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THE REACTIONS OF ANTI-TUMOUR ALKYLATING COMPOUNDS

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I. INTRODUCTION

Of the tremendous number of substances that have been tested for anti-tumour activity, a large group of compounds with alkylating properties is outstanding; these compounds attack tumour cells to a somewhat greater degree than normal cells.

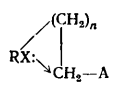
At present these preparations are widely used in the chemotherapy of some types of malignant tumours[1]. There are a number of reviews and monographs dealing with the biological properties of alkylating agents and also with the dependence of biological activity on chemical structure[2-7].

Since the selectivity of anti-tumour activity in these compounds is closely connected with the chemical properties of their electrophilic centres, it is of interest to examine in one review the most important features of the reactivity of those types of alkylating agent which have shown anti-tumour activity clinically or in animal experiments.

II. REACTIVITY OF ALKYLATING COMPOUNDS

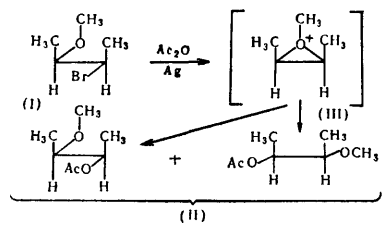
Table 1 gives a list of various types of compounds that have yielded preparations with anti-tumour activity.

In about half the compounds in Table 1 (the first ten) a heteroatom with a free pair of electrons is situated in position 2 or 4 to the electrophilic centre. This structure makes possible interaction between the electron pair and the electrophilic centre of the molecule, which facilitates the intramolecular removal of the substituent A on the carbon atom:

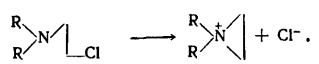


where X is N, O, or S and n = 1 or 3.

When the substituent A is eliminated by an intramolecular reaction, a cyclic cation is usually formed[24,25]. For example, in acetolysis of *D*-2-bromo-3-methoxybutane (I) with acetic anhydride in the presence of silver ions, the racemate of 2-acetoxy-3-methoxybutane (II) is formed; this can only be explained by the formation of an intermediate oxonium cation (III):



There are also direct proofs of the formation of cyclic products in the reactions of some alkyl halides. Thus when aliphatic 2- or 4-chloroalkylamines are dissolved in water, at first rapid separation of chloride ions without material change in the pH of the solution is observed[8,26,27]:



Moreover, stable salts of aziridinium have been separated from 2-chloroethylamines[28]. The rate of formation of the aziridinium ion is directly related to the basicity of the nitrogen atom[29-31] (see Table 2).

The question of the cyclisation of the 2-chloroethylamines is considered in detail in reviews[2,5,33]. According to polarographic results[34], a cyclic cation is also first formed in the hydrolysis of 2-mesylethylamines. Opening

TABLE 1.

Type of compounds	Formula	References
2-Halogenoalkylamines*	$R_2NCH_2CH_2X$	1, 5, 8, 9
2-Aminoethyl methanesulphonates*	$R_2NCH_2CH_2OSO_2CH_3$	10, 11
2-Aminoethyl toluene-*p*-sulphonates	$R_2NCH_2CH_2OSO_2C_6H_4CH_3$-*p*	12
Polyalcohol derivatives		
a. halogeno-derivatives*	$R(CHOH)_nCH_2X$	13
b. esters of methanesulphonic acids	$R(CHOH)_nCH_2OSO_2CH_3$	13
c. alkyl sulphates	$R(CHOH)_nCH_2OSO_2OR$	11
2-Halogeno-thioethers*	$RSCH_2CH_2X$	14
4-Halogenoethers	$RO(CH_2)_4X$	15, 16
α-Halogenocarbonyl compounds*	$RCOCHXR'$	17
N-Alkylaziridines*	$RN(CH_2)_2$	5
1,2-Epoxides*	$RCHCH_2O$	18, 19
Esters of methanesulphonic acid*	$ROSO_2CH_3$	1
Esters of *N*-arylsulphamic acids	$ArNRSO_2OR'$	20, 21
Diazo-compounds(aliphatic)*	RN_2	22
N-Nitrosoalkylurea derivatives	$RN(NO)CONR'_2$	23
Allyl alcohol derivatives*	$CH_2=CHCHROH$	24

* The anti-tumour activity of representatives of this type has been clinically studied.

TABLE 2. Basicity constants and rate of cyclisation of 2-chloroethylamines[5,32].

Amine	pK_a	K, min^{-1} at 15°
$(C_2H_5)_2NCH_2CH_2Cl$	8.82	0.145
$C_2H_5N(CH_2CH_2Cl)_2$	6.57	0.128
$N(CH_2CH_2Cl)_3$	4.39	0.073
$C_6H_5N(CH_2CH_2Cl)_2$	2.2	0

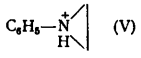
of the aziridinium cation proceeds comparatively slowly in the main by a bimolecular mechanism (S_N2),[35] and in agreement with this, stronger nucleophilic agents react more rapidly with the aliphatic chloroethylamines[26,36].

Aromatic 2-chloroethylamines differ somewhat from their aliphatic analogues. Hydrolysis of these compounds proceeds by an S_N1-mechanism[37-40]. An intramolecular mechanism is also indicated by the fact that the hydrolysis of nuclear-substituted 2-chloroethylamines of type (IV):

$$p\text{-}RC_6H_4N(CH_2CH_2Cl)_2 \qquad (R = OCH_3,\ CH_3,\ H,\ Cl,\ COOC_2H_5)$$
(IV)

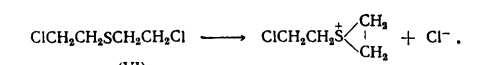
increases with increasing electron-donor properties of R [5] and falls sharply when the chloride ion is added to the solution[41].

The formation of the aziridinium ring in reactions of these compounds has not been proved[2,5,10], since the stability of rings of type (V)


(V)

must be greatly reduced because of the electron-attracting properties of the phenyl nucleus. Aromatic 2-chloroethylamines react faster with acid anions the greater the nucleophilic properties of the anion[37,42]. In this case, in contrast to hydrolysis, the reaction apparently proceeds by an S_N2-mechanism.

2-Halogeno-thioethers are very similar in properties to chloroethylamines. A study of the kinetics of hydrolysis of Yperite [mustard gas, bis-(2-chloroethyl) sulphide (Ed. of Translation)] (VI) showed that the intramolecular elimination of chlorine is also the rate-determining step[43,44]:


(VI)

As in the case of the aromatic chloroethylamines, the relative rate of reaction of bis-(2-chloroethyl) sulphide with acid anions increases with the nucleophilic properties of the anion; this probably also explains the change in substitution mechanism[45-47].

The dependence of the reaction mechanism on the nucleophilic properties of the attacking agent is particularly clearly shown in aromatic 2-chloroethyl sulphides[48]. Thus, in the hydrolysis of type (VII) compounds:

$$p\text{-}RC_6H_4SCH_2CH_2Cl \qquad (R = CH_3O,\ CH_3,\ H,\ I,\ Br,\ Cl,\ NO_2),$$
(VII)

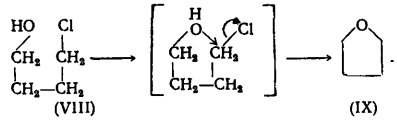
the rate of reaction increases with increasing electron-donor properties of R (S_N1-mechanism), but in the reaction with the iodide anion, which is a stronger nucleophilic agent than water, the reaction rate decreases with increasing electron-donor properties of nuclear substituents (S_N2-mechanism).

2-Halogenoalcohols, $HOCH_2CH_2X$, behave differently from most of the foregoing compounds in neutral and acid aqueous solutions.

The rate of hydrolysis of these compounds is ten or a hundred times less than the rate of hydrolysis of alkyl halides, which are similar in structure[49]. Reduction in the mobility of the chlorine by the introduction of a hydroxy-group in position 2 is observed both for the primary alkyl halides[50,51], reacting with water mainly by an S_N2-mechanism, and for the tertiary alkyl halides[52,53], decomposing under these conditions by an S_N1-mechanism.

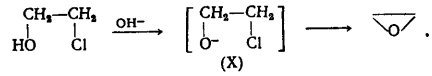
At present it is difficult to give an acceptable explanation of this effect. In any case, the reduction in the rate of the intramolecular reaction under the influence of an atom of oxygen in the β-position indicates the absence of interaction of the latter with the electrophilic centre of the molecule.

However, in 4-halogenoalcohols such an interaction exists between the oxygen and the δ-carbon atom. Streitwieser[33] explains this by the fact that a five-membered ring is formed more easily than a three-membered one. Hydrolysis of 4-chlorobutanol (VIII) gives tetrahydrofuran(IX):


(VIII) (IX)

In this reaction the rate of elimination of the halide ion is 1000 times greater than in the hydrolysis of 2-chloroethanol[59].

Kinetic data indicate[60,61], that in strongly alkaline solutions of 2-halogenoalcohols the alkoxide ion (X) is formed, and as a result, rapid intramolecular cyclisation takes place:

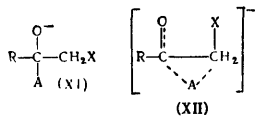

(X)

The rate of elimination of halide from the halogenoalcohols is substantially higher than the rate of elimination from the alkyl halides[62,63].

4-Halogenoethers, $RO(CH_2)_4X$,[15,16] appear to be similar to the corresponding alcohols in their behaviour on acid hydrolysis.

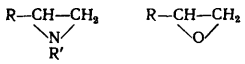
α-Halogenoketones, $O{=}C(R)CH_2X$, are close in structure to the types of compounds described above (Table 1), but steric considerations make the interaction of the lone pair of oxygen electrons with the α-carbon atom (β-position to the oxygen) impossible.

α-Halogenoketones react with nucleophilic agents by a bimolecular mechanism[64-66]. At first it was assumed that replacement of the halogen proceeds through a polar transition complex (XI).[67] However, such a scheme does not explain the significant difference

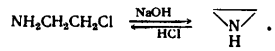

(XI) (XII)

in the rates of reaction of α-bromo- and α-chloro-ketones with the same reagent[68]. It was therefore postulated that the slow stage of the reaction is the formation of the intermediate (XII).[70,71]

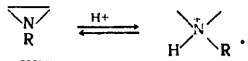
Derivatives of aziridine and ethylene oxide [oxiran]:



are closely related genetically to 2-halogenoalkylamines and 2-halogenoalcohols and are formed by treating the latter two compounds with alkalis[60,72,73]. On the other hand, 2-halogenoalkylamines and 2-halogenoalcohols may be prepared by treating the corresponding cyclic compounds with hydrohalogen acids[42,74,75]:



Aziridine itself is a weak alkylating agent, but it readily adds on a proton to give the reactive aziridinium ion (XIII):



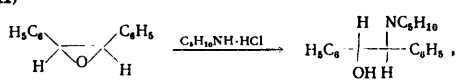
(XIV) (XIII)

The nitrogen atom of the aziridine ring (XIV) having a substituent with electron-acceptor properties should be considered separately. The equilibrium of the protonation reaction will here be strongly displaced to the left in contrast to aziridines not substituted on the nitrogen; however, the cation formed (XIII) must be more reactive because of the greater positive charge on the nitrogen atom.

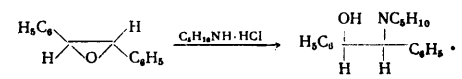
According to Ross[2], these compounds can react by a bimolecular mechanism without the participation of protons as catalyst.

The reactions of oxiran and the reactions of aziridine are catalysed by acids, but oxiran, in contrast to aziridine readily reacts with nucleophilic agents also in neutral and alkaline solutions. The mechanisms of these reactions change according to the conditions. Data on the rate of hydrolysis of substituted 1,2-epoxides in neutral and acid media (Table 3) show that replacement of an electron-donor substituent (CH_3–) by an electron acceptor ($ClCH_2$–) in neutral medium leads to an increased rate of reaction, but in an acid medium the opposite effect is observed[5,76]. This shows that in a neutral medium the reaction proceeds by an S_N2-mechanism and in acid medium by an S_N1-mechanism. This view is supported by a large number of papers[77,83].

A deeper study of some reactions of 1,2-epoxides catalysed by acids showed that the reactions are stereospecific. For example, when *cis*-$\alpha\alpha'$-epoxybibenzyl (XV) is opened by hydrogen halides[84], or by piperidine in the presence of piperidinium chloride[85], the *threo*-isomer is formed (XVI), but *trans*-$\alpha\alpha'$-epoxybibenzyl (XVII) gives the *erythro*-isomer (XVIII)



(XV) (XVI)



(XVII) (XVIII)

TABLE 3. Rate constants for the hydrolysis of 1,2-epoxides in neutral and acid solutions.

1,2-Epoxides	k_{H_2O}, s^{-1} (S_N2)	k_{H^+}, s^{-1} (S_N1)
CH_3–	$2.2\cdot10^{-6}$	$123\cdot10^{-3}$
$ClCH_2$–	$5.1\cdot10^{-6}$	$1.9\cdot10^{-3}$

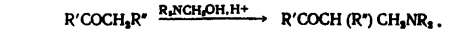
These facts contradict interpretations according to which a free carbonium ion is formed in these reactions (S_N1-mechanism).

An explanation of these contradictions has been given on the basis of the "push–pull" mechanism proposed by Swain[86]. According to this scheme, the reaction is regarded as a synchronous approach of the new substituent and removal of the old one; the rate of reaction is determined, on the one hand, by the strength of the interaction of the attacking substituent with the electrophilic centre of the molecular ("push component" of the reaction) and, on the other hand, by the ease of elimination of the old substituent ("pull component"). This mechanism explains[64,87,88] both the stereospecificity of the 1,2-epoxide (a consequence of the synchronism of the substitution), and the characteristics of a unimolecular mechanism shown by these reactions; these are particularly marked in acid solutions since protonation favours the rupture of the C–O bond of the oxide ring.

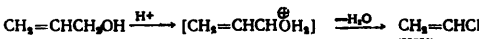
Aminomethanols, R_2NCH_2OH, form a characteristic type of alkylating agent. In the presence of acids they form the so-called carbimmonium cation[89]



which is the real alkylating agent[90–92]. Thanks to high activity, this cation can enter into an electrophilic substitution reaction at the carbon atom, for example, in the Mannich reaction[93]:

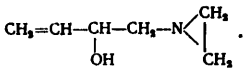


Derivatives of allyl alcohol, CH_2=CHCHROH, apparently cannot be regarded as typical alkylating compounds; however, in the presence of acids these compounds can sometimes form a carbonium ion (XIX)[94] which may behave as an alkylating agent:



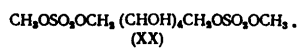
(XIX)

The allyl alcohol residue forms part of the molecule of the anti-tumour compound "Etoksen" ("Tetramin")[95]



Derivatives of esters of methanesulphonic acid, $ROSO_2CH_3$, not containing atoms with a lone pair of electrons are typical alkylating compounds. They react with nucleophilic reagents by a bimolecular mechanism[96–98].

Esters of sulphuric acid, $ROSO_2OR'$, are well known as compounds with pronounced alkylating properties[99]. It has been shown by experiments with labelled water (^{18}O) that the hydrolysis of these compounds proceeds simultaneously by S_N1- and S_N2-mechanisms[100]. There are indications that the mannitol derivative (XX) has anti-tumour activity[11,101]:

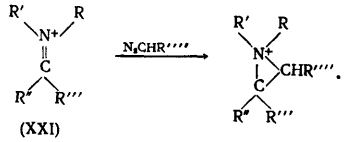


(XX)

Esters of *N*-arylsulphamic acids, $ArNRSO_2$.OR', are hydrolysed in alkaline medium at the same rate as 2-chloroethylamines[102]. The mechanism of this reaction has not been studied.

Diazo-compounds (aliphatic), RN_2, as alkylating agents have the important characteristic that they alkylate by a radical mechanism[103].

Reactions of diazo-compounds with the carbimmonium cation (XXI) leading to the formation of aziridine derivatives are interesting[104]:



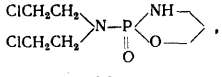
(XXI)

As can be seen from the scheme shown, this reaction *in vivo* might lead to the formation of new alkylating compounds[89,92].

Derivatives of nitrosoalkylurea, $R_2NCON.(NO)R'$, serve as starting materials for the preparation of diazo-compounds[105]. It has been shown in direct experiments that, under biological conditions in the organism, they behave as alkylating agents[23,106], reacting with purine basis of nucleic acids[107].

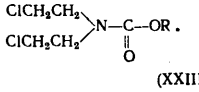
An important property of anti-tumour alkylating agents, which should be pointed out, is their ability to react with nucleophilic centres under relatively mild conditions. The high reactivity of a number of alkylating agents is shown by data on the hydrolysis of chloroethylamines[6,108-110], aziridines[76,111,112] and esters of methanesulphonic acid[2,98]; by data on the reaction of chloroethylamines[8,26] and 1,2-epoxides with the thiosulphate ion[76]; and also by the relative rates of reaction of bis-(2-chloroethyl sulphide and chloroethylamines with different anions[36,42,45,46].

In addition to these, there is a large group of anti-tumour compounds with very low alkylating activity[113]. However, partial decomposition of the molecule of such compounds, which may occur in the body, gives products with relatively high alkylating power. Because of this property, these compounds have been called alkylating compounds with latent activity[114,115]. They include such an effective preparation as the cyclophosphamide "Tsikolfosfan" ("Endoxan")(XXII)[114]:



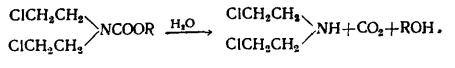
(XXII)

and also a large number of derivatives of carbamic acid of type (XXIII)[115-120]:

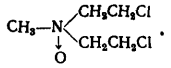


(XXIII)

In compounds of type (XXII) or (XXIII) the electron-acceptor substituent sharply reduces the basicity of the nitrogen, and, as a result, participation of the nitrogen in activating the substitution reaction at the β-carbon atom becomes impossible (see above). Hydrolytic or enzymic decomposition of these compounds under conditions in the body gives bis-(2-chloroethyl)amine[119]:



Bis-(2-chloroethyl)methylamine *N*-oxide ("Nitromin") obviously belongs to the type mentioned above[121]:



The relatively low alkylating properties of this compound should increase when it is reduced under biological conditions in the body to give bis-(2-chloroethyl)methylamine ("Embichin"†).

III. BIFUNCTIONALITY

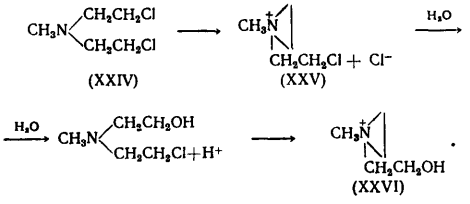
Highly effective anti-tumour alkylating compounds have an important structural feature: as a rule, their molecules contain two electrophilic groups. Compounds with one electrophilic centre have only one-tenth of the anti-tumour activity or they are entirely inactive‡.

This principle was first observed in aromatic[122] and aliphatic[123] chloroethylamines. Later the bifunctional rule was also noted in 1,2-epoxides[124,125], aziridines[124], and methanesulphonic acid esters[126]. Table 4 shows that not one unifunctional compound among the many classes of alkylating compounds has found practical application as a pharmaceutical preparation.

1. Mutual Effect of Electrophilic Centres in Bifunctional Alkylating Groupings

The cause of the higher biological activity of multifunctional compounds may lie, in principle, in the change in reactivity of the electrophilic centres as a result of the mutual influence of alkylating residues. It is therefore of interest to examine special features in the behaviour of this type of compound in comparison with the corresponding unifunctional compounds.

Aliphatic bis-(2-chloroethyl)amines, for example (XXIV) when dissolved in water form an aziridinium cation (XXV) at a slower rate then monochloroethylamines[32] (see Table 2)



(XXIV) (XXV) (XXVI)

It is also significant that the second electrophilic centre in such compounds can only enter into reaction after opening of the aziridinium ion (XXV).

The aziridinium ion

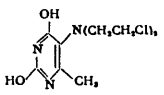
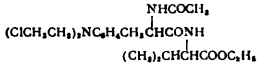
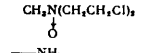
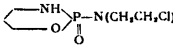
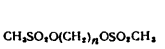
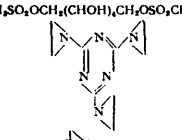
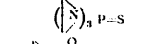
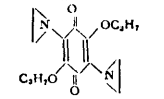
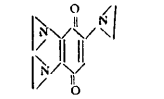
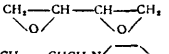
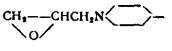


may be regarded as a symmetrical structure with four alkyl substituents on the nitrogen atom; it may therefore

† This is the hydrochloride, also known as Mechlorethamine hydrochloride, etc. (Ed. of Translation).

‡ This rule has a number of exceptions[5]. The main exceptions are compounds which contain, in addition to one alkylating centre, one residue without alkylating properties but nevertheless capable of interacting with some cell components (see p.542).

TABLE 4. Anti-tumour preparations used in practice* (from the Report of Committee of Experts on the Chemotherapy of Cancer [1]).

Compound	Structural formula
Bis-(2-chloroethyl)methylamine	$CH_3N(CH_2CH_2Cl)_2$
Bis-(2-chloroethyl)-2-chloropropylamine	$CH_3CHClCH_2N(CH_2CH_2Cl)_2$
γ-*p*-Bis-(2-chloroethyl)aminophenyl-butyric acid	$HOOC(CH_2)_3C_6H_4N(CH_2CH_2Cl)_2$
β-*p*-Bis-(2-chloroethyl)aminophenyl-α--alanine	$HOOCCH(NH_2)CH_2C_6H_4N(CH_2CH_2Cl)_2$
4-Methyl-5-[bis-(2-chloroethyl)amino]-uracil	
Ethyl ester of *N*-acetyl-β-*p*-[bis-(2--chloroethyl)amino]phenyl-α--alanylvaline	
1,6-Bis-(2-chloroethylamino)-1,6-di-deoxy-*D*-mannitol	$ClCH_2CH_2NHCH_2(CHOH)_4CH_2NHCH_2CH_2Cl$
Bis-(2-chloroethyl)methylamine *N*-oxide	
2-[Bis-(2-chloroethyl)amino]perhydro--2-oxo-1,3,2-oxazaphosphorine	
Tetramethylene bismethanesulphonate ($n = 4$)	
Nonamethylene bismethanesulphonate ($n = 9$)	
Mannitol 1,6-bismethanesulphonate	$CH_3SO_2OCH_2(CHOH)_4CH_2OSO_2CH_3$
2,4,6-Triaziridinyl-*sym*-triazine	
Triaziridinylphosphine sulphide	
3,6-Diaziridinyl-2,5-di-propoxy-*p*--benzoquinone	
2,3,5-Triaziridinyl-*p*-benzoquinone	
1,2 : 3,4-Diepoxybutane	
1,1′-Bis-(2,3-epoxypropyl)-4,4′--bipiperidyl	

* The table contains only the preparations that have been most studied clinically.

be supposed that in reactions of this compound there will be rupture not only of the three-membered ring, but in some measure also rupture of one of the bonds of the nitrogen atom with the substituent R or R′.

When two β-chloroethyl residues are present on the nitrogen atom, as for example in compound (XXIV), the probability of elimination of a CH_3 group must greatly increase because an aziridinium cation (XXV), (XXVI) is formed twice in the course of the reaction.

Thus, the amine (XXIV) when introduced into the animal body is almost completely destroyed with elimination of a methyl group[127]. According to Warwick, under the same conditions aromatic bis-(2-chloroethyl)amines do not eliminate a phenyl nucleus[128,129].

Bifunctional aromatic 2-chloroethylamines also show some special features connected with the presence of two 2-chloroethyl residues on one nitrogen atom. Because of the lower basicity of the nitrogen atom (Table 2), the rate of hydrolysis of chlorine in these compounds is appreciably less than in the corresponding monochloroethylamines. According to Ross, *N*-2-chloroethyl-*N*-ethylaniline ($pK_a = 3.5$) is hydrolysed under standard conditions to the extent of 58%, but *NN*-di-(2-chloroethyl)-aniline ($pK_a = 2.2$) is hydrolysed only to the extent of 20%.[5] The basicity of some 2,2′-iminodiethanols is nearly 1000 times greater than the basicity of the corresponding bis-(2-chloroethyl)amines[108]. It follows from these facts that after the first chlorine atom in dichlorodiethylamine is replaced by a hydroxyl group, the basicity of the nitrogen atom increases sharply, and this, in turn, should lead to hydrolysis of the second chlorine atom at a faster rate. In fact, with some examples[109] an inflection is observed on the curves of the concentration of chloride ion (Figure); this unambiguously indicates that the hydrolysis is accelerated at a definite stage.

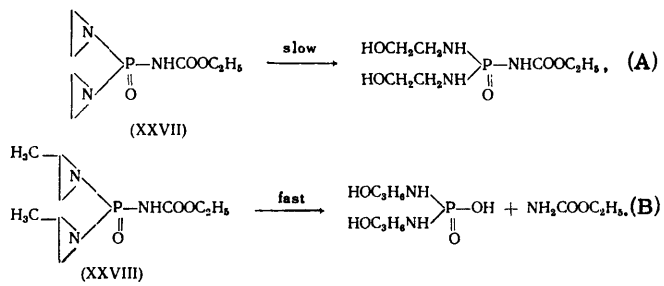
A similar effect should occur in the reactions of bis--(2-chloroethyl)amines with primary and secondary amines. Thus, the bifunctional aromatic chloroethylamines, in contrast to the unifunctional compounds, have a latent reactivity in some reactions; after the first alkylating residue enters into reaction the reactivity of the second increases.

When di-(2-chloroethyl)amines react with tertiary amines the quaternary ammonium salts,

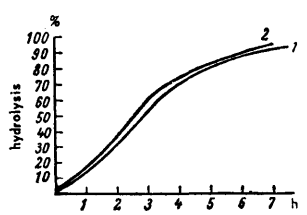
$$ClCH_2CH_2NRCH_2CH_2\overset{+}{N}R'R''R'''$$

are formed; the central nitrogen atom hardly changes its basicity and therefore in such reactions there should be no increase in the reactivity of the second electrophilic centre.

The mutual influence of aziridine residues in different di- and tri-aziridines has not been specially studied. However, it is interesting to note the work of Bardos, in which it was shown that (XXVII) is hydrolysed by Scheme (A), but (XXVIII) is hydrolysed by Scheme (B).[130]

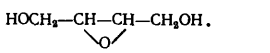


The mutual influence of two oxide rings in bifunctional compounds has also been little studied. Their mutual arrangement in the molecule is of great importance.

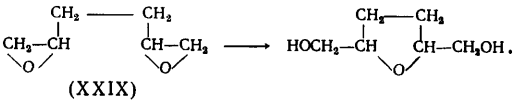


Curves showing the concentration of chloride ion in the hydrolysis of aromatic bis-(2-chloroethyl)amines:
1) *p*-bis-(2-chloroethyl)aminophenylalanine;
2) bis-(2-chloroethyl)aniline.

Ross has noted the fall in the rate of hydrolysis constant of diepoxybutane with time[76]. This may be connected with the formation of a less active intermediate product:



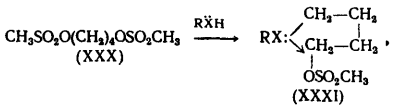
If the oxide rings are separated by two methylene groups (XXIX) a stable tetrahydrofuran ring is formed[131,132]:



(XXIX)

Compound (XXIX) reacts with only one molecule of water; it behaves as a unifunctional compound.

Esters of methanesulphonic acids, for example "Mialosan" ("Mileran") (XXX), containing two alkylating centres, react with strong nucleophilic reagents to form compounds of type (XXXI) in the first stage:

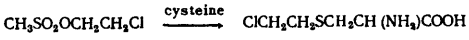


(XXX) (XXXI)

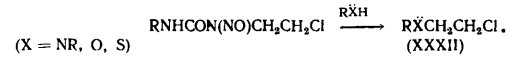
X = NR, O, S)

in which the character of the reactivity of the alkylating residue is materially changed by interaction of the electrophilic centre with the lone pair of electrons of the atom X.

There is indirect information to show that the 2-chloroethyl ester of methanesulphonic acid reacts *in vivo* with cysteine with the formation of 2-chloroethylcysteine, which has high alkylating power[2]:



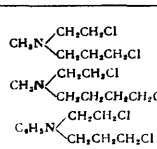
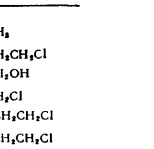
Among derivatives of nitrosoalkylureas the most active are derivatives of 2-chloroethylurea[106]. When such compounds react *in vivo*, biologically active compounds of type (XXXII) may also be formed:



(X = NR, O, S) (XXXII)

Anti-tumour compounds with unsymmetrical bifunctional groupings, in particular the halogenoalkylamines have the interesting property that one of their alkylating centres is very weakly reactive[8,133]. Table 5 shows that the replacement of an alkylating residue with weak reactivity by a residue having no alkylating properties at all leads to disappearances of activity. Compounds in which both electrophilic centres are slightly reactive are devoid of anti-tumour activity.

TABLE 5. Unsymmetrical dihalogenoalkylamines.

Preparations having anti-tumour activity	Preparations having no anti-tumour activity
	

Alkylating compounds containing one alkylating centre and a metabolite residue, for example, serine diazoacetate or diazooxonorleucine,

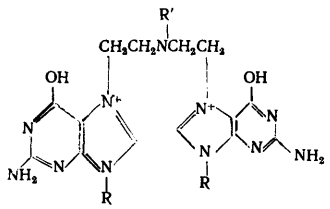
may also be regarded as unsymmetrical bifunctional compounds.

According to Baker[134-137], the natural parts of such molecules are capable of being adsorbed on the active centre of an enzyme, while the alkylating residue, reacting with a neighbouring nucleophilic group, may fix the whole molecule in this position.

2. A Special Feature in the Structure of Bifunctional Alkylating Compounds

The overwhelming majority of bifunctional alkylating compounds with pronounced anti-tumour activity are characterised by an important structural feature; the electrophilic centres of the bifunctional compounds are connected together by a chain of atoms which is preserved in the alkylating reaction. Because of this property, such compounds are capable of "stitching" DNA molecules, or cell nucleoproteins, forming so-called cross-linked bonds (cross-linking effect), which, in the opinion of some workers should lead to a sharp dislocation of the mitotic process[138-140].

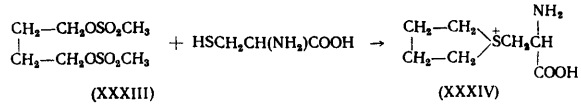
Several experimental results confirming this view have been obtained. Price and coworkers[141] proved indirectly that cross-linked bonds are formed between the DNA chains when DNA is treated with a derivative of bis-(2-chloroethyl)amine; cross-linked bonds are formed between the DNA chains because of the formation of N_7 derivatives of guanine:



Lowley and Brookes hydrolysed DNA that had been treated with bis-(2-chloroethyl) sulphide or Embichin, and they separated and identified cross-linked pairs of the bases guanine–guanine and guanine–cytosine[142]. Similar products are formed by the action of bis-(2-chloroethyl) sulphide on nucleic acids *in vivo*.[143]

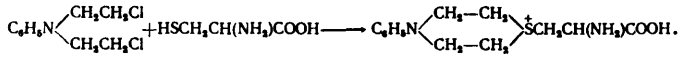
Earlier work of Alexander[144] indicates the possibility of cross-linking chains of DNA by the reaction of bifunctional compounds with phosphate residues.

The property of non-interaction between the two electrophilic centres in bifunctional compounds also favours the reaction of both centres with the same atom to form specific cyclic products. At present it may be considered proved that when "Mileran" (XXXIII) is introduced into the blood of animals, it reacts with cysteine to form a cyclic compound (XXXIV)[4,151-153]

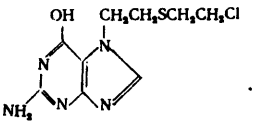


(XXXIII) (XXXIV)

There are also indications that, under biological conditions, the chloroethylamines are capable of forming unstable cyclic products of the thiazan type [128]:

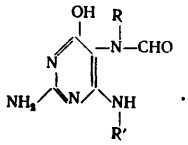


When only one of the two alkylating centres reacts with a molecule of metabolite, compounds termed alkylating metabolites are formed. Timmis showed that an alkylating derivative of guanine is formed in tissue treated with bis-(2-chloroethyl) sulphide [154,155]:



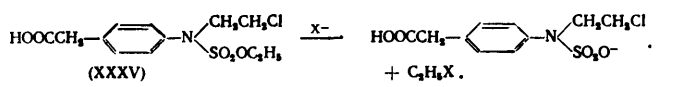
The formation under biological conditions in the body of (2-chloroethyl)cysteine [2] with alkylating properties has been mentioned above.

However, some workers cast doubt on the biological significance of the effect of cross-linking DNA chains [145,146]. Alkylation of DNA is accompanied by degradation of the molecule. Thus alkylation of guanine on the N_7-position may lead to the depurinisation of DNA, [141,147] or it is accompanied by the oxidative cleavage of the imidazole ring with the formation of the formyl derivative of an aminopyrimidine [148]:



In the reactions mentioned above, the system of hydrogen bonds in the doubly stranded DNA molecule is destroyed and this facilitates rupture of the DNA; the viscosity of the DNA gradually falls [149]. Brookes and Lowley consider that the high biological activity of bifunctional alkylating agents is due to their ability to destroy simultaneously the structure of both strands of DNA. [150]

Recently, in animal experiments, the anti-tumour activity of a compound containing a new unsymmetrical bifunctional alkylating group has been observed; this is the ester of an *N*-(2-chloroethyl)arylsulphamic acid (XXXV): [20,21]



(XXXV)

The electrophilic centres of compound (XXXV) are separated in the alkylation reaction. The result of this work is a direct proof that non-interaction of the electrophilic centres of bifunctional alkylating agents is not a necessary condition for anti-tumour activity.

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The property of alkylating compounds of attacking tumerous tissues cannot be connected with any definite alkylation mechanism. Different types of alkylating compounds with anti-tumour activity differ materially in reactivity. They include compounds capable of reacting with nucleophilic agents by both S_N1- and S_N2-mechanisms and also compounds that react by a mixed mechanism. Diazo-compounds and derivatives of nitrosoalkylurea react with nucleophilic centres through a stage in which free radicals are formed.

It is interesting to note that the reaction of acylation products does not lead to selective attack on tumour cells (there is not a single anti-tumour "acylating" agent), although acylating compounds (for example, anhydrides, acid chlorides, etc.), like alkylating compounds, contain strong electrophilic centres and are capable of reacting with nucleophilic centres in the living cell.

Examination of the structure and chemical properties of intermediate products of the conversion of alkylating agents under living conditions in the body shows that many of these may be active biologically. This shows that the anti-tumour activity of alkylating compounds is not necessarily the result of their direct action on the cell, but may be connected, at least in some instances [156], with subsequent reactions of the primary interaction products of these compounds with metabolites of the living organism.

It is important to note that the anti-tumour activity of alkylating compounds is connected with their ability to act selectively precisely on those cell processes which determine the difference between tumour cells and healthy cells, and this property must be directly related to the specific chemical behaviour of alkylating agents. The elucidation of this specificity will open the way to further progress in the development of anti-tumour preparations.

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