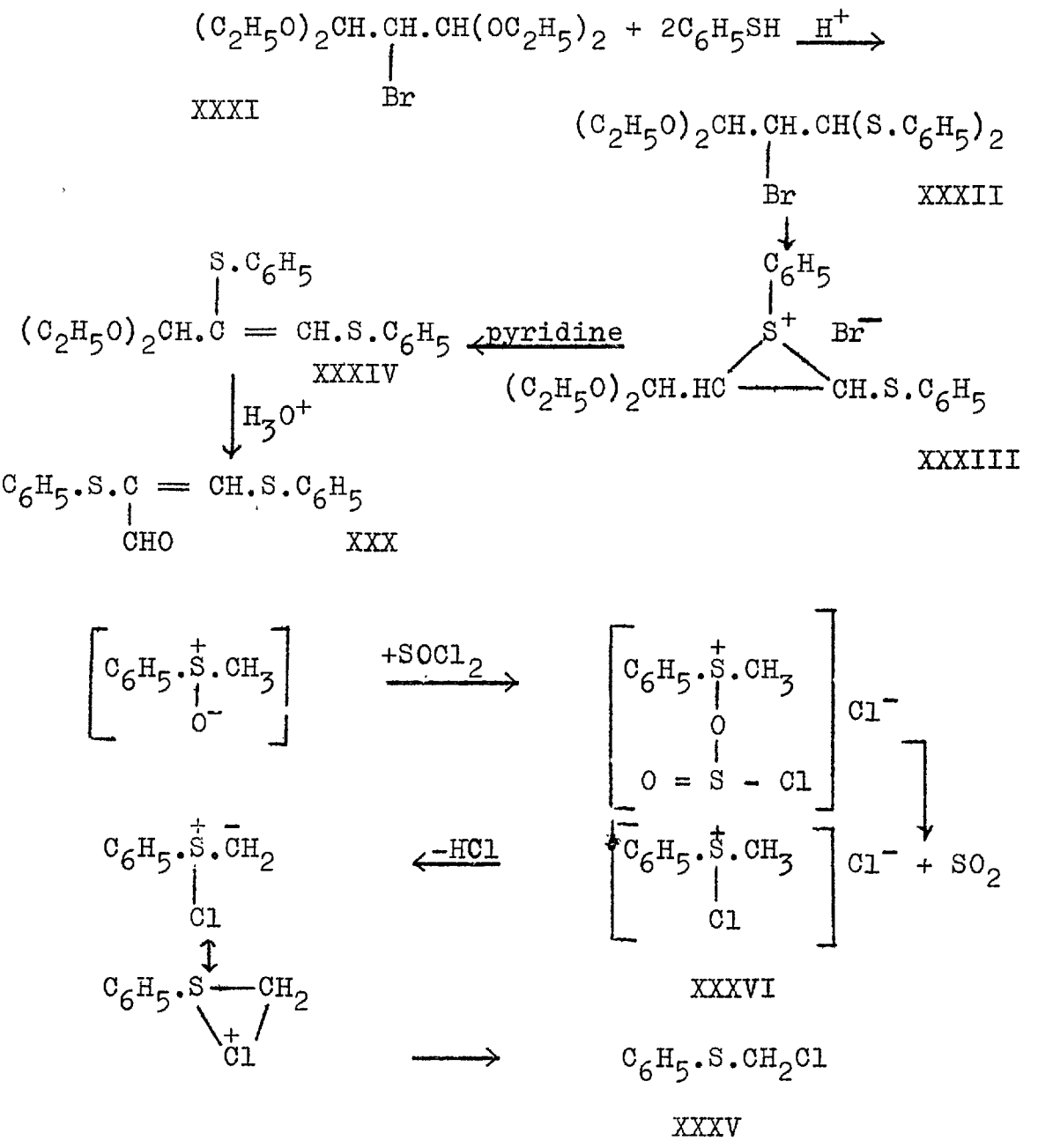
Rothstein and Whiteley[15] proposed the formation of ketenemercaptals (XXVII) after a spontaneous elimination of hydrogen halide from α-halomercaptals. But there is

a greater probability that such elimination reactions lead directly to the formation of 1,2-bis-(alkylmercapto)-ethylenes (XXIX) by molecular rearrangement involving a sulphonium intermediate[14] (XXVIII).



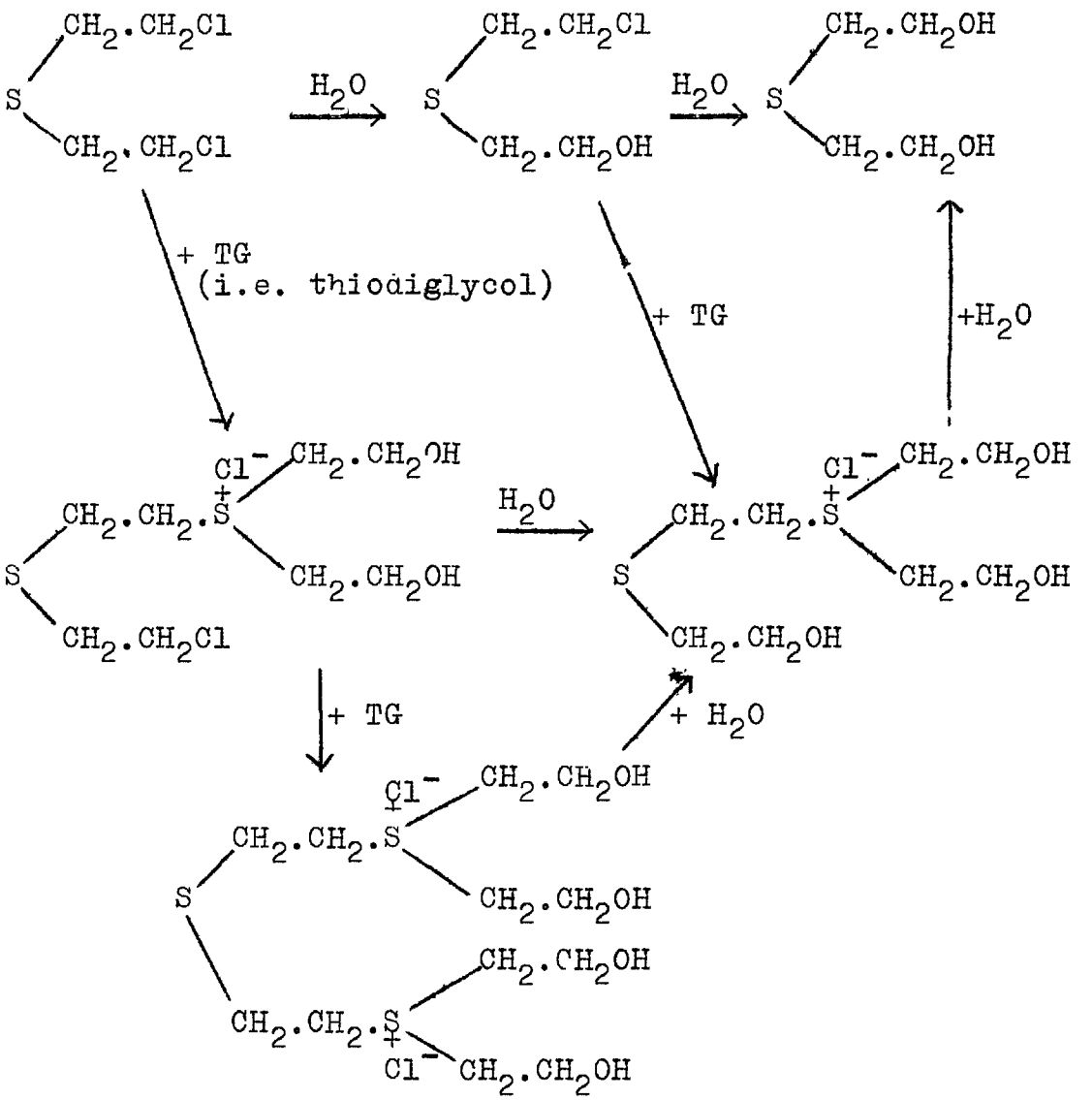
Parham and Heberling[16] described a synthesis of the aldehyde (XXX) by a route which provides convincing evidence that molecular rearrangement, involving sulphonium intermediates, occurred during the reaction of (XXXII) with pyridine.

Bordwell and Pitt[17] prepared phenyl chloromethyl sulphide (XXXV), by the action of thionyl chloride on phenyl methyl sulphoxide and one of the intermediates in their proposed mechanism was a chlorosulphonium chloride (XXXVI).



Finally it may be mentioned that acyclic sulphonium salts are also of biological interest. The physiological

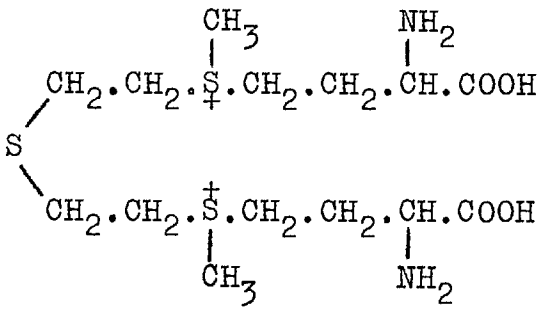
effects of mustard gas are the consequence of chemical processes which the agent initiates by its reaction with body constituents. The reactions given below indicate the importance of sulphonium salts as intermediates when mustard gas is hydrolysed in the presence of moderate quantities of water[18].



XXXVII

The formation of sulphonium salts during the hydrolysis of mustard gas is of physiological as well as chemical interest, since these salts possess a relatively great toxicity[18]. The toxicity of (XXXVII) is destroyed only when its solution is heated at 100° for 1-2 hours. It is established that the tendency to form sulphonium salts is much greater with the group $HO.CH_2.CH_2S$— than it is with the group $ClCH_2.CH_2S$—.

Stein and Moore[19] reported in 1946 the first example of transthiomethylation or methylmercapto migration. It was found that mustard gas reacts with methionine-sulphur more readily than it does with either the amino or carboxyl groups. From one molecule of mustard gas and two molecules of methionine, on treatment in 0.31 N hydrochloric acid, the sulphonium base (XXXVIII) has been isolated by formation of its crystalline azobenzene sulphonate, which is analogous in structure to the sulphonium salt (XXXVII).



XXXVIII

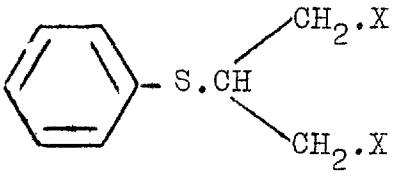
This is interesting since methionine is a protein constituent and plays an important role in biological transmethylation reactions. It is also well established that ATP plays an essential role in the transmethylation reactions which utilize methionine as the methyl donor[20].

Many other examples can be found in the literature[21] where the formation of sulphonium intermediates has been postulated to account for the products obtained during the reactions concerned, but these do not differ in principle from those discussed above.

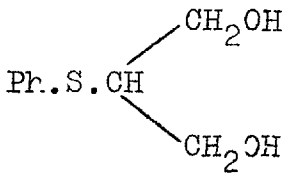
CHAPTER 2

Discussion

It has been stated in the preceding chapter that compounds with structure (III) might exhibit cytotoxic activity, and also allow structural modifications to be made by substitution in the benzene ring. Hence a research programme was undertaken which involved synthetic and reactivity studies of compounds of the type (III). An essential stage of this programme was the synthesis of 2-(phenylthio)propane-1,3-diol (XXXIX).

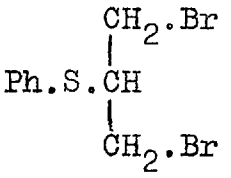


III

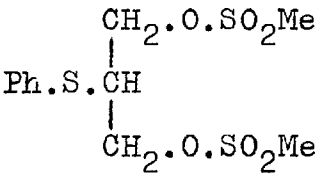


XXXIX

While attempting to prepare the sulphur mustard (XL), it was found convenient to synthesise the dimethanesulphonate derivative (XLI) and it was expected that this might act as an alkylating agent. During the investigations of its alkylating properties, the effect of the attack of various nucleophiles on it was thoroughly studied.

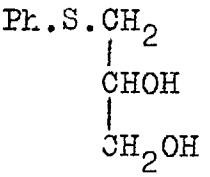


XL



XLI

The data available in the field of nuclear magnetic resonance spectrography on organic sulphur compounds were insufficient to be of any immediate help in solving the rearrangement problems that arose during the present investigations, and hence a good deal of information on this subject had to be recorded during the course of the present studies. The programme also included a parallel study on 3-(phenylthio)propane-1,2-diol (XLII) and its derivatives.

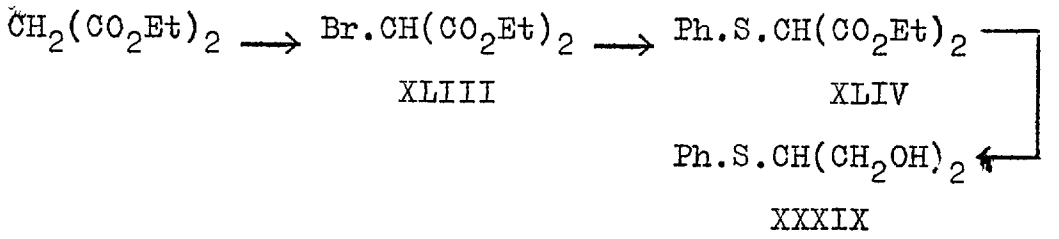


XLII

2-(PHENYLTHIO)PROPANE-1,3-DIOL AND ITS DERIVATIVES

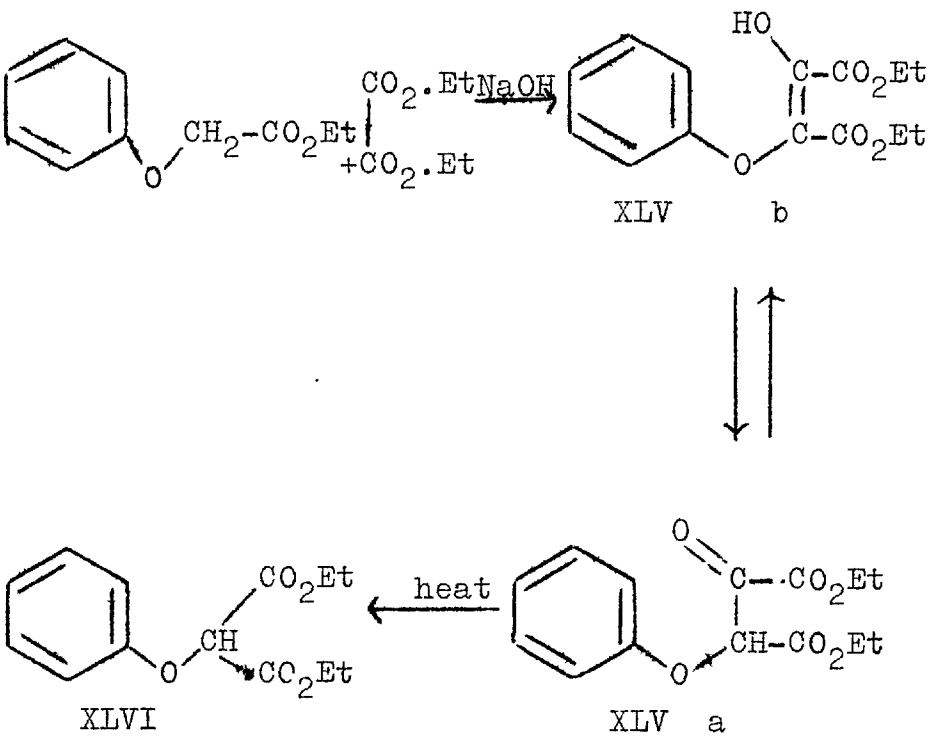
Synthesis of 2-(phenylthio)propane-1,3-diol.

It was presumed that the following synthetic scheme might lead to the production of the desired 1,3-diol.

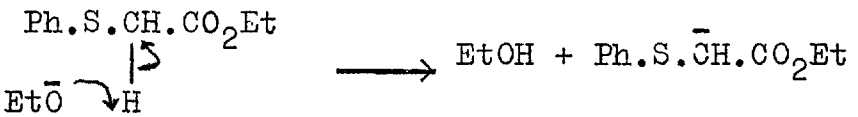


The ethyl bromomalonate (XLIII) was prepared[22] in 72% yield by bromination of diethyl malonate. (XLIII) was then converted into the diethyl (phenylthio)malonate (XLIV) by the action of sodium thiophenate in dry ethanol but the yield was poor and so the next stage for the synthesis of (XXXIX) was not attempted.

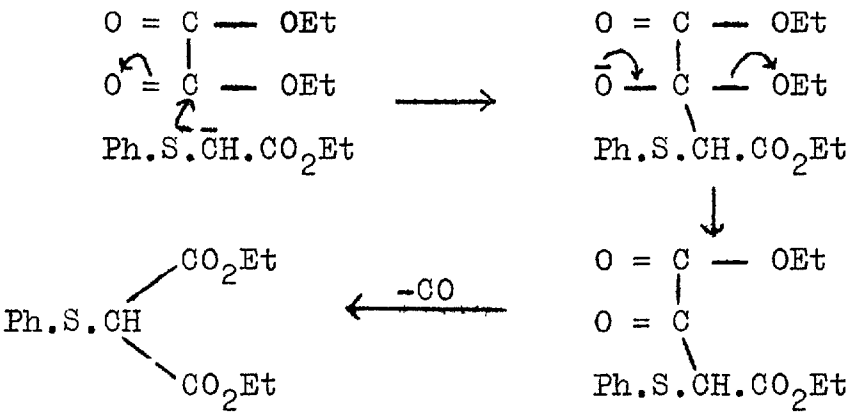
During the course of studies on the preparation of various thianaphthene derivatives, Huntress and Olsen[23] prepared the Koelsch and Whitney intermediate[24] which Huntress and Olsen[23] preferred to designate as diethyl oxalo-phenoxyacetate (XLV). Upon heating, the ketonic tautomer (XLV a) suffered a loss of carbon monoxide with consequent formation of diethyl phenoxy-malonate (XLVI).



The same workers also reported the preparation of diethyl (phenylthio)malonate (XLIV) according to the following scheme.



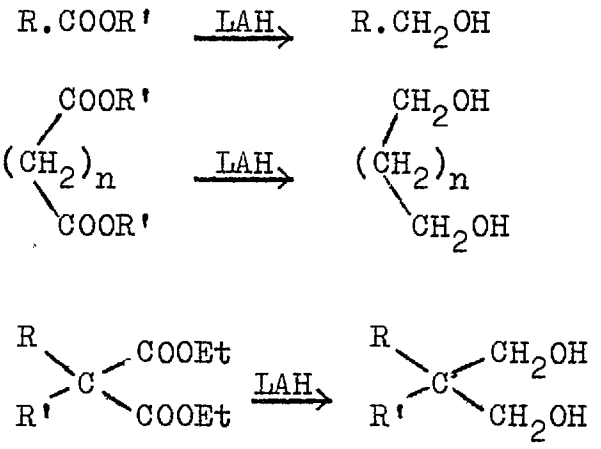
XLVII



XLIV XLVIII

The present author also has followed the latter scheme. Ethyl (phenylthio)acetate (XLVII) was prepared in 63% yield from ethyl chloroacetate ($ClCH_2.CO_2Et$) by the action of sodium thiophenate in ethanol. The acetate (XLVII) was then converted into diethyl oxalo(phenylthio)acetate (XLVIII) in 90% yield using Huntress and Olsen's method[23] with certain modifications. The oxaloacetate (XLVIII), however, could not be distilled without decomposition and was used directly. On heating this (XLVIII) at 170° and 50 mm. pressure, carbon monoxide was readily evolved to give diethyl (phenylthio)malonate (XLIV) in fairly good yield. The reduction of the malonate (XLIV) was expected to give the desired final product viz. 2-(phenylthio)propane-1,3-diol (XXXIX).

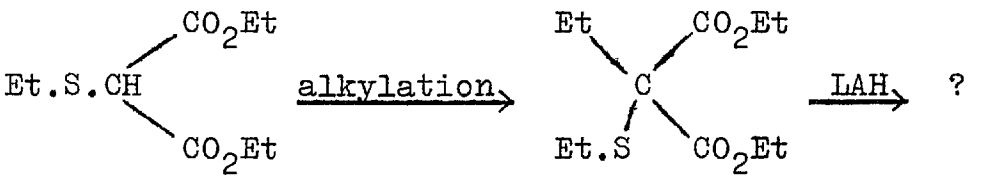
The conversion of esters to primary alcohols represents a very important application of lithium aluminium hydride (LAH) reductions. The reduction consumes one-half mole of the hydride for each mole of mono ester reduced. This method has been widely used for the synthesis of monofunctional as well as difunctional compounds. It gave satisfactory yields with malonic esters[25] of the type $RR'C(CO_2Et)_2$ where R was hydrogen or alkyl and R' was alkyl or aryl producing 1,3-propane diols. The succinic esters are converted to 1,4-butane diols. Yields were poorer when



the ester was of the type $RR'C(CO_2Et)_2$ or $RR'SC(CO_2Et)_2$.

All the present efforts to reduce diethyl (phenylthio)malonate (XLIV) to the 1,3-diol (XXXIX) under various conditions by using LAH in ether or THF,

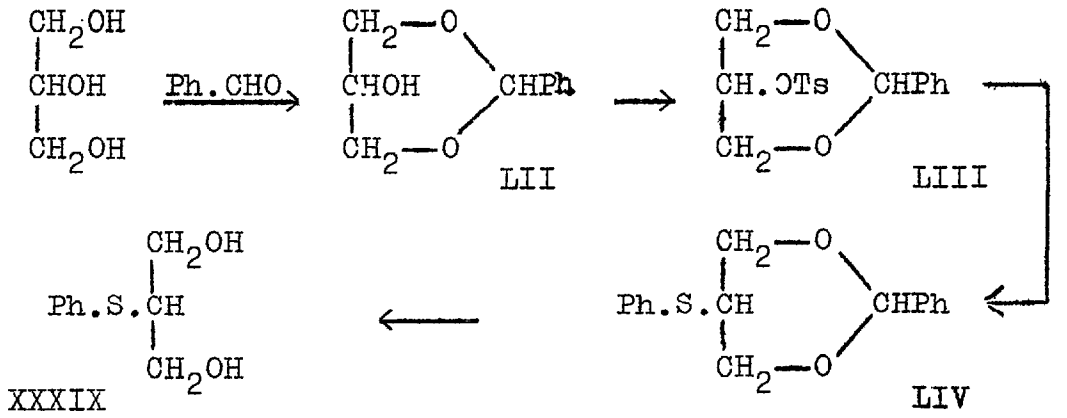
or by Bouveault and Blanc's method, were disappointing. The best results were obtained under mild conditions (at 19° over 96 hours) with LAH, which gave the desired 1,3-diol (XXXIX) in 15% yield (still rather impure). It was characterized as its bis-p-nitrobenzoate. Yale et al.[25] have reported similar discouraging results; after alkylation of diethyl ethylmercaptomalonate (L) with ethyl bromide to obtain diethyl ethyl-ethylmercaptomalonate (LI), they subjected the latter to LAH reductions, which appeared to proceed normally, but no material was obtained boiling above 100° probably due to degradation to volatile products.



L LI

As the yield and the purity of the 1,3-diol (XXXIX) obtained by the scheme mentioned earlier was not satisfactory, a third route was planned which can be claimed as of a novel type. (See page 123)

Hibbert et al.[26] reported in 1928 the preparation of 1,3-O-benzylideneglycerol (LII), the starting material

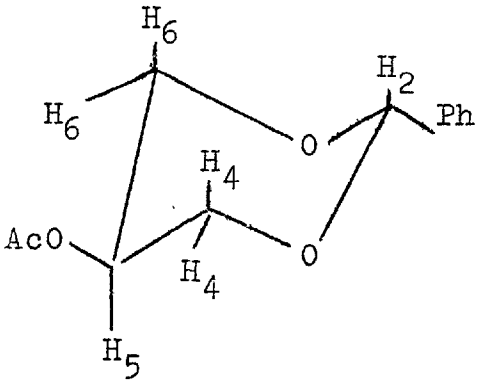


of the present series of reactions, by the condensation of glycerol with benzaldehyde in an atmosphere of carbon dioxide. Then in 1929, Hibbert and Carter[27] repeated the above condensation reaction in the presence of concentrated hydrochloric acid and found these conditions to be better than the former one. Evans and Owen[28] claimed to have improved the yield of the product by maintaining a slow stream of the hydrogen chloride gas through the mixture of glycerol and benzaldehyde during the course of the reaction. Consequently the method described by Evans and Owen was followed in the present case also. A mixture of 1,2- and 1,3- compounds was obtained as an oil from which the solid 1,3-isomer was separated and recrystallised from benzene-light petroleum (b.p.60-80°). It melted over a wide range of 69-76° presumably owing

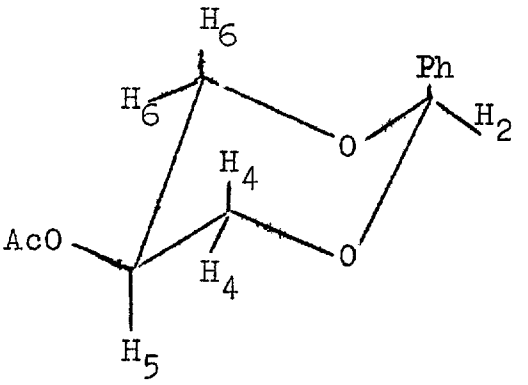
to the existence of the two forms, m.p.s 63° and 83.5°, already described in the literature[29].

1,3-O-Benzylideneglycerol (LII) was also prepared[30] by the condensation of glycerol and benzaldehyde in the presence of 60% perchloric acid. This method gave a cleaner product in better yield. It required great care for recrystallization, as the presence of traces of perchloric acid catalyses the re-establishment of the 1,2- $\rightleftharpoons$ 1,3- equilibrium, with consequent heavy loss of the 1,3-isomer. This loss was kept to its minimum by not allowing the temperature to rise above 50° during recrystallisation and by cooling the warm solution immediately.

The nuclear magnetic resonance spectrum in deuterochloroform showed a complex pattern, the shielding values, based on the τ scale, being 2.55 (5H; aromatic), 4.33 (1H; >CHPh), 5.88 (3H), 6.33 (2H), 7.22 (1H, -OH). These values were comparable with those recorded by Baggett *et al.* for cis- and trans- 2-O-acetyl-1,3-O-benzylideneglycerol as shown.



trans- form



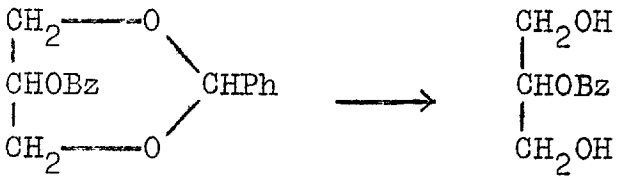
cis- form

	(complex coupling pattern)	(no resolvable coupling pattern)
τ,	2.65 (5H; aromatic)	2.65 (5H; aromatic)
	4.58 (1H; H_2)	4.51 (1H; H_2)
	5.0 to 7.0 (5H; H_5, $H_{4a,e}$, $H_{6a,e}$)	5.37 (1H; H_5)
		5.82 (4H; $H_{4,6}$)
	8.04 (3H; CH_3CO)	7.88 (3H; CH_3CO)

The tosylation of 1,3-O-benzylideneglycerol (LII) was carried out according to Matheson and Angyal's method[32] which gave 1,3-benzylidenedioxy-2-toluene-p-sulphonyloxypropane (LIII) in 75% yield. It was treated with sodium thiophenate in NN-dimethylformamide to give 1,3-benzylidenedioxy-2-(phenylthio)propane (LIV) in 71% yield.

Hibbert and Carter[27] found that 2-O-benzoyl-1,3-O-benzylideneglycerol (LV) readily undergoes hydrolysis of

its acetal ring under the influence of a trace of acid, to give glycerol monobenzoate (LVI).

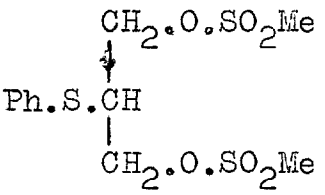


LV LVI

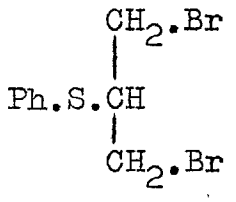
Therefore, the hydrolysis of 1,3-benzylidenedioxy-2-(phenylthio)propane (LIV) was carried out with normal sulphuric acid at 50-55°. Neutralization of the reaction mixture with ammonia gave 2-(phenylthio)propane-1,3-diol (XXXIX) in excellent yield (92%). The nuclear magnetic resonance spectrum was consistent with its structure. Its bis-p-nitrobenzoate derivative did not show any depression in melting point when admixed with the previous sample.

Synthesis of derivatives of the 1,3-diol.

It has already been stated in the beginning of this chapter that the dimethanesulphonate derivative (XLI) might act as an alkylating agent. In part I of this thesis, the alkylating agents have been shown to be very effective in their injurious action on the mitotic mechanism and thus arresting the cell multiplication which is responsible for malignant growth. Consequently the 1,3-dimethanesulphonate was regarded as equivalent



XLI

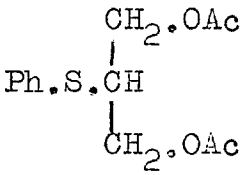


XL

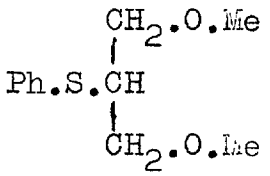
to the sulphur mustard (XL). This supposition led to the synthesis and detailed investigation of the alkylating properties of the 1,3-dimethanesulphonate (XLI). It was synthesised by the action of methanesulphonyl chloride on the 1,3-diol (XXXIX) in dry pyridine at low temperature. The yield of crystalline product was 75 to 91% with various batches. It was found to be unstable and decomposed gradually on being stored, and hence for each reaction a freshly prepared and purified sample was used. Authentic samples of the diacetate and the dimethyl ether

of the 1,3-diol were prepared as follows.

It is known that the hydroxyl groups of a polyhydric material react readily with acetic anhydride in the presence of an acidic catalyst (zinc chloride, hydrogen chloride, sulphuric acid or perchloric acid) or a basic catalyst (pyridine).[36] In the present case it was advisable to avoid acid conditions, which might cause rearrangement, and the 1,3-diol was therefore acetylated with acetic anhydride and pyridine to produce the diacetate (LVII) in 80% yield.



LVII

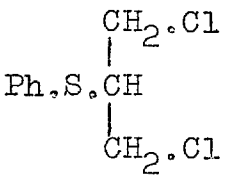


LVIII

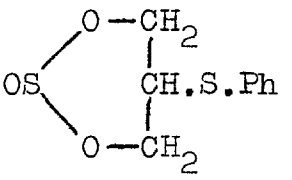
Prior to 1915, the methylation of a hydroxyl group was usually based upon the use of the expensive reagents methyl iodide and silver oxide.[37] Later, in a search for a suitable solvent for methylating sugars, W.N. Haworth[38] discovered that more satisfactory initial methylations could be accomplished in aqueous solution with the less expensive reagent dimethyl sulphate. Methylation with dimethyl sulphate and pulverized sodium hydorxide in tetrahydrofuran solution was first reported by Falconer

and Adams[39], and this latter procedure proved most suitable in the present studies for the preparation of the dimethyl ether derivative (LVIII) of the 1,3-diol. It was obtained in 83% yield and showed well defined characteristic absorption bands in the region $\nu_{max.}$ 3060-2810 $cm.^{-1}$

An attempt was made to prepare the mustard 1,3-dichloro-2-(phenylthio)propane (LIX) directly from the 1,3-diol by treatment with thionyl chloride in chloroform. It resulted in the formation of a mixture of a dichloro derivative and a cyclic sulphite (LX) in almost equal proportions. The product gave a positive Beilstein



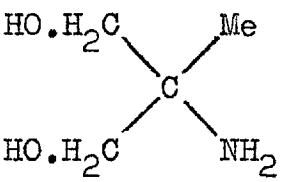
LIX



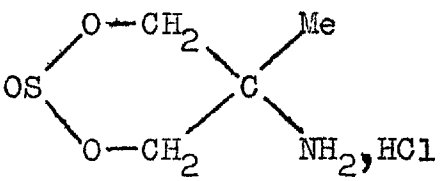
LX

test for chlorine, and the presence of the cyclic sulphite was indicated by the analytical results (a high value for S) and confirmed by the presence of strong absorption bands in the region $\nu_{max.}$ 1250-1200 $cm.^{-1}$ for S = O stretching vibrations characteristic for sulphites $(RO)_2SO$. To provide further evidence for the formation

of the cyclic sulphite, the product was hydrolysed with dilute hydrochloric acid, but the liberation of sulphur dioxide could not be detected as it was masked by the smell of thiophenol also produced during the hydrolysis, probably due to the cleavage of the S-C bond under acid conditions. Jones and Wilson[40] also reported the formation of a cyclic sulphite, i.e. 2-amino-2-methyl-propane-1,3-diol sulphite hydrochloride (LXII), when they treated the amino glycol (LXI) with thionyl chloride in chloroform. Similar cyclic sulphites have



LXI

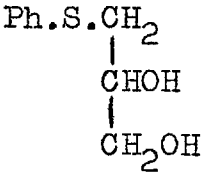


LXII

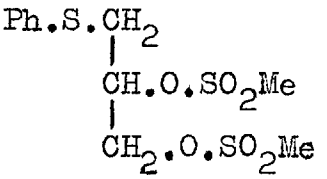
been reported[41] from other 1,3-glycols, e.g., pentaerythritol.

Synthesis of 3-(phenylthio)propane-1,2-diol and its derivatives

3-(Phenylthio)propane-1,2-diol (XLII) was synthesised by the method described by Hutchison and Smiles[42], by refluxing a mixture of sodium thiophenate and glycerol α-monochlorohydrin in ethanol. Its dimethanesulphonate (LXIII), diacetate (LXIV), and dimethyl ether (LXV)

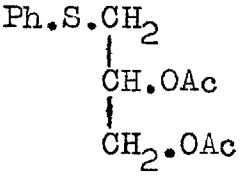


XLII

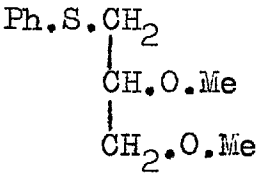


LXIII

derivatives were prepared in exactly the same manner as has been described for the 1,3-analogues. These were obtained in excellent yields.



LXIV



LXV

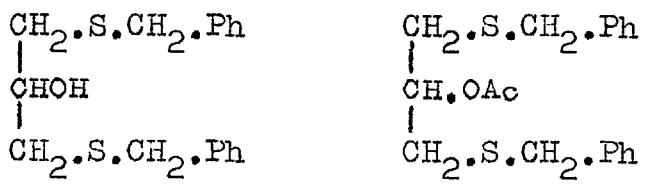
The 1,2-dimethanesulphonate (LXIII) was found to undergo gradual decomposition when stored and so whenever it was required for a reaction a freshly prepared and purified sample was used.

Other authentic compounds

As has been pointed out on the earlier pages, due to the lack of information in the field of nuclear magnetic resonance spectroscopy on organic sulphur compounds, it was necessary to provide more data on this subject.

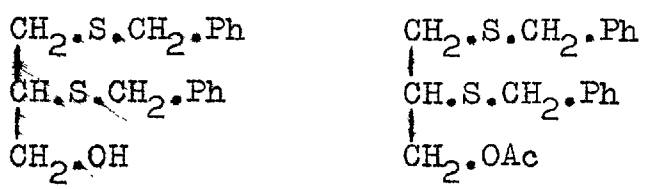
Samples of 1,3-bisbenzylthiopropan-2-ol (LXVI),

2-acetoxy-1,3-bisbenzylthiopropane (LXVII) and 2,3-bisbenzylthiopropanol (LXVIII), which were



LXVI LXVII

prepared by N.S. Johary[30] in this department, were available. 2,3-Bisbenzylthiopropyl acetate (LXIX) was then prepared from the alcohol (LXVIII) using the method of Johary et al.[30]



LXVIII LXIX

Allyl phenyl sulphide (LXX) was prepared[43] by the action of sodium thiophenate on allyl bromide. It was then brominated in dry carbon tetrachloride to give 1,2-dibromo-3-(phenylthio)propane (LXXI).

LXX LXXI

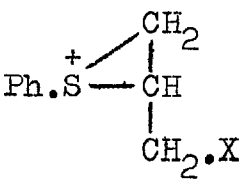
The nuclear magnetic resonance spectra were recorded on all the authentic samples described so far,

and were used for a comparative study to decide the structure of the reaction products, that will be described on the forthcoming pages of this thesis. The n.m.r. spectrum on cinnamyl acetate (LXXII) was also recorded, because it has some structural similarity to certain unsaturated products which were obtained.

$$Ph.CH=CH.CH_2.O.COCH_3 \qquad \text{LXXII}$$

Reactions of the dimethanesulphonates of 2-(phenylthio)propane-1,3-diol and 3-(phenylthio)propane-1,2-diol

The reactivity studies involved investigation of the effect of various nucleophiles on the above dimethanesulphonates, under various conditions, to determine whether any rearrangement occurred; this might be expected to take place via an ethylene sulphonium intermediate of the type (LXXIII).



LXXIII

The results obtained, and the evidence for the structures of the products, will now be described.

(a) Reaction with dry acetic acid

The solvolysis of the 1,3-dimethanesulphonate with acetic acid in the presence of acetic anhydride afforded only the rearranged product, i.e. the diacetate of 3-(phenylthic)propane-1,2-diol identified conclusively by its n.m.r. spectrum, similar to that of the authentic compound and quite different from the 1,3-diacetate. Solvolysis of the 1,2-dimethanesulphonate under similar conditions gave the same 1,2-diacetate.

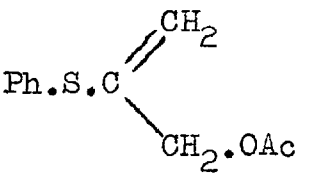
(b) Reaction with potassium acetate in acetic anhydride

When the reaction was carried out with freshly fused anhydrous potassium acetate in acetic anhydride, both the 1,3- and the 1,2-dimethanesulphonates gave a mixture of the 1,3- and the 1,2-diacetates which, as judged from the n.m.r. spectrum, were formed in almost equal proportions.

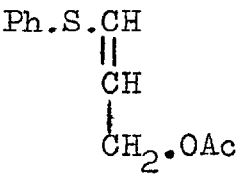
(c) Reaction with tetraethylammonium acetate in acetone

Roberts, Young and Winstein[44] in their studies on the replacement reactions of the butenyl chlorides, found that anionotropic rearrangement was inhibited with tetraethylammonium acetate in acetone, reaction occurred exclusively by the S_N2 mechanism to give the normal acetates. In the present experiment when the solvolysis with acetic acid or the reaction with potassium acetate in acetic anhydride failed to produce 1,3-diacetate from the 1,3-dimethanesulphonate, it was thought that tetraethylammonium acetate in acetone might behave differently. Tetraethylammonium acetate was therefore prepared by the method of Steigman and Hammett,[45] and its action on the 1,3- and the 1,2-dimethanesulphonates was studied separately in dry acetone. However, in both cases an unsaturated ethylenic compound was the main product, accompanied by the 1,3- and the 1,2-diacetates in small amounts. This conclusion was derived on the basis of the n.m.r. spectrum. The mechanism which has been proposed for the production of the methoxy propenes (see below) also holds good to explain the formation of 3-O-acetoxy-2-(phenylthio)-propene (LXXIV) and 3-O-acetoxy-1-(phenylthio)propene

(LXXV) from the 1,3-dimethanesulphonate and 1,2-dimethanesulphonate respectively, by the action of tetraethylammonium acetate in acetone.



LXXIV



LXXV

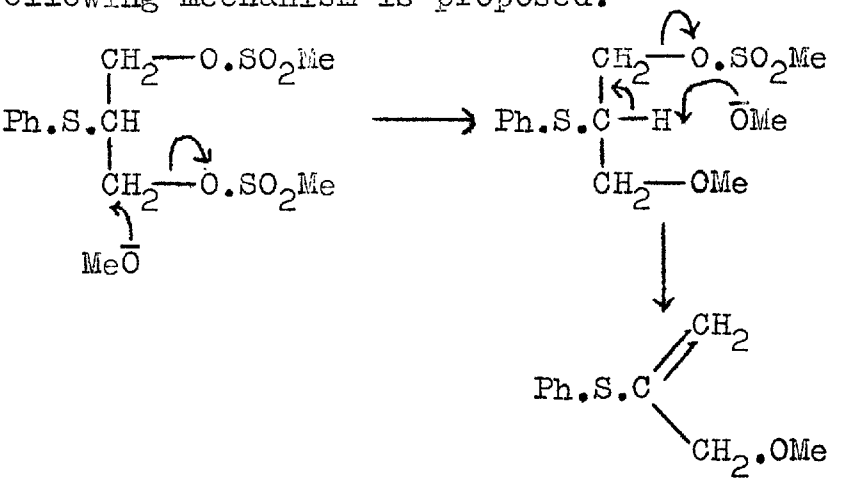
(d) Reaction with dry methanol

Both the 1,3- and the 1,2-dimethanesulphonates when solvolyzed in dry methanol produced a mixture of 1,3- and 1,2-dimethyl ethers in almost equal proportions, as shown by the n.m.r. spectrum.

(e) Reaction with sodium methoxide in methanol

It was expected that reaction of the 1,3-dimethanesulphonate with sodium methoxide in methanol would produce the normal 1,3-dimethyl ether and/or the rearranged 1,2-dimethyl analogue. But it was surprising to find that the nuclear magnetic resonance spectrum of the reaction product could account for only 12 instead of 16 protons. The molecular formula calculated on the basis of the analytical data was $C_{10}H_{12}OS$ and therefore

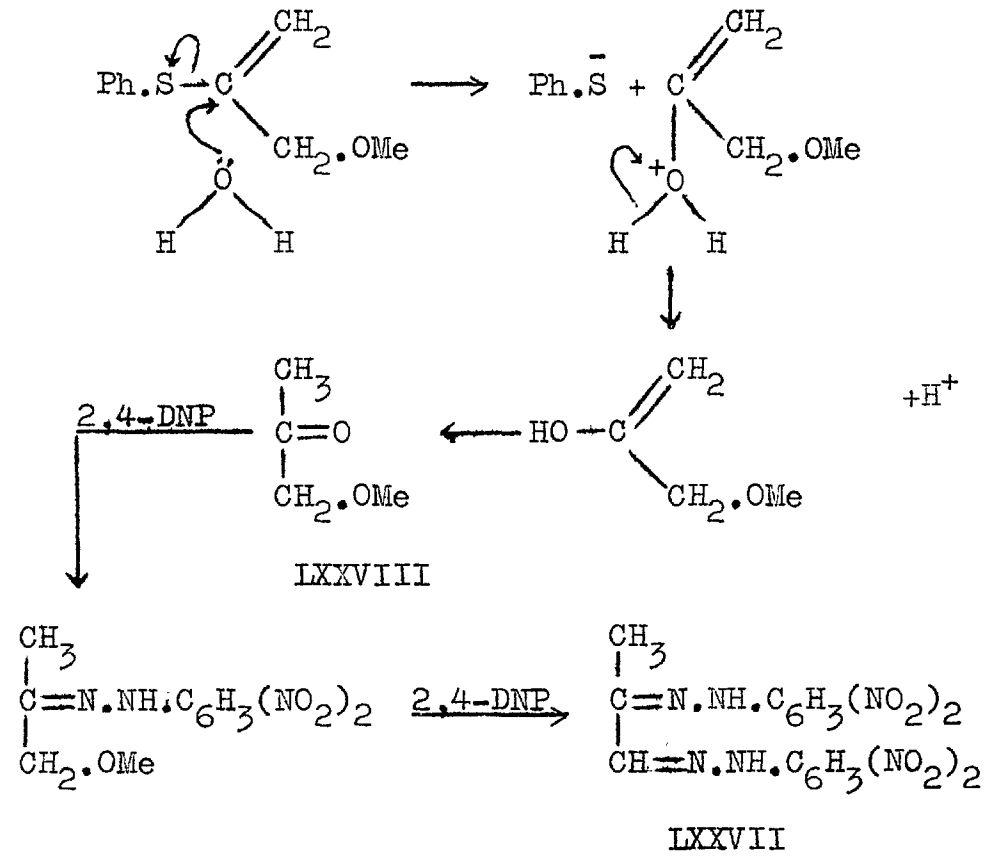
the structure (LXXVI) seemed likely. To explain the formation of 3-methoxy-2-(phenylthio)propene (LXXVI), the following mechanism is proposed.



LXXVI

The structure (LXXVI) was confirmed by reaction with acidic 2,4-dinitrophenylhydrazine to give methylglyoxal bis-(2,4-dinitrophenyl)-osazone (LXXVII). It was presumed that the propene (LXXVI) would undergo hydrolysis first to give a ketonic compound (LXXVIII) which would then react with 2,4-dinitrophenylhydrazine to give the osazone (LXXVII). (See the next page).

Similarly when the 1,2-dimethanesulphonate was treated with sodium methoxide in methanol, an unsaturated compound, 3-methoxy-1-(phenylthio)propene (LXXIX) was obtained, the structure being based on analytical and n.m.r. evidence. Its formation can be explained by



LXXVIII

LXXVII

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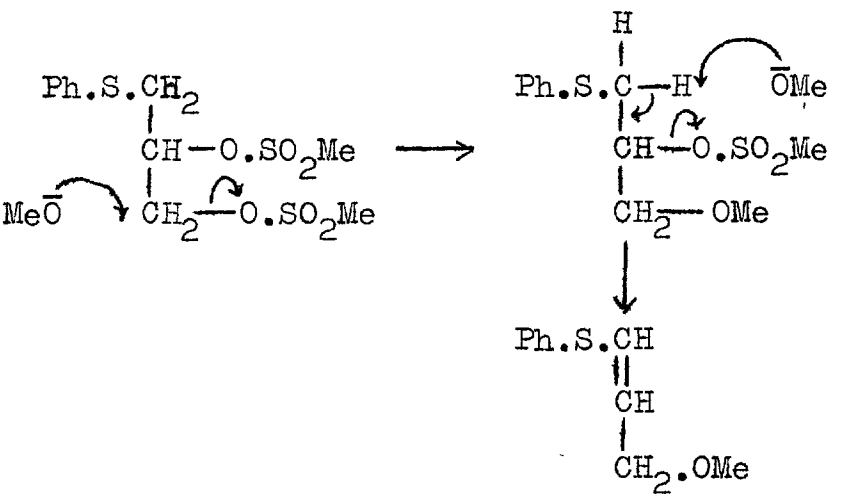
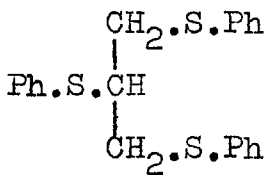
the following mechanism.



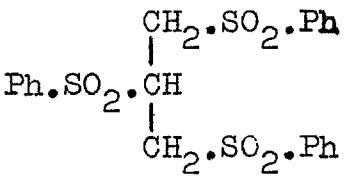
LXXIX

(f) Reaction with sodium thiophenate

With this reagent, in methanol, the 1,3-dimethanesulphonate and the 1,2-analogue both produced the same expected product, i.e. 1,2,3-trisphenylthiopropane (LXXX). Rearrangement, even if it occurred, would not be detected in this system, but there was no evidence of any unsaturated product. The trisulphide thus obtained was oxidised to 1,2,3-trisphenylsulphonylpropane (LXXXI) by the action of 30% hydrogen peroxide and acetic acid. The trisulphone (LXXXI) was obtained in 80% yield. The trisulphone was also completely free



LXXX



LXXXI

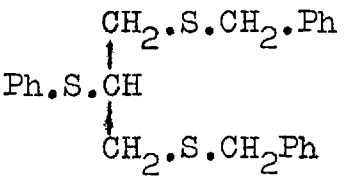
from any contamination with unsaturated compound as evidenced by its n.m.r. spectrum.

(g) Reaction with benzyl sodium sulphide

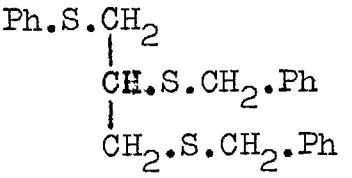
The nucleophilic attack of benzyl sodium sulphide in methanol on the 1,3- and the 1,2-dimethanesulphonates gave trisulphides. Both were oily products and their n.m.r. spectra did not give a clear indication about

their structure. Hence their crystalline trisulphones were prepared by oxidation with hydrogen peroxide and acetic acid. Although the n.m.r. spectra of these trisulphones also failed to throw any clear light on their exact structure, they were undoubtedly two different compounds; their melting points were sharp and different from one another. The trisulphone obtained from the trisulphide which was itself derived from the 1,3-dimethanesulphonate melted at 170-172° whereas the one prepared starting from the 1,2-analogue had m.p. 230-233°. One most important feature of the n.m.r. spectra of these trisulphides and their oxidation products was the complete absence of the absorption bands at low field specific for unsaturated protons. In the light of the above facts it is meaningless to think that the 1,3-dimethanesulphonate would have produced the rearranged 1,2-bisbenzylthio-3-(phenylthio)propane and the 1,2-dimethanesulphonate had given the rearranged 1,3-bisbenzylthio-2-(phenylthio)propane. Therefore it is most reasonable to conclude that the nucleophilic attack of a powerful nucleophile like benzyl sodium sulphide proceeded as a straightforward nucleophilic displacement reaction without formation of any cyclic

sulphonium intermediate. Thus the action of benzyl sodium sulphide on the dimethanesulphonates almost certainly afforded the normal expected trisulphide; the 1,3-bisbenzylthio-2-(phenylthio)propane (LXXXII) being produced from the 1,3-dimethanesulphonate and the 1,2-bisbenzylthio-3-(phenylthio)propane (LXXXIII) from the 1,2-dimethanesulphonate. Their oxidation

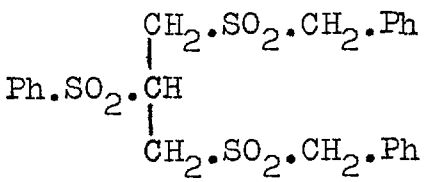


LXXXII

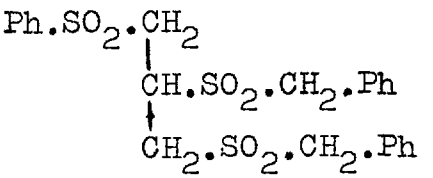


LXXXIII

with 30% hydrogen peroxide and acetic acid furnished 1,3-bisbenzylsulphonyl-2-phenylsulphonylpropane (LXXXIV) and 1,2-bisbenzylsulphonyl-3-phenylsulphonylpropane (LXXXV) respectively.



LXXXIV

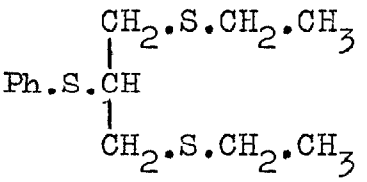


LXXXV

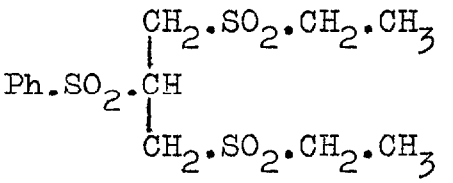
(h) Reaction with ethyl sodium sulphide

As was expected, 1,3-bisethylthio-2-(phenylthio)-propane (LXXXVI) was formed as a result of the reaction

between ethyl sodium sulphide and the 1,3-dimethanesulphonate. The n.m.r. spectrum was consistent with



LXXXVI

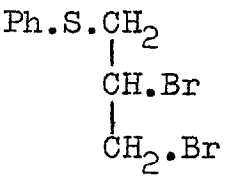


LXXXVII

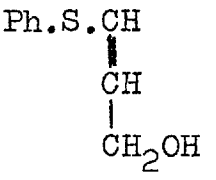
its structure. When it was subjected to oxidation by hydrogen peroxide and acetic acid, a trisulphone was obtained, a well resolved n.m.r. spectrum of which was in complete agreement with the structure of 1,3-bisethylsulphonyl-2-(phenylsulphonyl)propane (LXXXVII). On the other hand, treatment of the 1,2-dimethanesulphonate with ethyl sodium sulphide gave a mixture of 1,2-bisethylthio-3-(phenylthio)propane and a small proportion of an ethylenic compound. The formation of this unsaturated compound was indicated by the appearance of a weak absorption for olefinic protons at low field in the nuclear magnetic resonance spectrum and by the low analytical value found for sulphur.

(i) Reaction with lithium bromide in dry acetone

When the 1,3-dimethanesulphonate was refluxed with lithium bromide in dry acetone the 1,2-dibromo derivative (LXXI) was obtained, having an n.m.r. spectrum identical with that of the authentic compound described earlier. The same product was also obtained when the 1,2-dimethanesulphonate was treated with lithium bromide in anhydrous acetone. Hydrolysis of the dibromide with sodium hydroxide in aqueous acetone gave an elimination product (LXXXVIII) containing hydroxyl and ethylenic groups which from n.m.r. evidence constituted about 50% of the reaction product.



LXXI

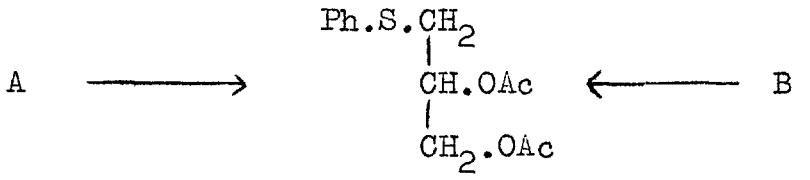


LXXXVIII

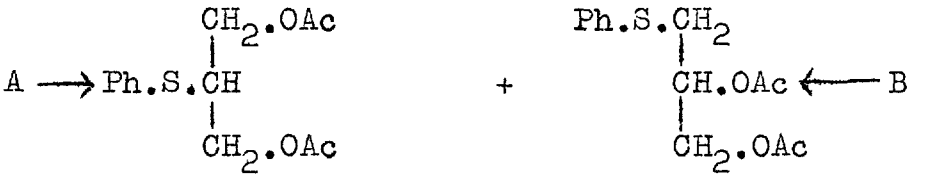
Summary of the reactions of the dimethanesulphonates

The reactions of the 1,3-di-O-methanesulphonate (A) and of the 1,2-di-O-methanesulphonate (B) are summarised below.

(a) Reaction with dry acetic acid in acetic anhydride.

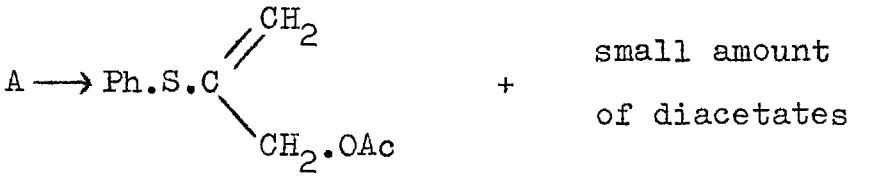


(b) Reaction with potassium acetate in acetic anhydride.



50% 50%

(c) Reaction with tetraethylammonium acetate in acetone.



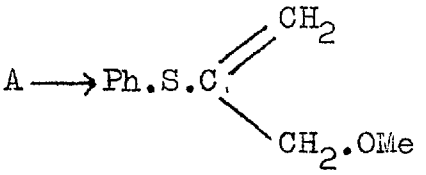
small amount of diacetates

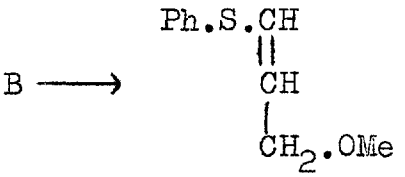
small amounts of diacetates

(d) Reaction with dry methanol

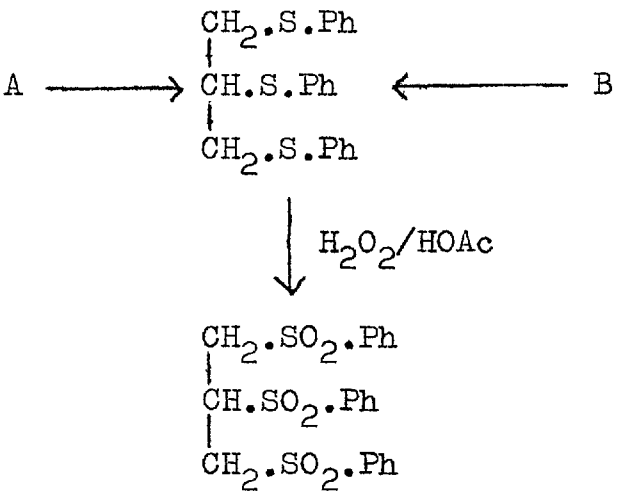
50% 50%

(e) Reaction with sodium methoxide in methanol.

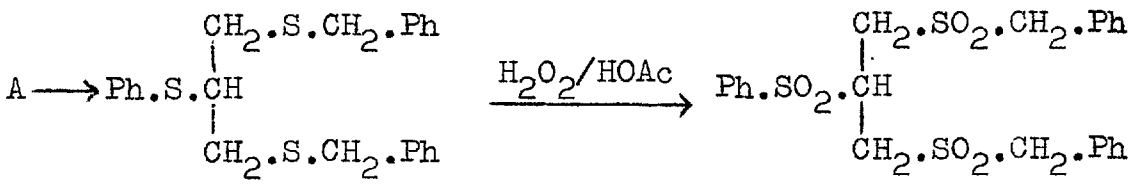


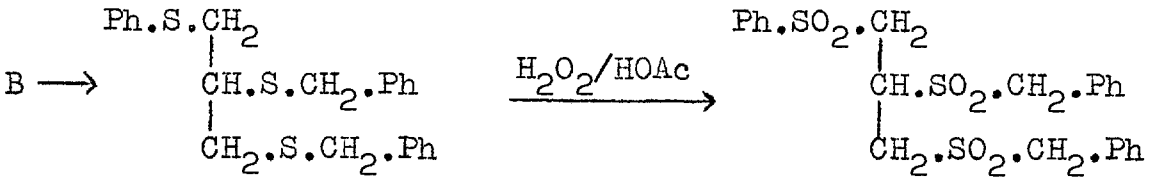


(f) Reaction with sodium thiophenate.

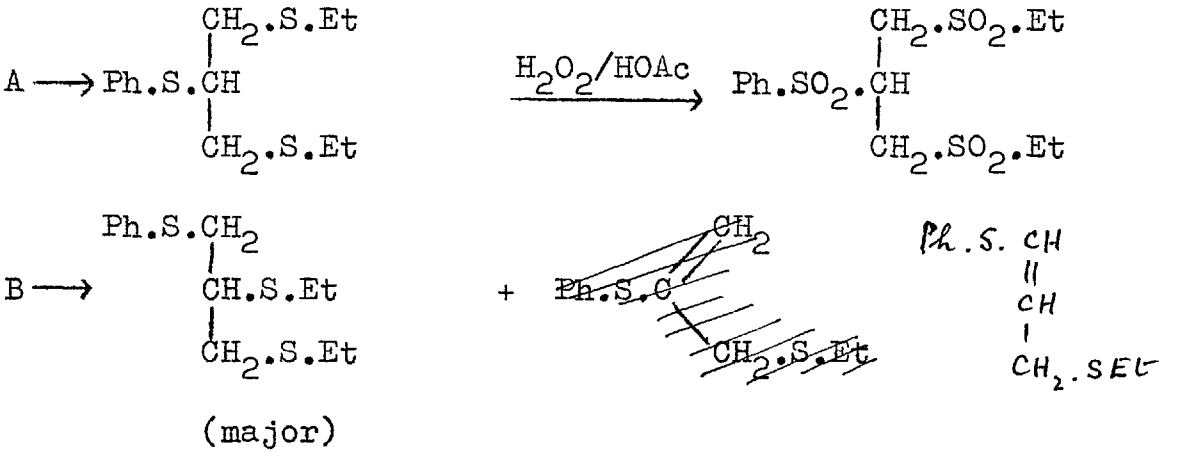


(g) Reaction with benzyl sodium sulphide.



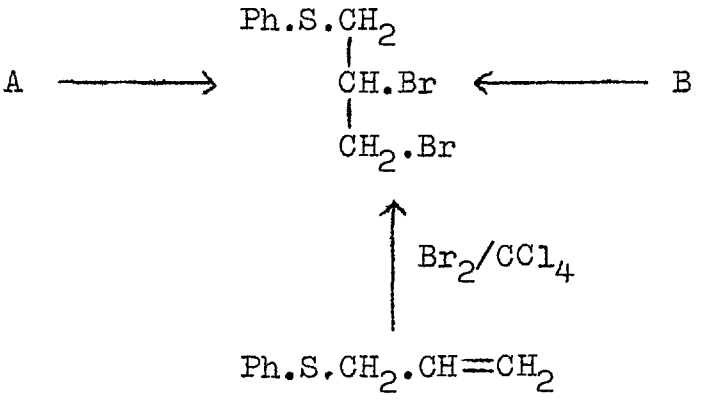


(h) Reaction with ethyl sodium sulphide.



(major)

(i) Reaction with lithium bromide in acetone.



The most significant feature of the above reactions is the way in which the nature of the products depends on the variety of the nucleophiles used. Some

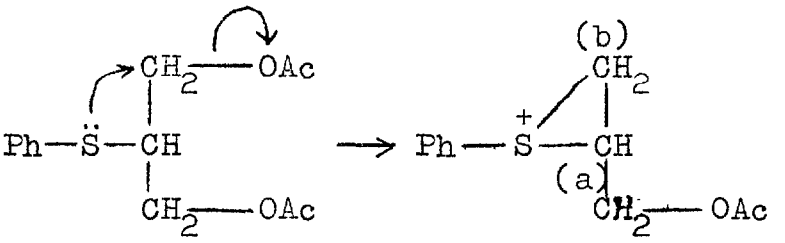
of them were solvents (weakly acidic or neutral) acting as weak nucleophiles, e.g. acetic acid and methanol; some were strong bases, e.g. sodium methoxide; and others were very powerful nucleophiles, $Ph.S^-$, $Ph.CH_2.S^-$ and $Et.S^-$. The selection of nucleophiles for the purpose of the reactivity studies was such that there was a gradual gradation from weak nucleophiles on one side to the very powerful nucleophiles on the other. It was expected that with the use of weak nucleophiles, the solvolysis of the dimethanesulphonates would proceed according to the S_N1 type of mechanism via the formation of the cyclic sulphonium ion intermediate, hence leading to the possibility of rearranged reaction products. The powerful nucleophiles were also to play an important role in the above reactions. It was thought that their attack would be more likely to follow the S_N2 type of route. Direct nucleophilic substitution reactions were thus expected, with the least possibility of rearrangement, and this in fact is what was observed.

It has been found during the course of the present studies that the direct acetylation of the 1,3-diol gave the normal 1,3-diacetoxy derivative, but when the solvolysis of the 1,3-dimethanesulphonate was carried

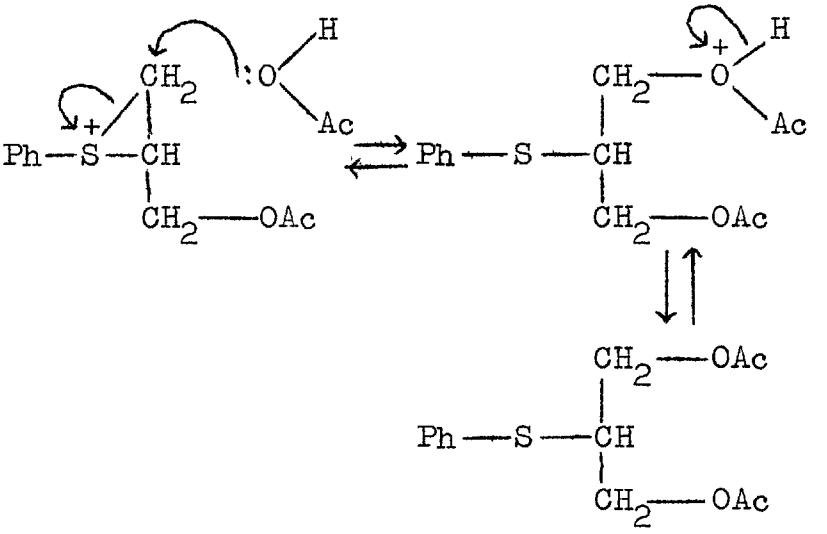
out with dry acetic acid in the presence of acetic anhydride, molecular rearrangement occurred resulting in the formation of the 1,2-diacetoxy compound. In order to provide information on the relative stabilities of the two diacetates under equilibrating conditions, the solvolysis of the 1,3-diacetoxy compound (derived by the direct acetylation of the 1,3-diol) was performed with acetic acid and acetic anhydride in the presence of a strong acid, i.e. methanesulphonic acid. (The experimental conditions were essentially the same as were maintained during the solvolysis of the 1,3-dimethanesulphonate with dry acetic acid and acetic anhydride, in which, of course, methanesulphonic acid was liberated). The 1,3-diacetoxy compound underwent complete molecular rearrangement, producing the 1,2-diacetoxy derivative, the structure of which was established by comparative study of its nuclear magnetic resonance spectrum and infrared spectrum with those of the authentic 1,2-diacetoxy compound (LXIV) (derived by the direct acetylation of the 1,2-diol).

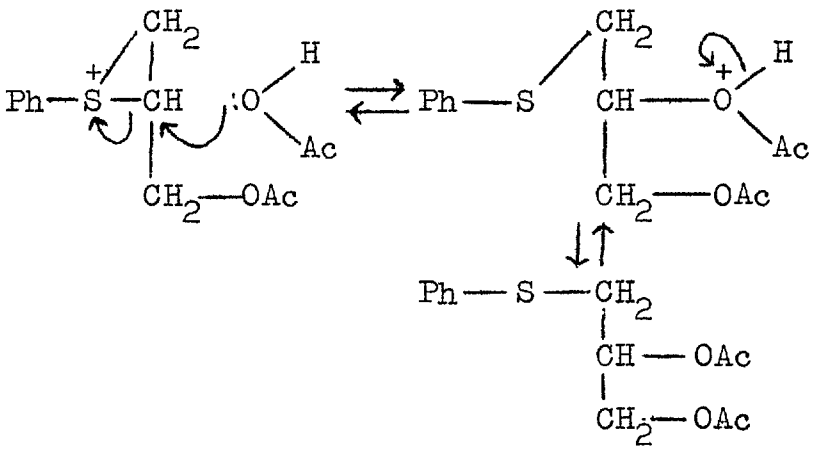
It is clear that the molecular rearrangement is preceded by the formation of a sulphonium ion intermediate (LXXIII) which could be attacked at either (a)

or (b). The attack at (b) would give the normal product whereas the attack at (a) would give the rearranged product.



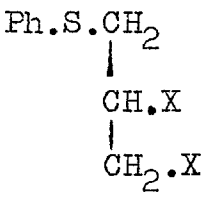
LXXIII



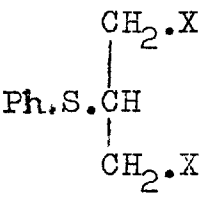


It can be seen that under the acidic conditions all these transformations are reversible, so that eventually an equilibrium is established between them. It is possible that as position (b) is more exposed than position (a) for nucleophilic attack, the 1,3-diacetate might be formed more easily, but, if the 1,2-diacetate is thermodynamically more stable, the equilibrium would finally result in conversion into the 1,2-diacetate.

In the light of the above results, it has become apparent that the reactivities of the dimethanesulphonates are not governed by any one specific principle. Arising out of these investigations, is the fact that compounds having the structure (LXXXIX) are, in general, thermodynamically more stable than those which are represented by the structure (III).



LXXXIX



III

Significance of N.M.R. Method

In many of the cases of rearrangement through sulphonium ions which have been described in the literature, the structures of the products were based on further chemical transformations, but such methods are not always free from criticism because it is conceivable that rearrangement could occur again. Evidence of structure based upon physical methods would in such cases be more authentic and dependable than those of a chemical type. Nuclear Magnetic resonance spectroscopy was considered to be the best choice because it was expected to decide between isomeric structures much more clearly than other spectroscopic methods. Its application in the present work appears to be the first time it has been used in settling problems of molecular rearrangement involving sulphonium intermediates, and it turned out to be most satisfactory. The results on all the compounds examined during the present research are tabulated in the following pages. They are given as tau values (tetramethylsilane, τ 10.0, as internal reference).

Abbreviations: s for singlet

d for doublet

t for triplet

qr for quartet

qn for quintet

m for multiplet

The integral curve for the aromatic protons (corresponding to 5H, 10H or 15H in various compounds) provided a check on the accuracy of this measurement. The integrals for the other protons are not quoted when they were in satisfactory agreement with the groups present; they are recorded only for mixtures or when assignments were not conclusive. Assignments to free hydroxyl groups were confirmed by disappearance of the particular peak in the presence of deuterium oxide.

AUTHENTIC COMPOUNDS

Spec. No.	Compound	Solv.	Arom	$-CH_2X$	Ph.S.CH	X	CH_2 in X	CHY	Y	Groups X, Y
3	$CH_2.OH$ $CH.S.Ph$ $CH_2.OH$	$CDCl_3$	2.72 5H	6.23 d	6.75 qn	6.45 s				X = OH
4	$CH_2.O.SO_2.CH_3$ $CH.S.Ph$ $CH_2.O.SO_2.CH_3$	$CDCl_3$	2.62 5H	5.57 d 4H	6.41 qn	6.97 s 6H				X = $O.SO_2.CH_3$
13	$CH_2.O.COCH_3$ $CH.S.Ph$ $CH_2.O.COCH_3$	CCl_4	2.72 5H	5.82 d 4H	6.54 qn	8.02 s 6H				X = OAc
17	$CH_2.O.CH_3$ $CH.S.Ph$ $CH_2.O.CH_3$	CCl_4	2.70 5H	6.50 d 4H	6.30 hump	6.70 s 6H				X = OMe
38b	$CH_2.S.CH_2.C_6H_5$ $CH.OH$ $CH_2.S.CH_2.C_6H_5$	$CDCl_3$	2.71 10H	7.44 4H			6.31 4H	6.31	6.73 1H	X = $S.CH_2.Ph$ Y = OH
34	$CH_2.S.CH_2.C_6H_5$ $CH.O.COCH_3$ $CH_2.S.CH_2.C_6H_5$	CCl_4	2.80 10H	7.48 d 4H			6.38 s 4H	5.05 qn	8.00 s 3H	X = $S.CH_2.Ph$ Y = OAc

AUTHENTIC COMPOUNDS

Spec. No.	Compound	solv.	Arom.	CHX	CH_2X	$Ph.S.CH_2$	X	Unsaturated protons	Miscellaneous	Group X
7	$CH_2.S.Ph$ $CH.OH$ $CH_2.OH$	$CDCl_3$	2.72 5H	6.35 m		6.98 d	7.32 s 2H			OH
9, 14	$CH_2.S.Ph$ $CH.O.SO_2.CH_3$ $CH_2.O.SO_2.CH_3$	$CDCl_3$	2.70 5H	5.20 m	5.55 t	6.70 d 6.80 d	6.98 s 6H			$O.SO_2.CH_3$
12	$CH_2.S.Ph$ $CH.O.COCH_3$ $CH_2.O.COCH_3$	CCl_4	2.72 5H	4.95 m	5.80 t	6.95 d	8.02s 8.08s 6H			OAc
21	$CH_2.S.Ph$ $CH.O.CH_3$ $CH_2.O.CH_3$	CCl_4	2.78 5H	6.35 m	6.62	6.98 m	6.65s 6.72s 6H			OMe
6	$CH_2.S.Ph$ $CH.Br$ $CH_2.Br$ from allyl phenyl sulphide	CCl_4	2.70 5H						5.86, 1Hm 6.15, 1Ht 6.30, 2Hd 6.54, 1H 2d	Br
43	$Ph.CH =$ $CH.CH_2.OAc$	CCl_4	2.75 5H		5.37 d		8.01 s 3H	3.21-4.09 m 2H		OAc

REACTIONS OF THE 1,3-DIMETHANESULPHONATE

Spec. No.	Reaction product/s	Solv.	Arom.	>CHX	$-CH_2X$	Ph.S.CH	Ph.S.CH_2	X	Unsaturated protons	Miscellaneous	Group X
16	(Dry HOAc) 1,2-Diacetate	CCl_4	2.70 5H	5.00 qn	5.80 t		6.95 d	8.03s 8.09s 6H			OAc
46	(Solvolysis of 1,3-diacetate) 1,2-Diacetate	CCl_4	2.72 5H	4.98 m	5.80 t		6.95 d	8.02s 8.08s 6H			OAc
19	(KOAc in Ac_2O) 1,2- + 1,3-diacetates 50% each	CCl_4	2.70 5H	5.00 qn 1/2H	5.83 m 3H	6.54 qn 1/2H	6.93 d 1H	8.03s 8.09s 6H			OAc
26	($Et_4N.OAc$ in Me_2CO) $Ph.S.C(=CH_2)-CH_2.OAc$ + small amount of 1,2- and 1,3-diacetates	CCl_4	2.71 5H		5.83 m 1/2H	6.42 s 1/3H		8.02 m 3H	4.56t 1H 4.81d 1H	5.29 1/2H 5.48 1½H	OAc
22	(Dry MeOH) 1,2- and 1,3-dimethyl ethers 50% each	CCl_4	2.75 5H				7.00 m 1H	6.68s 6.73s 6H		6.33-6.60 m 4H	OMe

Spec. No.	Reaction Product/s	Solv.	Arom.	>CHX	$-CH_2X$	Ph.S.CH	Ph.S.CH_2	X	Unsat. protons	CH_2 in Group X	Group X
23	(CH_3ONa in MeOH) Ph.S.C(=CH_2)-CH_2.OMe	CCl_4	2.70 5H		6.15 t			6.75 m 3H	4.55t 1H 4.88s 1H		OMe
28	(Ph.S.Na) CH_2.S.Ph CH.S.Ph CH_2.S.Ph	CCl_4	2.85 15H			6.81 s 5H					S.Ph
36	oxidation $CH_2.SO_2.Ph$ $CH.SO_2.Ph$ $CH_2.SO_2.Ph$	CF_3CO_2H	2.24 15H	5.62 m 1H	5.91 m 4H						SO_2.Ph
32	($Ph.CH_2.S.Na$) $CH_2.S.CH_2.Ph$ CH.S.Ph $CH_2.S.CH_2.Ph$	CCl_4	2.82 15H		7.33 d 4H	6.86 qn				6.41 t 4H	S.CH_2.Ph
39	oxidation $CH_2.SO_2.CH_2.Ph$ $CH.SO_2.Ph$ $CH_2.SO_2.CH_2.Ph$	CF_3CO_2H	2.55 15H		5.95 m 2H 6.15 m 2H	5.45 t				5.45 t 4H	$SO_2.CH_2$.Ph

Spec. No.	Reaction Product/s	Solv.	Arom.	$.CH_2X$	$Ph.S.CH$	CH_2 in gr. X	CH_3 in gr. X	Unsat. protons	Miscel-laneous	Group X
31	(Et.S.Na) $CH_2.S.CH_2.CH_3$ $CH.S.Ph$ $CH_2.S.CH_2.CH_3$	CCl_4	2.71 5H	7.15 d 4H	6.70 m	7.47 qr 4H	8.78 t 6H			S.Et
	↓ oxidation									
45	$CH_2.SO_2.CH_2.CH_3$ $CH.SO_2.Ph$ $CH_2.SO_2.CH_2.CH_3$	$CHCl_3$	1.88-2.38 5H	6.32 d 4H	5.76 qn	6.85 qr 4H	8.65 t 6H			$SO_2.Et$
5	(LiBr in Me_2CO) 1,2-Dibromide	CCl_4	2.68 5H						5.89, 1H m 6.13, 1H t 6.29, 2H d 6.54, 1H2d	Br
	↓ hydrolysis									
10	$Ph.S.CH$ $\|\|$ CH $\|$ $CH_2.OH$	CCl_4	2.73 5H	7.95 m	5.98 s			4.45-4.90 m 1H	7.62 m OH 5.12 s weak absorption	OH.

REACTIONS OF 1,2-DIMETHANESULPHONATE

Spec. No.	Reaction Product/s	Solv.	Arom.	>CHX	$.CH_2X$	Ph.S.CH	Ph.S.CH_2	X	Unsat. protons	Miscellaneous	Group X
15	(Dry HOAc) 1,2-Diacetate	CCl_4	2.70 5H	4.96 m	5.80 t		6.94 d	8.04s 8.07s 6H			OAc
20	(KOAc in Ac_2O) 1,2- + 1,3-diacetates 50% each	CCl_4	2.72 5H	5.06 m 1/2H	5.83 m 3H	6.52 m 1/2H	6.97 d 1H	8.03s 8.09s 6H			OAc
27	($Et_4N.OAc$ in Me_2CO) Ph.S.CH ‖ CH | $CH_2.OAc$ + small amount of 1,2- and 1,3-diacetates	CCl_4	2.74 5H		5.32 d 5.50 d 2H			8.03 2d 3H	3.55 m 1H 4.26 m 1H	weak absorptions for $Ph.S.CH_2$ Ph.S.CH $CH_2.OAc$ CH.OAc	OAc
24	(Dry MeOH) 1,2- + 1,3-dimethyl ethers 50% each	CCl_4	2.79 5H		6.55 t 4H	6.30 weak	6.98 m 1H	6.68s 6.73s 6H			OMe

Spec. No.	Reaction Product/s	Solv.	Arom.	>CHX	$-CH_2X$	Ph.S.CH_2	Ph.S.CH	X	Unsat. protons	Misc-ella-neous	CH_2 in gr.X	Group X
25	(MeONa in MeOH) Ph.S.CH ‖ CH | CH_2.OMe	CCl_4	2.78 5H					6.53 m 3H	3.80-6.00 complex 3H	6.70 m 1/2H		OMe
29	(Ph.S.Na) CH_2.S.Ph | CH.S.Ph | CH_2.S.Ph	CCl_4	2.85 15H			6.79 s 5H						SPh
33	(Ph.CH_2.S.Na) CH_2.S.Ph | CH.S.CH_2.Ph | CH_2.S.CH_2.Ph	CCl_4	2.82 15H	7.32 m 3H		6.89 m					6.42 t 4H	S.CH_2.Ph
41	↓ oxidation CH_2.SO_2.Ph | CH.SO_2.CH_2.Ph | CH_2.SO_2.CH_2.Ph	CF_3CO_2H	2.0-2.4 5H 2.55 10H	5.15——5.65 hump 5H							5.80-6.30 hump 4H	SO_2.CH_2.Ph

Spec. No.	Reaction Product/s	Solv.	Arom.	>CHX	$-CH_2X$	Ph.S.CH_2.	Ph.S.CH	CH_2 in gr.X	Unsat. protons	CH_3 in gr.X	Miscellaneous	Group X
30	(Et.S.Na) $CH_2.S.Ph$ $CH.S.CH_2.CH_3$ $CH_2.S.CH_2.CH_3$ + $CH.S.Ph$ CH $CH_2.S.CH_2.CH_3$	CCl_4	2.74 5H	5.00 weak absorption 7.14 s 3H		6.80 m $1\frac{1}{2}$	6.52 $\frac{1}{2}$H	7.48 two qr .4H	4.30 weak absorption	8.78 t 6H		$SO_2.CH_2.CH_3$
11	(LiBr in Me_2CO) 1,2-Dibromide	CCl_4	2.70 5H								5.84,1Hm 6.14,1Ht 6.30,2Hd 6.55,1H2d integration was not satisfactory	Br

CHAPTER 3

Experimental

2-(PHENYLTHIO)PROPANE-1,3-DIOL AND ITS DERIVATIVES

Synthesis of 2-(phenylthio)propane-1,3-diol

Ethyl bromomalonate (186)

This was prepared[22] from diethyl malonate, by bromination in carbon tetrachloride. The yield of pure ester was 72%; b.p. 72-76°/0.2 mm. (lit.73-75%; b.p. 132-136°/33 mm., 121-125°/16 mm.). $\nu_{max.}$ (liquid film), 1740 $cm.^{-1}$ with a shoulder at 1760 $cm.^{-1}$ ($-CO_2Et$).

Diethyl (phenylthio)malonate from ethyl bromomalonate (189,195)

Freshly cut sodium (0.766 g.) was dissolved in anhydrous ethanol (25 ml.), to which was added thiophenol (3.85 g.) followed by ethyl bromomalonate (7.96 g.). A reaction took place immediately. The mixture was refluxed gently for about 2 hours and then evaporated.

The residue, after mixing with water, was extracted with chloroform (100 ml.), washed with 5% sodium carbonate (3 x 20 ml.), and water (3 x 20 ml.), dried ($MgSO_4$) and concentrated to give a light brown oil. This was distilled and the fraction with b.p. 110-118°/0.075 mm. was collected. On standing, colourless needles separated and had m.p. 55-58°. (c.f. diphenyl disulphide, m.p. 61°). These were removed and the residual oil was redistilled to give a liquid, b.p. 116-118°/0.05 mm., n_D^{20} 1.5251. (lit.[23] b.p. 203-205°/23 mm., $n_D^{24.5}$ 1.5207). The yield was poor. $\nu_{max.}$ (liquid film), 1750-1720 $cm.^{-1}$ ($-CO_2Et$).

Ethyl (phenylthio)acetate (198)

Freshly cut sodium (6.9 g.) was dissolved in anhydrous ethanol (140 ml.) to which was added redistilled thiophenol (37.4 g = 34.5 ml.) followed by redistilled ethyl chloroacetate (36.75 g., 31.7 ml.). The mixture was refluxed in an atmosphere of nitrogen for one hour, at the end of which the ethanol was removed. The residue was mixed with water and extracted with chloroform (250 ml.), washed with 5% sodium carbonate (3 x 50 ml.), water (2 x 50 ml.), dried ($MgSO_4$),

and concentrated to give a yellow oil. The distilled pure colourless acetate (63%) had b.p. 100-106°/0.7 mm., $n_D^{23.5}$ 1.5445. (lit.[33] b.p. 265°/754.4 mm., n_D^{25} 1.5429. lit.[34] b.p. 147.6°/12 mm.).

Diethyl oxalo(phenylthio)acetate. (204,220)

The method described by Huntress and Olsen[23] was followed with modifications.

To a cooled solution of 50% sodium hydride (11.54 g., 0.24 mole) in dry ether (100 ml.) and anhydrous ethanol (3 ml.), was slowly added a solution of diethyl oxalate (30 g. ≡ 28.7 ml., 0.2 mole) in dry ether (50 ml.). The mixture was refluxed for 20 minutes. Maintaining gentle reflux, a solution of ethyl (phenylthio)acetate (30 g., 0.153 mole) in dry ether (100 ml.) was added dropwise during 5 hours. The mixture was then refluxed for a further 17 hours, at the end of which anhydrous ethanol (3 ml.) was added and refluxing continued for a further 90 minutes. The resulting mixture was then poured onto ice (125 g.), the aqueous layer separated, and the ether layer extracted with water (3 x 20 ml.). The combined aqueous extracts were acidified, under

fresh ether (150 ml.), with concentrated hydrochloric acid (15 ml.). The ether layer was separated and the aqueous phase extracted with ether (3 x 25 ml.). The dried ($MgSO_4$) combined ethereal solution was evaporated below 40° to give a light brown oil, which was heated (oil-bath at 80-90°) under 0.15 mm. for 30 minutes, and the resulting oil (40.5 g., 90%), $n_D^{19.5}$ 1.5365 was used directly for the next stage without being distilled. (lit.[23] 91%, n_D^{24} 1.5353).

Diethyl (phenylthio)malonate from Diethyl oxalo(phenylthio)-acetate (208, 223)

This compound was prepared essentially by the method described by Huntress and Olsen.[23] The yield of the pure diester was 87%, b.p. 129-130°/0.25 mm., n_D^{21} 1.5223. (lit. 90%, b.p. 203-205°/23 mm., $n_D^{24.5}$ 1.5207). $\nu_{max.}$ (liquid film), 1730 $cm.^{-1}$ ($-CO_2Et$).

2-(Phenylthio)propane-1,3-diol from diethyl (phenylthio)-malonate (212,225,231,250)

(a) A solution of diethyl (phenylthio)malonate (3.142 g.) in dry ether (30 ml.) was added slowly during

40 minutes to a well stirred slurry of powdered lithium aluminium hydride (0.9 g., large excess) in ether (30 ml.) and refluxed gently for a further hour. Then it was worked up to give a pale yellow oil, b.p. 130°/ 0.2 mm., $n_D^{22.5}$ 1.5639, $\nu_{max.}$ (liquid film), 3500 (-OH), 1730 ($-CO_2Et$) $cm.^{-1}$ The oil was found, by quantitative saponification, to contain 62.6% unchanged diester).

(b) When the reduction of diethyl (phenylthio)malonate was carried out with lithium aluminium hydride in dry tetrahydrofuran at reflux temperature during 5 hours, the product was an oil, a major portion of which crystallized. Recrystallization from ethanol gave diphenyl disulphide, m.p. 55-58°, not depressed on admixture with an authentic specimen of m.p. 60-61°. The remaining oil had b.p. 96-98°/0.2 mm., and was a mixture of the diol and the diester. $\nu_{max.}$ (liquid film), 3450 (-OH), 1725 ($-CO_2Et$) $cm.^{-1}$

(c) Diethyl (phenylthio)malonate (5 g.) in dry ether (75 ml.) was added dropwise to a slurry of powdered lithium aluminium hydride (3.12 g., 4.4 moles for each

mole of the diester) in dry ether (75 ml.) during 3 hours. The mixture was maintained at 19° and kept well stirred for 96 hours, at the end of which, the excess of the reagent was decomposed by cautious addition of water. The precipitated complex was then decomposed by cold 6N hydrochloric acid (65 ml.). The ether layer was separated and the aqueous phase extracted with ether (3 x 25 ml.). The combined ether layer was washed with sodium bicarbonate and then with water, dried ($MgSO_4$) and concentrated to give a pale yellow oil (1.3 g., 38.2%). Distillation gave a lower-boiling fraction, b.p. 24-28°/0.15 mm. with a smell of thiophenol, and then impure 2-(phenylthio)propane-1,3-diol (0.5 g., 15%), b.p. 132-136°/0.15 mm., n_D^{25} 1.5831. $\nu_{max.}$ in carbon tetrachloride, 3450 $cm.^{-1}$ (-OH). (Found: S, 19.29. $C_9H_{12}O_2S$ requires S, 17.40%). It was characterized by reaction with p-nitrobenzoyl chloride and pyridine to give the bis-p-nitrobenzoate which was recrystallised from methanol and had m.p. 95-96°. (Found: C, 56.79; H, 3.47; N, 5.75. $C_{23}H_{18}N_2O_8S$ requires C, 57.25; H, 3.77; N, 5.81%).

(d) The reduction of diethyl (phenylthio)malonate

by Bouveault and Blanc's method gave the diol as a yellow oil in very poor yield. $\nu_{max.}$ in chloroform, 3400 $cm.^{-1}$ (-OH).

1,3-O-Benzylideneglycerol. (268,277,462,583)

This compound was prepared by two methods.

(a) The method described by Evans and Owen,[28] in which glycerol was condensed with benzaldehyde in the presence of hydrogen chloride, gave an oil (mixture of 1,2- and 1,3- isomers), b.p. 140°/0.6 mm. (lit.139-142°/0.6 mm.), $n_D^{22.5}$ 1.5388. The solid 1,3- compound which crystallised out was separated and recrystallised from benzene-light petroleum (3:4) in 77% yield. It melted over a wide range 69-76° (lit. m.p. 65-75°). The nuclear magnetic resonance spectrum in deuterochloroform showed τ, 2.55 (5H; aromatic), 4.33 (1H; >CHPh), 5.88 (3H), 6.33 (2H), 7.22 (1H; -OH).

(b) In the second method, 1,3-O-benzylideneglycerol was prepared essentially by the method described by Johary and Owen.[30] A mixture of glycerol, benzaldehyde and 60% perchloric acid was heated at 75-87° under 55-75 mm. to give a mixture of 1,2- and 1,3- isomers.

The 1,3- isomer was separated from the oil and recrystallised from benzene-light petroleum (3:4) and had m.p. 70-78° (lit. m.p. 65-67°).

1,3-Benzylidenedioxy-2-toluene-p-sulphonyloxypropane (254,284,349,482)

This compound was prepared by the tosylation of 1,3-O-benzylideneglycerol in dry pyridine, using the method of Matheson and Angyal.[32] When recrystallised from methanol, shining colourless prisms in 75% yield were obtained and had m.p. 125-127° (lit.62.0%, m.p. 125°; Iqbal[35] gives m.p. 117-122°). $\nu_{max.}$ in chloroform, 1380 with shoulder at 1405, and 1240-1180 $cm.^{-1}$ (-OTs). $\lambda_{max.}$ in ethanol 225 mμ ($\varepsilon_{max.}$ 11052).

1,3-Benzylidenedioxy-2-(phenylthio)propane. (256,289,353,484)

To a solution of sodium ethoxide (prepared by the action of ca. 1.9 g. freshly cut sodium on 30 ml. dry ethanol), was added a solution of thiophenol (12.91 g. ≡ 12.0 ml.) in ethanol (20 ml.). The solvent was then removed and the dry residue thus obtained was mixed with 1,3-benzylidenedioxy-2-toluene-p-sulphonyloxypropane (25 g.) and dry NN-dimethylformamide (70 ml.). The

mixture was kept at 95-100° for 17 hours in an atmosphere of nitrogen. It was then cooled and NN-dimethylformamide was stripped off to give 1,3-benzylidenedioxy-2-(phenylthio)-propane. It was purified by recrystallisation from methanol (charcoal) when colourless shining needles (14.5 g., 71%) were obtained, m.p. 92-92.5°. The nuclear magnetic resonance spectrum in deuterochloroform showed τ, 2.60 (10H; aromatic), 4.55 (1H; >CH.Ph), 5.60 (2H; $-CH_2-$), 6.20 (1H; Ph.S.CH), 6.33 (2H; $-CH_2-$). (Found: C, 70.29; H, 5.62; S, 11.60. $C_{16}H_{16}O_2S$ requires C, 70.56; H. 5.92; S, 11.77%).

2-(Phenylthio)propane-1,3-diol from 1,3-benzylidenedioxy-2-(phenylthio)propane. (315,320,357,486)

To a well stirred and hot (50-55°) solution of 1,3-benzylidenedioxy-2-(phenylthio)propane (35 g.) in methanol (610 ml.), was added N-sulphuric acid (525 ml., preheated to 55°) in one lot. Stirring was continued and the temperature maintained at 50-55°, when after one hour the mixture became homogeneous; 90 minutes later it was quickly cooled to 0°. The cold solution was neutralized with ammonia, filtered and concentrated to a small volume. It was then extracted with ether

(5 x 150 ml.), dried ($MgSO_4$), and concentrated to give 2-(phenylthio)propane-1,3-diol (21.5 g., 92%), b.p. $144^{\circ}/10^{-4}$ mm., n_D^{18} 1.5928. $\nu_{max.}$ in chloroform, 3500 (-OH), 1100, 1080, 1050 ($-CH_2OH$) $cm.^{-1}$ The nuclear magnetic resonance spectrum in deuterochloroform showed τ, 2.72 (5H; aromatic), 6.23 (d, 4H; two $-CH_2-$), 6.45 (s, 2H; two -OH), 6.75 (q, 1H; Ph.S.CH). The n.m.r. spectrum was also run on another sample after mixing with D_2O and showed τ, 2.72 (5H), 6.22 (d, 4H), 6.70 (q, 1H). (Found: C, 58.57; H, 6.44; S, 17.40. $C_9H_{12}O_2S$ requires C, 58.66; H, 6.56; S, 17.40%).

The bis-p-nitrobenzoate had m.p. 94-96°, not depressed when mixed with the previous sample (page 166).

Derivatives of 2-(phenylthio)propane-1,3-diol

Dimethanesulphonate (364,380,391,501,533,566,574,621, 638,656,680)

An ice-cooled solution of methanesulphonyl chloride (3.72 g. ≡ 2.6 ml.) in dry pyridine (14.5 ml.), was added to a cold solution of 2-(phenylthio)propane-1,3-diol (2 g.) in 13.5 ml. dry pyridine, and the mixture was stored in the refrigerator for 16 hours, at the end of which it was poured onto 200 g. crushed ice and stirred. The solid dimethanesulphonate of the 1,3-diol was thus obtained (yield 75% to 91% with various batches). it was recrystallized from ether-petroleum (b.p. 30-40°) and had m.p. 55-56°, $\nu_{max.}$ in chloroform 1375, 1370 and 1180 cm.$^{-1}$ ($R_1.O.SO_2.R_2$). The nuclear magnetic resonance spectrum in deuterochloroform showed τ, 2.62 (5H, aromatic), 5.57 (d; 4H, two $-CH_2-$), 6.41 (qn; 1H, Ph.S.CH), 6.97 (s; 6H, two - $O.SO_2.CH_3$). (Found: C, 38.72; H, 5.03; S, 28.14. $C_{11}H_{16}O_6S_3$ requires C, 38.81; H, 4.74; S, 28.26%).

Diacetate (455)

An ice-cooled mixture of acetic anhydride (3.5 ml.) and dry pyridine (5 ml.), was added to 2-(phenylthio)-propane-1,3-diol (1.674 g.) at 0°. On shaking the mixture, a clear solution was obtained which was left at ca. 20° for 19½ hours, at the end of which it was poured onto crushed ice (40 g.), extracted with chloroform (3 x 25 ml.), washed with cold 2N sulphuric acid (2 x 15 ml.), saturated sodium bicarbonate (3 x 15 ml.) and water (4 x 15 ml.), dried ($MgSO_4$) and concentrated. Distillation gave the diacetate of the 1,3-diol as a colourless oil (1.95 g., 80%), b.p. 114-116°/10^{-4} mm., n_D^{21} 1.5262, $\nu_{max.}$ in chloroform, 1745, 1390, 1375 and 1275 cm.$^{-1}$ (-OAc). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.72 (5H, aromatic), 5.82 (d; 4H, two $-CH_2-$), 6.54 (qn; 1H, Ph.S.CH), 8.02 (s; 6H, two $-COCH_3$). (Found: C, 58.42; H, 6.27. $C_{13}H_{16}O_4S$ requires C, 58.19; H, 6.01%).

Dimethyl Ether (552)

To a solution of 2-(phenylthio)propame-1,3-diol (2 g.) in dry tetrahydrofuran (35 ml.), was added

pulverised sodium hydroxide (ca. 3 g.). To this mixture, stirred at ca. 30°, was added a solution of dimethyl sulphate (6.9 g. ≡ 5.2 ml.) in tetrahydrofuran (2 ml.) during 95 minutes. The mixture, after being stirred at 30-45° for a further 19 hours, was cooled to room temperature, sufficient water was added and tetrahydrofuran expelled in a stream of nitrogen at 65°. It was then neutralized with 2N sulphuric acid at room temperature, extracted with chloroform (100 ml.), washed with water (3 x 50 ml.), dried ($MgSO_4$) and concentrated to give 1,3-dimethoxy-2-(phenylthio)propane. The pure colourless oil (1.9 g., 83%) had b.p. 86-88°/ 10^{-4} mm., $n_D^{21.5}$ 1.5340, $\nu_{max.}$ in carbon tetrachloride 3060, 2960, 2910, 2860, 2810 (Ph-, $-CH_3$, $>CH_2$, $\equiv C-H$, $-O.CH_3$), 1130 and 1070 ($-CH_2.O.CH_2-$) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.70 (5H, aromatic), 6.30 (hump; 1H, Ph.S.CH), 6.50 (d; 4H, two $-CH_2-$), 6.70 (s; 6H, two $-O.CH_3$). (Found: C, 62.81; H, 7.68; S, 15.04. $C_{11}H_{16}O_2S$ requires C, 62.23; H, 7.60; S, 15.10%).

Attempted preparation of 1,3-dichloro 2-(phenylthio)-propane (331)

A solution of redistilled thionyl chloride (3 ml.≡ 4.9 g., ca. 3 times in excess) in chloroform (5 ml.) was added to a solution of 2-(phenylthio)propane-1,3-diol (0.976 g.) in chloroform (2 ml.). The mixture was gradually heated to 70-75° in 30 minutes and maintained for a further 90 minutes. Chloroform and excess of thionyl chloride were then distilled off to give a light yellow oil (1.18 g.). It was purified by refractionation to give a colourless oil which had b.p. 94-102°/10^{-4} mm., $n_D^{19.5}$ 1.5819 and gave a positive Beilstein's test for halogen. $\nu_{max.}$ in chloroform, 1590 (Ph-) and 1250-1200 $cm.^{-1}$ [S = O stretching vibration for sulphite $(RO)_2SO$]. (Found: S, 21.06. $C_9H_{10}Cl_2S$ requires S, 14.50% and the cyclic sulphite $C_9H_{10}O_3S_2$ requires S, 27.85%). The product was probably a mixture of a dichloro-derivative and the cyclic sulphite in almost equal proportions. On hydrolysis with 2N hydrochloric acid the characteristic smell of sulphur dioxide could not be detected as it was masked by the smell of thiophenol, produced during the hydrolysis.

Solvolysis of the 1,3-diacetate with dry acetic acid in presence of a strong acid (761)

A mixture of the authentic 1,3-diacetate (0.54 g.), acetic acid (10 ml.), acetic anhydride (1.5 ml.), and methanesulphonic acid (ca. 0.4 g., i.e. 2 mole equivalents), was heated under nitrogen at 120° for 7 hours and then left overnight at room temperature. Acetic acid and acetic anhydride were distilled off when a dark brown viscous oily residue was obtained. It was chromatographed [silica gel, ether - petroleum (40-60°), 1:1]. The first component which was eluted was concentrated to give a yellow oil which was further purified by distillation, and had b.p. 150-160° (oil-bath)/10^{-3} mm., n_D^{16} 1.5348. $\nu_{max.}$ in carbon tetrachloride, 1745 and 1240 $cm.^{-1}$ (-OAc), and 1588 $cm.^{-1}$ (Ph-). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.72 (5H, aromatic), 4.98 (m; 1H, >CH.OAc), 5.80 (t; 2H, $-CH_2OAc$), 6.95 (d; 2H, $Ph.S.CH_2-$), 8.02 and 8.08 (two s; 6H, $.O.CO.CH_3$). It was identical with that of the authentic 1,2-diacetate.

3-(PHENYLTHIO)PROPANE-1,2-DIOL AND ITS DERIVATIVES

3-(Phenylthio)propane-1,2-diol (421,628)

This compound was prepared, in 70% yield, by refluxing a mixture of sodium thiophenate and glycerol α-monochlorohydrin in ethanol. It had m.p. 70-71° (benzene). The method used was essentially the same as that described by Hutchison and Smiles,[42] who give m.p. 65-67°. (Centre d'études pour l'industrie and Michel Roussons[46] give m.p. 69.5-70°). $\nu_{max.}$ in chloroform, 3625-3475 (-OH), 1090, 1070 and 1030 ($-CH_2OH$ and >CHOH) $cm.^{-1}$ The nuclear magnetic resonance spectrum in deuterochloroform showed τ, 2.72 (5H, aromatic), 6.35 (m; 3H, $-C\underline{H}O\underline{H}-C\underline{H}_2OH$), 6.98 (d; 2H, $Ph.S.C\underline{H}_2.$), 7.32 (s; 2H, two -OH).

Dimethanesulphonate (426,468,543,585,591,631,648,666,674)

To an ice-cooled solution of 3-(phenylthio)propane-1,2-diol (4 g.) in dry pyridine (10 ml.), was added a cooled solution of methanesulphonyl chloride (7.44 g. ≡ 5.2 ml.) in pyridine (29 ml.), and the mixture was stored

in the refrigerator for 16 to 19 hours. It was then poured onto 400 g. of crushed ice and stirred when solid dimethanesulphonate of the 1,2-diol was obtained (yield 96% to 98% with various batches). It was recrystallized from benzene-petroleum (b.p. 40-60°) as fine silky needles, m.p. 60-61°. $\nu_{max.}$ in chloroform, 1375, 1228, and 1183 ($R_1.O.SO_2.R_2$) $cm.^{-1}$ The nuclear magnetic resonance spectrum in deuterochloroform showed τ, 2.70 (5H, aromatic), 5.20 (m; 1H, :CHOMs), 5.55 (t; 2H, CH_2.OMs), 6.70 and 6.80 (two d; 2H, Ph.S.CH_2.), 6.98 (S; 6H, two .O.SO_2.CH_3). (Found: C, 38.62; H, 4.52; S, 28.13. $C_{11}H_{16}O_6S_3$ requires C, 38.81; H, 4.74; S, 28.26%).

Diacetate (448)

3-(Phenylthio)propane-1,2-diol (3.68 g.) was added to an ice-cooled mixture of acetic anhydride (8.25 g. ≡ 7.6 ml.) and dry pyridine (10 ml.) and the solution was left at ca. 20° for 17 hours, at the end of which it was poured onto 60 g. of crushed ice, extracted with chloroform (3 x 25 ml.), washed with 2N sulphuric acid (2 x 25 ml.), saturated sodium bicarbonate (4 x 25 ml.), water (3 x 25 ml.), dried ($MgSO_4$) and concentrated to

give the diacetate of the 1,2-diol (4.7 g., 88%), b.p. 124-126°/10^{-4} mm., n_D^{21} 1.5268. $\nu_{max.}$ in chloroform, 1750, 1380, 1270 (-OAc) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.72 (5H, aromatic), 4.95 (m; 1H, >CH.OAc), 5.80 (t; 2H, $-CH_2OAc$), 6.95 (d; 2H, $Ph.S.CH_2-$), 8.02 and 8.08 (s; 6H, two $.O.CO.CH_3$). (Found: C, 58.49; H, 6.04. $C_{13}H_{16}O_4S$ requires C, 58.19; H, 6.01%).

Dimethyl ether (559)

Pulverized sodium hydroxide (ca. 2 g.) was added to a solution of 3-(phenylthio)propane-1,2-diol (1.3 g.) in dry tetrahydrofuran (25 ml.) and the mixture was kept well stirred at 30-35°. To this was added dropwise a solution of dimethyl sulphate (ca. 4.5 g. ≡ 3.4 ml.) in tetrahydrofuran (2 ml.) during one hour. The mixture was stirred at 30-40° for 7 hours and then at ca. 20° for 13 hours, at the end of which sufficient water was added and excess of tetrahydrofuran expelled in a stream of nitrogen at 65°. The mixture was then neutralized with 2N sulphuric acid, extracted with chloroform (125 ml.), washed with water (3 x 50 ml.),

dried ($MgSO_4$) and concentrated to give 1,2-dimethoxy-3-(phenylthio)propane (1.19 g., 80%), b.p. 76-78°/10^{-4} mm., $n_D^{17.5}$ 1.5381. $\nu_{max.}$ in carbon tetrachloride 3040, 2960, 2890, 2865, 2790 (Ph-, $-CH_3-$, $-CH_2-$, = C-H, $-OCH_3$), 1130, 1105 ($-CH_2.O.CH_2-$) cm^{-1}. The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.78 (5H, aromatic), 6.35 (m; 1H, >CH.OMe), 6.62 (t; 2H, $-CH_2$.OMe), 6.65 and 6.72 (two s; 6H, two $-CH_3$), 6.98 (m; 2H, Ph.S.CH_2-). (Found: C, 62.50; H, 7.76; S, 15.16. $C_{11}H_{16}O_2S$ requires C, 62.23; H, 7.60; S, 15.10%).

OTHER AUTHENTIC COMPOUNDS

The first two of the following compounds were prepared by N.S. Johary[30] in 1954 in the Armstrong Laboratory of this department. These samples were kindly provided by Professor L.N. Owen.

1,3-Bisbenzylthiopropan-2-ol (691)

It was purified by redistillation and the pure oil had b.p. 182-183°/10^{-4} mm., n_D^{18} 1.6116. The nuclear magnetic resonance spectrum in deuterochloroform showed τ, 2.71 (10H, aromatic), 6.31 (5H, 4H of two .CH_2.Ph group and 1H of >CH-), 6.73 (1H, -OH), 7.44 (4H, two -CH_2-).

2-Acetoxy-1,3-bisbenzylthiopropane (689)

This compound was purified by redistillation and had b.p. 180-186°/10^{-4} mm., n_D^{15} 1.5849. The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.80 (10H,aromatic), 5.05 (qn; 1H, >CH.OAc), 6.38 (s; 4H, two -CH_2.Ph), 7.48 (d; 4H, two -CH_2-), 8.00 (s; 3H, -O.CO.CH_3).

Allyl phenyl sulphide (405)

It was prepared essentially by the method of Hurd and Greengard[43]. It had b.p. 44-46°/ 2 x 10^{-4} mm., $n_D^{21.9}$ 1.5732 (lit.[43] b.p. 48-49°/0.43 mm., n_D^{25} 1.5732.)

2,3-Dibromo 1-(phenylthio)propane (411)

A solution of bromine (1.1 ml., previously washed with concentrated sulphuric acid) in dry carbon tetrachloride (10 ml.) was added dropwise to a cooled, vigorously stirred solution of allyl phenyl sulphide (3 g.) in dry carbon tetrachloride (20 ml.) during $2^1/2$ hours. Stirring was continued for one further hour allowing the solution to warm up to room temperature. It was then washed quickly with ice water (3 x 50 ml.), dried ($MgSO_4$) and concentrated to give 2,3-dibromo 1-(phenylthio)propane (3.6 g., 60%), b.p. 118-120°/10^{-4} mm., n_D^{20} 1.6255. The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.70 (5H, aromatic), 5.86 (m; 1H), 6.15 (t; 1H), 6.30 (d; 2H), 6.54 (two ds; 1H).

Cinnamyl acetate (767)

After purification by refractionation, it had b.p. 90°/2 mm., n_D^{13} 1.5461 (lit. n_D^{12} 1.54415). $\nu_{max.}$ in carbon tetrachloride, 1735 $cm.^{-1}$ (OAc). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.75 (5H, aromatic), 3.21 - 4.09 (m; 2H, CH = CH), 5.37 (d; 2H, $-CH_2OAc$), 8.01 (s; 3H, $.O.COCH_3$).

REACTIONS OF THE DIMETHANESULPHONATE OF 2-(PHENYLTHIO)PROPANE-1,3-DIOL

The freshly prepared dimethanesulphonate derivative of 2-(phenylthio)propane-1,3-diol was used in all the following experiments.

(a) Reaction with dry acetic acid (519)

The 1,3-dimethanesulphonate (3.2 g.) was mixed with acetic acid (37.5 ml.) and acetic anhydride (1.5 ml.) and slowly heated to 120° (oil-bath) and maintained at this temperature for 7 hours. Acetic acid was removed by distillation and the residue was taken up in chloroform (80 ml.), washed with saturated sodium bicarbonate and then water, dried ($MgSO_4$) and concentrated to give the rearranged product, the diacetate of 3-(phenylthio)-propane-1,2-diol (0.91 g., 58%), b.p. 120-124°/10^{-4} mm., n_D^{21} 1.5270. $\nu_{max.}$ in chloroform, 1740, 1383 and 1275 (-OAc) cm.$^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.70 (5H, aromatic), 5.00 (qn; 1H, >CH.OAc), 5.80 (t; 2H, $-CH_2$.OAc), 6.95 (d; 2H, Ph.S.CH_2-), 8.03 and 8.09 (two s; 6H, two $-OCOCH_3$).

(b) Reaction with potassium acetate in acetic anhydride (535)

A mixture of the 1,3-dimethanesulphonate (2.77 g.), anhydrous potassium acetate (4 g.), and redistilled acetic anhydride (15 ml.) was gradually heated to 140-150° (oil bath). It was maintained at this temperature for 4 hours and then left at room temperature for 13 hours. It was then cautiously diluted with ice-water, extracted with chloroform (100 ml.), washed with saturated sodium bicarbonate and then with water, dried ($MgSO_4$) and concentrated to give a dark brown oil. The distilled oil (1.8 g., 83%) was chromatographed (silica gel, ether-light petroleum 1:1), and redistilled to give a light yellow oil with b.p. 120-124°/10^{-4} mm., n_D^{19} 1.5268. $\nu_{max.}$ in carbon tetrachloride, 1740, 1378 and 1260 - 1210 (-OAc) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetradhloride showed τ, 2.70 (5H, aromatic), 5.00 (qn., 1/2H), 5.83 (not well resolved, 3H), 6.54 (qn; 1/2H), 6.93 (d; 1H), 8.03 and 8.09 (two s; 6H). The product was thus a mixture of the 1,2- and the 1,3-diacetates in approximately equal proportions.

(c) Reaction with tetraethylammonium acetate in acetone (622)

(i) Preparation of silver oxide: It was prepared[47] in 99% yield by the action of aqueous sodium hydroxide on aqueous silver nitrate.

(ii) Preparation of tetraethylammonium acetate: This was prepared, essentially by the method of Steigman and Hammett[45] as its monohydrate $Et_4N.OAc, H_2O$ by the action of silver oxide on an aqueous solution of tetraethylammonium bromide and then neutralising the product with acetic acid.

(iii) To a suspension of tetraethylammonium acetate (2.5 g.) in dry acetone (10 ml.), was added dropwise a solution of the 1,3-dimethanesulphonate (1.58 g.) in 10 ml. dry acetone during 90 minutes. The mixture was maintained at ca. 50° for 6 hours and then left at room temperature for 12 hours, at the end of which it was concentrated, diluted with ice-water (100 ml.), extracted with chloroform (3 x 50 ml.), washed with saturated sodium bicarbonate (50 ml.), water (3 x 50 ml.), dried ($MgSO_4$), and concentrated to give a dark brown oil. Distillation gave a pale yellow oil (0.57 g.), b.p. 85-90°/10^{-4} mm. $n_D^{20.5}$ 1.5551. $\nu_{max.}$ (liquid film),

1740 (-OAc), 1643 and 1618 (unsaturated), 1375 and 1225 (-OAc). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.71 (5H), 4.56 (t; 1H), 4.81 (d; 1H), 5.29 ($^1/_2$H), 5.48 ($1^1/_2$H), 5.83 (m; $^1/_2$H), 6.42 (s; $^1/_3$H), 8.02 (m; 3H). (Found: C, 60.27; H, 5.70. The diacetate $C_{13}H_{16}O_4S$ requires C, 58.19; H, 6.01% and the elimination product $C_{11}H_{12}O_2S$ requires C, 63.43; H, 5.81%).

(d) Reaction with dry methanol (568)

The 1,3-dimethanesulphonate (2.43 g.) in dry methanol (25 ml.) was boiled under reflux for $9^1/_2$ hours and then left at room temperature for 12 hours. Methanol was then distilled off and the residue taken up in chloroform (75 ml.), washed with saturated sodium bicarbonate (30 ml.) and then with water (3 x 50 ml.), dried ($MgSO_4$) and concentrated to give a light brown oil which on distillation gave a colourles oil (1.5 g., 26%), b.p. $74^\circ/10^{-4}$ mm. $\nu_{max.}$ in carbon tetrachloride, 3060, 2980, 2925, 2900, 2825 (Ph-, $-CH_3$, $-CH_2-$, =C-H, $-OCH_3$), 1130 and 1105 ($-CH_2-O-CH_2-$) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride

showed τ, 2.75 (5H, aromatic), 6.33 to 6.60 (m; 4H), 6.68 (s; 3H, $-CH_3$), 6.73 (s; 3H, $-CH_3$), 7,00 (m; 1H), indicating a mixture of the 1,2- and 1,3-dimethoxy compounds in almost equal proportions.

(e) Reaction with sodium methoxide in methanol (576)

To a solution of sodium methoxide, prepared by dissolving sodium (ca. 1.6 g.) in anhydrous methanol (30 ml.), was added the 1,3-dimethanesulphonate (2.282 g.). The solution, which became dark, was boiled under reflux for 30 minutes and then maintained at ca. 50° for 7 hours. It was then cooled and concentrated. The residue was diluted with water (100 ml.), extracted with chloroform (3 x 50 ml.), washed with saturated sodium bicarbonate (50 ml.), water (3 x 50 ml.), dried ($MgSO_4$) and concentrated to give a dark brown oil. Distillation gave colourless 3-methoxy-2-(phenylthio)-propene (0.5 g., 42%), b.p. 92°/0.3 mm., n_D^{21} 1.5591. $\nu_{max.}$ in carbon tetrachloride, 3060, 2990, 2925, 2680, 2825 (Ph-, $-CH_3$, $-CH_2-$, =C-H, $O-CH_3$), 1615 (-C=C-), 1590 (aromatic), 1195 and 1115 ($-CH_2.O.CH_2-$) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.70 (5H), 4.55 (t; 1H), 4.88 (s;

1H), 6.15 (t; 2H), 6.75 (m; 3H). (Found: C, 67.02; H, 6.93; O, 9.03; Mol. wt., 182.4. $C_{10}H_{12}OS$ requires C, 66.63; H, 6.71; O, 8.87% and Mol. wt., 180.3).

Methylglyoxal bis-(2,4-dinitrophenyl)-osazone from 3-methoxy-2-(phenylthio)propene (597,601)

3-Methoxy-2-(phenylthio)propene (63.8 mg.) was added to a mixture of 2,4-dinitrophenylhydrazine (338.5 mg.), concentrated sulphuric acid (0.2 ml.) and ethanol (7 ml.). The mixture was refluxed under nitrogen on a steam-bath for 3 hours. The smell of thiophenol became more and more prominent and an orange-red material crystallized out. The crystals were filtered off, and washed several times with ethanol and then with water to give methylglyoxal bis-(2,4-dinitrophenyl)-osazone (0.14 g., 93%). It was purified by washing repeatedly with a hot mixture of ethanol and ethyl acetate (10:3) and then with ethanol and had m.p. 303-305° (decomposed). (Swoboda[48] gives m.p. 300-302° (d)). (Found: C, 41.90; H, 3.01; N, 25.34. Calc. for $C_{15}H_{12}N_8O_8$: C, 41.66; H, 2.79; N, 25.92%).

(f) Reaction with sodium thiophenate (640)

To a solution of sodium methoxide (prepared by dissolving 1.01 g. sodium in 25 ml. dry methanol), was added thiophenol (5.9 g. ≡ 5.5 ml.) followed by the 1,3-dimethanesulphonate (1.5 g.). The mixture was slowly heated and maintained at ca. 55° in an atmosphere of nitrogen for 4 hours and then at room temperature for 18 hours. It was then concentrated; the residue was diluted with water (75 ml.), extracted with chloroform (3 x 50 ml.), washed with saturated sodium bicarbonate (50 ml.), and water (3 x 50 ml.), dried ($MgSO_4$) and evaporated to give a brownish oil. Distillation gave at first a lower-boiling oil, b.p. $96°/10^{-3}$ mm., which crystallized out, melting at 58-59° (c.f. diphenyl disulphide m.p. 61°) and later the higher-boiling oily 1,2,3-trisphenylthiopropane (0.945 g., 59%), b.p. $195°/10^{-4}$ mm., n_D^{19} 1.6535, $\nu_{max.}$ in carbon tetrachloride, 1587, 1487, 1445, 1420 (aromatic) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.85 (15H, aromatic), 6.81 (s; 5H), (Found: C, 68.19; H, 5.33; S, 26.13. $C_{21}H_{20}S_3$ requires C, 68.43; H, 5.47; S, 26.10%).

1,2,3-Trisphenylsulphonylpropane (743)

30% Hydrogen peroxide (2.5 ml.) was added to a solution of the trisulphide obtained above (0.4 g.) in acetic acid (2.5 ml.). The mixture was shaken and, after 5 minutes, more peroxide (1 ml.) was added, and heated on a steam-bath for 25 minutes. Some solid had then appeared, and more was precipitated on cooling and dilution with water. The solid was collected and washed with water. The trisulphone (0.4 g., 80%) was recrystallized from acetonitrile as fine needles, m.p. 227-230°, $\nu_{max.}$ (nujol mull), 1585 (aromatic), 1310 with shoulder at 1290, and 1150 with shoulders at 1170 and 1135 (sulphone) $cm.^{-1}$ The nuclear magnetic resonance spectrum in trifluoracetic acid showed τ, 2.24 (15H, aromatic), 5.62 (m; 1H, $>CH.SO_2Ph$), 5.91 (m; 4H, two $-CH_2-$). (Found: C, 54.89; H, 4.25; O; 20.18. $C_{21}H_{20}O_6S_3$ requires C, 54.29; H, 4.34; O, 20.66%).

(g) Reaction with benzyl sodium sulphide (658)

Sodium (1.15 g.) was dissolved in dry methanol (30 ml.). To this was added benzyl mercaptan (7.40 g.≡ 7.0 ml.) followed by the 1,3-dimethanesulphonate (1.70 g.).

Maintaining an atmosphere of nitrogen, the mixture was heated at 50-55° for 2 1/2 hours and then left at room temperature for 20 hours at the end of which it was concentrated, the residue diluted with water (75 ml.), and extracted with chloroform (3 x 50 ml.). The extract was washed with N sodium hydroxide (4 x 50 ml.), water (3 x 50 ml.), dried (Na_2SO_4) and concentrated to give an oil. Benzyl mercaptan and dibenzyl disulphide were removed by distillation and the residual oil was chromatographed (silica gel, L.F.C., benzene) to give 1,3-bisbenzylthio-2-(phenylthio)propane (0.95 g., 50%), n_D^{17} 1.5765, $\nu_{max.}$ in carbon tetrachloride, 1585 $cm.^{-1}$ (aromatic). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.82 (15H, aromatic), 6.41 (t; 4H, two $-CH_2Ph$), 6.86 (qn; 1H, >CH.SPh), 7.33 (d; 4H, two $-CH_2-$). (Found: S, 24.54. $C_{23}H_{24}S_3$ requires S, 24.25%).

1,3-Bisbenzylsulphonyl-2-phenylsulphonylpropane (747)

30% Hydrogen peroxide (2 ml.) was added to a solution of 1,3-bisbenzylthio-2-(phenylthio)propane (0.266 g.) in acetic acid (2 ml.). After shaking

for 5 minutes a further quantity (1 ml.) of peroxide was added and the mixture heated on a steam-bath for 30 minutes when a crystalline substance had appeared. Dilution of the mixture with water on cooling resulted in the precipitation of some more. The crystals were collected and washed with water. The trisulphone (0.25 g., 76%) was recrystallized from ethanol as small needles, m.p. 170-172°. The n.m.r. spectrum was recorded in trifluoracetic acid but the integration was not good (see the tables, spec. no. 39). (Found: C, 56.30; H, 4.79; O, 19.45. $C_{23}H_{24}O_6S_3$ requires C, 56.07; H, 4.91; O, 19.49%).

(h) Reaction with ethyl sodium sulphide (682)

A solution of sodium methoxide was prepared in methanol by dissolving sodium (1.15 g.) in 25 ml. dry methanol. Ethanethiol (5.84 g. ≡ 7 ml.) and then the 1,3-dimethanesulphonate (1.65 g.) were added and the mixture was heated at 40-50° for 7 hours and then left at room temperature for 15 hours. The solvent was then distilled off, the residue treated with ice-water (100 ml.), extracted with chloroform (3 x 50 ml.), washed with N sodium hydroxide (4 x 50 ml.), water (4 x 50 ml.),

dried (Na_2SO_4) and concentrated to give an oil. Distillation of the oil gave 1,3-bisethylthio-2-(phenylthio)propane (0.7 g., 53%), b.p. 122°/10^{-4} mm., n_D^{14} 1.5888. $\nu_{max.}$ in carbon tetrachloride, 1590 $cm.^{-1}$ (aromatic). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.71 (5H, aromatic), 6.70 (m; 1H, >CH.SPh), 7.15 (d; 4H, two .S.CH_2.Me), 7.47 (qr; 4H, two -CH_2-), 8.78 (t; 6H, two -CH_3). (Found: C, 57.42; H, 7.11; S, 34.98. $C_{13}H_{20}S_3$ requires C, 57.30; H, 7.40; S, 35.30%).

1,3-Bisethylsulphonyl-2-phenylsulphonylpropane (756)

A mixture of 1,3-bisethylthio-2-(phenylthio)propane (0.282 g.), acetic acid (5 ml.), and 30% hydrogen peroxide (5 ml.) was evaporated on a steam-bath until a white solid residue was obtained. The residue was washed several times with small quantities of water and the crystalline material was recrystallized from ethanol (0.29 g., 75%) and had m.p. 158-159°. $\nu_{max.}$ (nujol mull), 1300, 1128, 1123, 1148 $cm.^{-1}$ (R_2SO_2). The nuclear magnetic resonance spectrum in deuterochloroform

showed τ, 1.88 - 2.38 (m; 5H, aromatic), 5.76 (qn; 1H, >CHSPh), 6.32 (d; 4H, two $-CH_2.S.Et$), 6.85 (qr; 4H, two $-CH_2.Me$), 8.65 (t; 6H, two $-CH_3$). (Found: C, 42.41; H, 5.45. $C_{13}H_{20}O_6S_3$ requires C, 42.37; H, 5.47%).

(i) Reaction with lithium bromide in dry acetone (385,396)

Lithium bromide (19.15 g.) was dissolved in anhydrous acetone (100 ml.) at reflux temperature and the solution thus obtained was added dropwise but fairly rapidly to a solution of the 1,3-dimethanesulphonate (5 g.) in anhydrous acetone (ca. 10 ml.). The mixture was refluxed for 30 minutes and then cooled. The acetone was distilled off and the white residue was extracted with chloroform (150 ml.), washed quickly with ice-water (150 ml.), dried immediately over $MgSO_4$, and concentrated to give a light yellow oil. Distillation gave the 1,2-dibromide (4.1 g., 90%), b.p. 116-120°/10^{-4} mm., n_D^{18} 1.6242. $\nu_{max.}$ (liquid film), 1585 $cm.^{-1}$ (aromatic). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.68 (5H, aromatic), 5.89 (m; 1H), 6.13 (t; 1H), 6.29 (d; 2H),

6.54 (two d; 1H). (Found: C, 35.16; H, 3.37; Br, 52.19. $C_9H_{10}Br_2S$ requires C, 34.86; H, 3.25; Br, 51.55%).

Hydrolysis of 1,2-dibromo-3-(phenylthio)propane (432)

A mixture of 1,2-dibromo-3-(phenylthio)propane (0.99 g.), N sodium hydroxide (16.6 ml.), and acetone (16.6 ml.) was refluxed for 8 hours and then left at room temperature for 13 1/2 hours. Acetone was distilled off and the aqueous solution was neutralized with N hydrochloric acid. It was then extracted with ether (3 x 20 ml.), washed with water (2 x 20 ml.), dried ($MgSO_4$) and concentrated to give a pale yellow oil (0.43 g., 74%), $n_D^{21.5}$ 1.5670. $\nu_{max.}$ 3500 (-OH), 1670, 1620 (C=C), 1590 (aromatic) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.73, (5H, aromatic), 4.45 - 4.90 (m; 1H), 5.98 (s; 1H), 7.62 (m; 1H, -OH), 7.95 (m; 2H), 5.12 (s; weak absorption), indicating that the product of hydrolysis was an elimination product containing hydroxyl and ethylenic groups.

REACTIONS OF THE DIMETHANESULPHONATE OF 3-(PHENYLTHIO)PROPANE-1,2-DIOL

The freshly prepared dimethanesulphonate derivative of 3-(phenylthio)propane-1,2-diol was used in all the following experiments.

(a) Reaction with dry acetic acid (473)

A mixture of the 1,2-dimethanesulphonate (2.2 g.), acetic acid (25 ml.) and acetic anhydride (ca. 1 ml.) was heated slowly up to 120° (oil-bath) during 2 hours, maintained at this temperature for 11 hours and then left at ca. 18° for 13 hours. It was then concentrated and the residual oil taken up in chloroform (75 ml.), washed successively with saturated sodium bicarbonate (3 x 25 ml.), water (3x 25 ml.), dried ($MgSO_4$) and concentrated to give the 1,2-diacetate (1.5 g., 85%), b.p. 118-119°/10^{-4} mm., $n_D^{19.5}$ 1.5272. $\nu_{max.}$ in chloroform, 1740, 1380 and 1265 $cm.^{-1}$ (-OAc). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.70 (5H,aromatic), 4.96 (m; 1H, >CH.OAc), 5.80 (t; 2H, $-CH_2$.OAc), 6.94 (d; 2H, Ph.S.CH_2-), 8.04, and 8.07 (2S; 6H, two -O.CO.CH_3). These physical

constants are essentially the same as those described for the authentic 1,2-diacetate obtained by the acetylation of the 1,2-diol.

(b) Reaction with potassium acetate in acetic anhydride (545)

A mixture of the 1,2-dimet anesulphonate (2.1 g.) freshly fused anhydrous potassium acetate (3.7 g.) and redistilled acetic anhydride (30 ml.), was heated slowly to 140-150° (oil-bath) and maintained for 3 hours. The temperature was then lowered to 120° and maintained for 2 hours at the end of which the mixture was left at room temperature for about 15 hours. Acetic anhydride was then distilled off. Ice-water (100 ml.) was added to the residue and, after stirring vigorously for one hour, the mixture was extracted with chloroform (3 x 50 ml.), washed with saturated sodium bicarbonate (50 ml.), water (4 x 50 ml.), dried ($MgSO_4$) and concentrated to give a dark brown oil. The oil was distilled, chromatographed (silica gel, ether-light petroleum 1:3) and redistilled to give a mixture of the 1,2- and 1,3-diacetates in almost equal proportions (1.1 g., 66%), b.p. 112-116°/ 10^{-4} mm., n_D^{20} 1.5252. $\nu_{max.}$ in carbon tetrachloride, 1740, 1380 and 1250 $cm.^{-1}$ (-OAc). The nuclear

magnetic resonance spectrum in carbon tetrachloride showed τ, 2.72 (5H, aromatic) 5.06 (m; 1/2H), 5.83 (not well resolved, 3H), 6.52 (m; 1/2H), 6.97 (d; 1H), 8.03 and 8.09 (2s; 6H).

(c) Reaction with tetraethylammonium acetate (633)

The 1,2-dimethanesulphonate (1.8 g.) in dry acetone (10 ml.) was added drop by drop to a suspension of tetraethylammonium acetate (4.4 g.) in 20 ml. dry acetone during 2 hours. The mixture was heated to 58°, maintained there for 7 hours and then left at room temperature for 13 1/2 hours. The residue, obtained on concentration, was treated with ice-water (100 ml.), extracted with chloroform (3 x 50 ml.), washed with saturated sodium bicarbonate (50 ml.), water (3 x 50 ml.), dried (Na_2SO_4) and then concentrated to give a brown oil. Distillation gave a mixture of the 1,2-diacetate and the unsaturated elimination product in almost equal proportions (0.72 g., 51%), b.p. 92-96°/10^{-4}mm., n_D^{18} 1.5615. $\nu_{max.}$ (liquid film), 1735 and 1230 (-OAc), 1630 and 1610 (C = C) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ,

2.74 (5H, aromatic), 3.55 (m; 1H), 4.26 (m; 1H), 5.32 and 5.50 (2d; 2H), 8.03 (2d; 3H). There were also weak absorptions for Ph.S.CH_2-, Ph.S.CH, .CH_2.OAc and >CH.OAc. (Found: C, 61.01; H, 5.60. $C_{13}H_{16}O_4S$ requires C, 58.19; H, 6.01% and $C_{11}H_{12}O_2S$ requires C, 63.43; H, 5.81%).

(d) Reaction with dry methanol (587)

The 1,2-dimethanesulphonate (1.79 g.) in dry methanol (25 ml.) was gently refluxed for 7 hours and then left at room temperature for 13 1/2 hours. Methanol was then distilled off and the residue was taken up in chloroform (60 ml.), washed with saturated sodium bicarbonate (25 ml.), water (3 x 25 ml.), dried ($MgSO_4$) and concentrated to give a light yellow oily mixture of the 1,2- and the 1,3-dimethyl ethers (0.51 g., 46%) in almost equal proportions; it had b.p. 110-112°/0.15 mm., $\nu_{max.}$ in carbon tetrachloride 3090, 3000, 2950, 2900, 2840 (Ph-, -CH_3, -CH_2-, =C-H, -O.CH_3), 1590 (aromatic), 1198, 1180, 1125, 1103, 1093 (-CH_2.O.CH_2-) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed, τ, 2.79 (5H), 6.55

(t; 4H), 6.68 and 6.73 (2s; 6H, two $-CH_3$), 6.98 (1H), 6.30 (weak absorption).

(e) Reaction with sodium methoxide in methanol (593)

To a solution of sodium methoxide, prepared by dissolving sodium (ca. 1.2 g.) in anhydrous methanol (25 ml.), was added the 1,2-dimethanesulphonate (1.77 g.). The mixture was maintained at 50° for 6 hours and then left at room temperature for 13 hours. It was then concentrated and the residue was diluted with water (75 ml.), extracted with chloroform (3 x 50 ml.), washed with saturated sodium bicarbonate (50 ml.), water (3 x 50 ml.), dried ($MgSO_4$) and concentrated to give a dark brown oil. Distillation gave a colourless oil 3-methoxy-1-(phenylthio)propene (0.48 g., 52%), b.p. 86-88°/0.3 mm., $n_D^{19.5}$ 1.5705. $\nu_{max.}$ in chloroform, 3025, 2975, 2825 (Ph-, =C-H, $-OCH_3$), 1650 (C=C), 1585 (aromatic), 1140, 1115 and 1090 ($-CH_2.O.CH_2-$) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.78 (5H), 3.80 - 6.00 (complex; 3H), 6.53 (m; 3H), 6.70 (m; 1/2H). (Found: C, 66.36; H, 6.55; S, 17.61. $C_{10}H_{12}OS$ requires

C, 66.63; H, 6.71; S, 17.79%).

Attempted preparation of 2,4-dinitrophenylhydrazone derivative from 3-methoxy-1-(phenylthio)propene (607)

A mixture of 2,4-dinitrophenylhydrazine (0.27 g.), concentrated sulphuric acid (ca. 0.2 ml.), anhydrous ethanol (6 ml.) and 3-methoxy-1-(phenylthio)propene (0.48 g.) was refluxed in an atmosphere of nitrogen on a steam-bath for 3 hours. It was then worked up as usual to give a brown solid (0.2 g.). It was chromatographed (mixture of bentonite and Kieselguhr 28 g. : 7 g., alcohol/chloroform 1 : 4) when an orange red crystalline material was collected (0.124 g.), but this was unchanged reagent. No reaction product could be found.

(f) Reaction with sodium thiophenate (650)

Sodium (1.22 g.) was dissolved in anhydrous methanol (25 ml.). To this solution of sodium methoxide, was added thiophenol (7.3 g ≡ 6.8 ml.) followed by the 1,2-dimethanesulphonate (1.81 g.). The mixture was maintained under nitrogen at ca. 55° for 7 hours and then at room temperature for 15 hours. Concentration gave

a solid residue which was treated with water (75 ml.), extracted with chloroform (3 x 50 ml.), washed with N sodium hydroxide (4 x 50 ml.), water (4 x 50 ml.), dried (Na_2SO_4) and then evaporated. Distillation of the residue yielded at first a lower-boiling oil (b.p. 94 - 108°/10^{-4} mm.) identified as diphenyl disulphide and then 1,2,3-trisphenylthiopropane (1.3 g., 67%), b.p. 192°/10^{-4} mm., n_D^{18} 1.6543. $\nu_{max.}$ in carbon tetrachloride; 1588 (aromatic) $cm.^{-1}$ The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.85 (15H, aromatic), 6.79 (S; 5H) and was identical with the spectrum of the same compound described on page 189.

(g) Reaction with benzyl sodium sulphide (676)

Benzyl mercaptan (8.1 g. ≡7.65 ml.), followed by the 1,2-dimethanesulphonate (1.85 g.), was added to a solution of sodium methoxide, prepared by dissolving sodium (1.25 g.) in dry methanol (30 ml.). The mixture was maintained at 50-55° for 5 1/2 hours and then at room temperature for 17 hours. The residue that was obtained on concentration, was treated with water (75 ml.), extracted with chloroform (3 x 50 ml.), washed with N sodium hydroxide (4 x 50 ml.), water (4 x 50 ml.),

dried ($MgSO_4$) and then concentrated to give a brown oil. On distillation, benzyl mercaptan (b.p. 42-44°/10^{-4} mm.) was followed by dibenzyl disulphide (b.p. 118-122°/10^{-4} mm.) and finally 1,2-bisbenzylthio-3-(phenylthio)propane (1.22 g., 57%), b.p. 224°/10^{-4} mm., n_D^{15} 1.6391. $\nu_{max.}$ in carbon tetrachloride, 1590 $cm.^{-1}$ (aromatic). The nuclear magnetic resonance spectrum in carbon tetrachloride showed τ, 2.82 (15H, aromatic), 6.42 (t; 4H), 6.89 (m; 2H), 7.32 (m; 3H). (Found: S, 24.23. $C_{23}H_{24}S_3$ requires S, 24.25%).

2,3-Bisbenzylsulphonyl-1-phenylsulphonylpropane (751)

30% Hydrogen peroxide (2.5 ml.) was added to a mixture of 2,3-bisbenzylthio-1-(phenylthio)propane (0.3 g.) and acetic acid (2.5 ml.). The mixture was shaken for 5 minutes, mixed with a further quantity (1.5 ml.) of peroxide and heated on a steam-bath. After 20 minutes a white crystalline substance had appeared which increased in quantity when cooled and treated with water. The crystals were collected and washed with water. **The trisulphone** (0.28 g., 76%) was purified by recrystallization from ethanol and had m.p. 230-233°. The nuclear magnetic resonance spectrum in

trifluoracetic acid showed τ, 2.0 - 2.40 (5H, aromatic), 2.55 (10H, aromatic), 5.15 - 5.65 (hump; 5H), 5.80 - 6.30 (hump; 4H). (Found: C, 56.22; H, 5.04; O, 19.55. $C_{23}H_{24}O_6S_3$ requires C, 56.07; H, 4.91; O, 19.49%).

(h) Reaction with ethyl sodium sulphide (668)

To a solution of sodium methoxide which was prepared by dissolving sodium (1.22 g.) in anhydrous methanol (30 ml.), was added ethanethiol (5.8 g. ≡ 7 ml.) and then the 1,2-dimethanesulphonate (1.8 g.). The air of the reaction vessel was replaced by dry nitrogen, and the mixture was heated at 30-35° for 6 hours and then left at room temperature (10°) for 13 hours. Solvent methanol was removed, the residue was diluted with water (75 ml.), and extracted with chloroform (3 x 50 ml.), which was washed with N sodium hydroxide (4 x 50 ml.), water (4 x 50 ml.), dried (Na_2SO_4) and concentrated. Distillation of the crude oil gave diethyl disulphide (b.p. 64°/0.8 mm.), and then 1,2-bisethylthio-3(phenylthio)propane (0.8 g., 55%), b.p. 128°/10^{-4} mm., $n_D^{15.5}$ 1.5889. $\nu_{max.}$ in carbon tetrachloride, 1588 $cm.^{-1}$ (aromatic). The nuclear magnetic resonance spectrum in

carbon tetrachloride showed τ, 2.74 (5H, aromatic), 4.30 (weak absorption), 5.00 (weak absorption); 6.80 (m; $1^1/2$H), 6.52 ($^1/2$H), 7.14 (s; 3H), 7.48 (two qr; 4H), 8.78 (t; 6H). There was weak absorption for olefinic protons at low field. (Found: S, 34.10. $C_{13}H_{20}S_3$ requires S, 35.30% and the elimination product $C_{11}H_{14}S_2$ requires S, 30.47%). The properties of the reaction product agree with those expected for a mixture of 1,2-bisethylthio-3-(phenylthio)propane (main product) and an ethylenic compound (in small proportion).

(i) Reaction with lithium bromide in dry acetone (441)

Lithium bromide (19.15 g.) was dissolved in dry acetone (100 ml.) at reflux temperature and added to a solution of the 1,2-dimethanesulphonate (5.8 g.) in dry acetone (10 ml.). The mixture was refluxed for 6 hours, then cooled, and the acetone was distilled off. The residual solid was extracted with chloroform (200 ml.), washed quickly with ice-water, dried ($MgSO_4$) and concentrated to give a light yellow oil (4.24 g., 80%). Distillation gave the 1,2-dibromide, b.p. 116-

$120^{\circ}/10^{-4}$ mm., n_D^{19} 1.6238. $\nu_{max.}$ 1585 $cm.^{-1}$ (aromatic). The nuclear magnetic resonance spectrum showed τ, 2.70 (5H, aromatic), 5.84 (m; 1H), 6.14 (t; 1H), 6.30 (d; 2H), 6.55 (two d; 1H). The integration of the spectrum was not good, and the values quoted are approximations. (Found: C, 34.95; H, 3.29. $C_9H_{10}Br_2S$ requires C, 34.86; H, 3.25%).

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