464. *The Reactions of Certain Epoxides in Aqueous Solutions.*

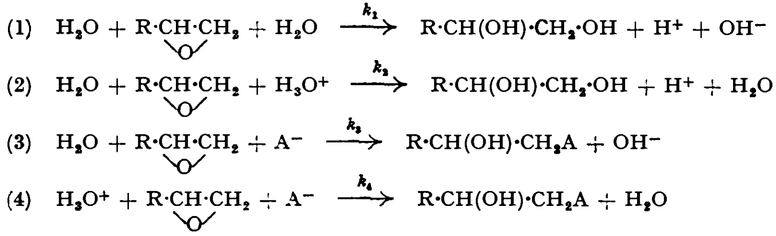
By W. C. J. Ross.

The reactions of several mono- and di-epoxides have been studied, a new method being used for the determination of epoxides in dilute aqueous solution. Evidence has been obtained for the view that the reactions proceed by the combined action of an acid and a base, and the effect of substituents upon the rate of reaction can be interpreted on this basis. The results are discussed in relation to the biological action of the various epoxides.

A NUMBER of attempts have been made to relate the biological effects of the mustard-gas types of compound with their chemical reactivity. The suggestions that mustard gas acts by the liberation of intracellular acid or that it is converted in the tissues into a reactive sulphone were once current but they have been rejected by Peters and Walker (*Biochem. J.*, 1923, **17**, 260). A more recent hypothesis was that of Barron, Bartlett, and Miller (*J. Expt. Med.*, 1948, **87**, 489) who believed that " nitrogen mustard-gas " derivatives could act as structural inhibitors of enzymes because of the similarity between choline and acetylcholine and the ethyleneimonium ions derived from these " mustards." It is doubtful whether the latter hypothesis covers the case of more complex aliphatic " nitrogen mustards " or of the aromatic " nitrogen mustards " which do not appear to yield ethyleneimonium ions (Ross, *J.*, 1949, 1972). It would now seem that the characteristic feature of all the mustard-gas types of compound which exhibit " radiomimetic " properties (Boyland, *Biochem. Symposia*, 1948, No. 2, p. 67) is their ability to act as difunctional electrophilic reagents under mild conditions in aqueous solution (Davis, Everett, and Ross, *J.*, 1950, 1331).

Epoxides are known to act as electrophilic reagents under these same conditions and it has been demonstrated that they react readily with proteins at physiological pH (Fraenkel-Conrat, *J. Biol. Chem.*, 1944, **154**, 227). This led to the testing of various diepoxides and the establishment of their radiomimetic properties (Loveless and Revell, *Nature*, 1949, **164**, 938; Davis, Everett, and Ross, *loc. cit.*; Bird, in the press).

In the case of the aromatic " nitrogen mustards " the efficiency of a compound as a tumour-growth inhibitor runs parallel with its relative reactivity towards nucleophilic centres, a property conveniently assessed by measuring the rate of hydrolysis in aqueous acetone (Haddow, Kon, and Ross, *Nature*, 1948, **162**, 824). Since this hydrolysis proceeds by an S_N1 mechanism, one is, in effect, measuring the rate of production of carbonium ions from the arylhalogenoalkylamine. One object of the present work was to discover whether a similar correlation between the biological activity of various diepoxides and their reactivity towards nucleophilic centres could be established. Brønsted, Kilpatrick, and Kilpatrick (*J. Amer. Chem. Soc.*, 1929, **51**, 428) have shown that when an epoxide reacts in aqueous solutions containing anions the following reactions take place :



$$(1)\quad H_2O + R{\cdot}\underset{\diagdown O \diagup}{CH{\cdot}CH_2} + H_2O \xrightarrow{k_1} R{\cdot}CH(OH){\cdot}CH_2{\cdot}OH + H^+ + OH^-$$

$$(2)\quad H_2O + R{\cdot}\underset{\diagdown O \diagup}{CH{\cdot}CH_2} + H_3O^+ \xrightarrow{k_2} R{\cdot}CH(OH){\cdot}CH_2{\cdot}OH + H^+ + H_2O$$

$$(3)\quad H_2O + R{\cdot}\underset{\diagdown O \diagup}{CH{\cdot}CH_2} + A^- \xrightarrow{k_3} R{\cdot}CH(OH){\cdot}CH_2A + OH^-$$

$$(4)\quad H_3O^+ + R{\cdot}\underset{\diagdown O \diagup}{CH{\cdot}CH_2} + A^- \xrightarrow{k_4} R{\cdot}CH(OH){\cdot}CH_2A + H_2O$$

(1) and (3) being the " spontaneous " addition of water and anion respectively and (2) and (4) the corresponding acid-catalysed reactions.

The reaction with anions (3) liberates hydroxyl ions and proceeds at a velocity which is independent of pH over the range 4—8·5, but Brønsted *et al.* (*loc. cit.*) have suggested that the reaction might be reversed at higher pH and have shown that more rapid acid-catalysed reactions occur when the pH falls below 4·2 (see also below). To measure the reactivity of an epoxide towards anions it is therefore necessary to be able to determine the amount of hydroxyl ion liberated and to keep the pH within the prescribed limits; both these conditions may be satisfied by the continous titration of the reaction mixture with a weak acid.

Most organic anions react slowly with epoxides so that in order to be able to make a rapid comparison of the relative reactivities of a range of compounds the strongly nucleophilic thiosulphate ion was used—compare the high reactivity of this ion towards the carbonium ions derived from di-2-chloroethyl sulphide (Ogston, *Trans. Faraday Soc.*, 1948, **44**, 45), the aliphatic " nitrogen mustards " (Golumbic, Fruton, and Bergmann, *J. Org. Chem.*, 1946, **11**, 518), and their aromatic analogues (Ross, *J.*, 1949, 2589).

Since a number of the epoxides examined were sparingly soluble in water it was necessary to measure the comparative reactivities in aqueous acetone. In the method finally adopted the epoxide was added to a boiling 0·2M-solution of sodium thiosulphate in 50% acetone containing phenolphthalein as indicator and the alkalinity which developed was continuously titrated with 0·2N-acetic acid; the rate of addition of acid was proportional to the rate of ester formtion. Under these conditions changes in the composition of the solvent caused by the addition of aqueous acid and the effect of added acetate ions were not significant, for the unimolecular rate coefficient calculated from equation (i) below was reasonably constant. With epoxides of the general formula  $R{\cdot}\underset{\diagdown O \diagup}{CH{\cdot}CH_2}$ the reaction with the thiosulphate ion was quantitative, negligible amounts of glycol being formed so that (viii) (see below) becomes simplified to :

$$k' = \frac{2{\cdot}303}{t}\log_{10}\frac{100}{100 - \%\ \text{ester formed}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (i)$$

For more heavily substituted epoxides some glycol was formed under the standard conditions and the ratio x/y was calculated from :

$$\frac{100 - \%\ \text{ester formed at}\ t = \infty}{\%\ \text{ester formed at}\ t = \infty} \qquad \text{(ii)}$$

and this value was employed to calculate k'(thio), equation (viii) being used. In some cases, particularly of unsymmetrical diepoxides, the value of k' was not constant throughout the whole course of the reaction and in these cases the figures given in Table I are those calculated for 20% and 80% reaction : the results are discussed below.

TABLE I.

Correlation of the chemical reactivity of various epoxides with their biological activity.

Compound.	100k' (thio), min.$^{-1}$.	Toxicity, LD$_{50}$ mg./kg.[a]	Tumour-growth inhibition.[b]
Methiodide of *NN*-di-(2 : 3-epoxypropyl)-*p*-anisidine	370	100	—
Epichlorohydrin	130	215	—
Glycidol	115	300	—
1 : 2-3 : 4-Diepoxybutane *	85	25	+
Di-(2 : 3-epoxypropyl) ether *	69	50	+
Ethylene oxide	67		
Bigot's " diallyl dioxide "	60		—
p-Di-(2 : 3-epoxypropoxy)benzene, m. p. 114° †	55	170	+
p-Di-(2 : 3-epoxypropoxy)benzene, m. p. 90° †	55	150	—
m-Di-(2 : 3-epoxypropoxy)benzene †	55	200	—
1 : 2-3 : 4-Diepoxy-2-methylbutane *	53—44	100	+
2 : 4 : 6-Tri(ethyleneimino)-1 : 3 : 5-triazine	44	2	+
1 : 2-5 : 6-Diepoxyhexane	40	97	+
Ethyl di-(2 : 3-epoxypropyl)acetate	38	1000	—
Propylene oxide	36	500	—
NN-Di-(2 : 3-epoxypropyl)-*p*-anisidine	26	200	—
Butadiene monoxide	24·6	160	—
1 : 2-5 : 6-Diepoxy-2 : 5-dimethylhexane *	23·4	800	—
p-Divinylbenzene dioxide *	19·8	180	—
1 : 2-3 : 4-Diepoxy-2 : 3-dimethylbutane *	21·2—14·3		—
1-Vinylhex-3-ene dioxide *	13·3—11·3	500	—
*cyclo*Hexene oxide	5·7	500	—
2 : 3-6 : 7-Diepoxy-2 : 6-dimethyloctane (dihydromyrcene dioxide) *	5·7—1·4	1000	—
Limonene dioxide *	3·9—2·1	1100	—
1 : 2-3 : 4-Diepoxy-1 : 4-dimethylbutane *	2·3—1·6		—
1 : 2-3: 4-Diepoxy-1 : 4-diphenylbutane *	0·4	1000	—
Divinyl sulphone	57—39		—
Acraldehyde	3·6—1·8		
Acrylonitrile	0·5		

* The preparation of these compounds will be described in a later communication by Prof. G. A. R. Kon and Mr. J. L. Everett.

† Werner and Farenhorst, *Rec. Trav. chim.*, 1948, **67**, 438.

[a] Administered in arachis oil to mice by an intraperitoneal injection. [b] Measured against the transplanted Walker rat carcinoma.

Since the reaction of simple epoxides with thiosulphate in 50% acetone is quantitative it provides a convenient method for the determination of such epoxides in aqueous solution. By adding an equal volume of acetone and then sodium thiosulphate to make the solution 0·2M. and titrating the alkalinity formed, the rate of disappearance of epoxide from aqueous solutions under a variety of conditions can be followed. Earlier methods for the quantitative determination of epoxides, such as those of Lubatti (*J. Soc. Chem. Ind.*, 1944, **63**, 133), Nicolet and Poulter (*J. Amer. Chem. Soc.*, 1930, **52**, 1186), and King (*Nature*, 1949, **164**, 706), are not easily adapted to the determination in dilute aqueous solutions.

The rates of reaction of several mono- and di-epoxides in completely aqueous solution have been studied by using this method of determination. Table II shows the rate at which these epoxides react (*a*) with water alone to form glycol and (*b*) with 0·1M-sodium acetate to form ester and glycol at 37°—these conditions approximate more closely to those encountered within cells than those used to determine the order of activity as in Table I, but unfortunately they can only be used for the more water-soluble compounds. There is a considerable difference in the relative amounts of reaction with water and with anion when the reaction proceeds in water alone, as compared with the reaction in 50% acetone—more ester being formed in the latter case. This is one of the reasons for adding acetone before determining the amount of epoxide in solution by the thiosulphate method.

TABLE II.

Reaction of epoxides with (a) *water and* (b) *water containing* 0·1M-*sodium acetate and* 0·1N-*acetic acid.* (Concn. of oxide, 0·1M. for monoepoxides and 0·05M. for diepoxides. Temp. 37°.)

	(*a*)		(*b*)			
Time, hrs.	% epoxide reacting.	k_1, hr.$^{-1}$.	% ester formed..	% epoxide reacting.	k_1, hr.$^{-1}$.	k' (Ac), hr.$^{-1}$.
Glycidol.						
24	15·6	0·0071	7·8	22·8	0·0071	0·0036
48	28·6	0·0070	13·4	39·2	0·0068	0·0035
72	40·0	0·0071	17·8	52·4	0·0068	0·0035
96	49·3	0·0071	22·0	64·0	0·0070	0·0037
Epichlorohydrin.						
24	36·5	0·019	20·6	52·0	0·0185	0·012
48	59·0	0·0185	30·0	76·6	0·0185	0·012
72	72·5	0·018	34·8	88·0	0·018	0·0115
100	84·5	0·0185	38·0	96·0	0·0185	0·013
169	96·5	0·020	39·6	100·0	—	—
1 : 2-3 : 4-*Diepoxybutane.*						
24	15·5	0·0070	11·1	26·3	0·0074	0·0055
48	28·2	0·0069	17·5	45·0	0·0075	0·0052
72	37·4	0·0065	22·1	55·6	0·0068	0·0045
96	43·5	0·0062	25·7	64·0	0·0065	0·0043
168	60·3	0·0055	30·7	78·5	0·0056	0·0037
264	68·6	0·0044	34·2	88·2	0·0050	0·0033
1 : 2-5 : 6-*Diepoxyhexane.*						
6	13·0	0·023	2·4	27·0	0·046	0·0043
24	42·0	0·023	6·05	71·0	0·047	0·0044
32	52·8	0·023	6·75	79·5	0·047	0·0044
48	68·0	0·024	7·75	90·2	0·045	0·0042
72	81·0	0·023	7·85	96·0	—	—
96	89·0	0·023	—	—	—	—
Di-(2 : 3-*epoxypropyl*) *ether.*						
24	15·5	0·0071	6·6	21·8	0·0071	0·0031
48	28·0	0·0069	12·2	40·1	0·0074	0·0032
75	39·6	0·0067	16·8	54·5	0·0072	0·0032
95	47·0	0·0067	19·8	62·5	0·0071	0·0033
119	53·0	0·0065	23·0	72·6	0·0074	0·0034
170	67·8	0·0067	27·0	83·3	0·0071	0·0034
1 : 2-5 : 6-*Diepoxyhexane.**						
24	5·5	0·0024	4·6	13·0	0·0037	0·0021
48	9·5	0·0023	9·2	24·0	0·0038	0·0022
72	—	—	12·8	35·4	0·0038	0·0022
96	19·0	0·0022	16·0	45·5	0·0041	0·0022
144	25·0	0·0020	—	—	—	—
173	—	—	21·1	65·5	0·0041	0·0020
192	30·0	0·0019	—	—	—	—
245	—	—	24·8	77·5	0·0041	0·0020
269	38·4	0·0019	—	—	—	—
342	44·5	0·0017	—	—	—	—

* In these experiments 50% acetone was used as solvent in place of water.

Brønsted *et al.* (*loc. cit.*) studied the reactions of several monoepoxides in aqueous solution, using a method similar to that now adopted, but they were not able to measure the rate of disappearance of epoxide in the same experiments as the rate of ester formation. The coefficient for the rate of reaction of the oxide with water was determined separately by a dilatometric method in the absence of salts and then in the presence of varying concentrations of a salt—sodium perchlorate—which had been shown not to react to form an ester. In this way the variation of the rate coefficient with rising salt concentration was determined and assumed to apply in other cases where the anion was not unreactive.

Although the accuracy attained in the present work is not as high as that of Brønsted *et al.*, largely because only limited quantities of the diepoxides were available, they are more satisfactory in that no assumptions were made regarding the rate coefficient for the reaction with water. As in the case of reaction with the thiosulphate ion, it was necessary to prevent the solution becoming alkaline because of reaction (3), and this was done by having an excess of acetic acid present; the buffering action of sodium acetate kept the pH of the solution within the required limits. The amount of ester formed was determined as in the experiments of Brønsted *et al.* by titrating the remaining acetic acid, and the epoxide content of the solution was determined in the same aliquot, after addition of acetone, by the thiosulphate method. As the acetic acid is little dissociated, practically all the acetate ions come from the sodium acetate, and as the oxide reacts by equation (3) the acid is neutralised, with the result that the acetate-ion concentration is kept constant. Thus for the reaction with water (1):

$$d[\text{oxide}]/dt = k_1[\text{oxide}] \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad \text{(iii)}$$

and for the reaction with anion (3):

$$d[\text{oxide}]/dt = k_3[\text{oxide}][\text{A}^-] = k'[\text{oxide}], \text{ where } k' = k_3[\text{A}^-] \quad . \; . \; . \; . \quad \text{(iv)}$$

It is assumed that the rate-determining step is the attack of the oxide by a water molecule or an anion (see below); k_1 includes the constant concentration of water. The acid-catalysed reactions (2) and (4) will not be significant at the pH of the acetate–acetic acid mixtures. Combining these equations and integrating, we have

$$(k_1 + k')t = 2{\cdot}303 \log_{10} \frac{100}{100 - (k_1 + k')x/k_1} \quad . \; . \; . \; . \; . \; . \; . \quad \text{(v)}$$

$$= 2{\cdot}303 \log_{10} \frac{100}{100 - (k_1 + k')y/k'} \quad . \; . \; . \; . \; . \; . \; . \quad \text{(vi)}$$

where x and y = % epoxide converted into glycol and into ester, respectively, in time t.

Since $x/y = k_1/k'$, equations (v) and (vi) may be re-written:

$$k_1 = \frac{2{\cdot}303}{t(1 + y/x)} \log_{10} \frac{100}{100 - (1 + y/x)x} \quad . \; . \; . \; . \; . \; . \; . \; . \quad \text{(vii)}$$

$$k' = \frac{2{\cdot}303}{t(1 + x/y)} \log_{10} \frac{100}{100 - (1 + x/y)y} \quad . \; . \; . \; . \; . \; . \; . \; . \quad \text{(viii)}$$

which can be solved since x, y, and t are determined in the experiment. The values of the coefficients k_1 and k' given in Tables II and III were calculated by using these equations. In Table III, which shows the effect of increasing acetate concentration on the extent of the reaction, $k_3 = k'[\text{A}^-]$ which should be constant if the reaction proceeds as in (3).

The first part of Table IV shows the amounts of ester and glycol formed when one of the most biologically active diepoxides—1 : 2-3 : 4-diepoxybutane—reacts in solutions containing different anions. The reactions were carried out in the presence of relatively small amounts of the corresponding organic acid in order to keep the pH within the required limits. In the case of the anions derived from strong acids, the corresponding acid could not be used since too great a decrease in pH would have resulted; therefore acetic acid was employed. Since the concentration of acetate ions was low compared with that of the anion being examined, no serious error was introduced by adopting this procedure.

Table IV also shows the result of allowing the diepoxide to react with several anions in the absence of added acid—these figures are discussed below.

An approximate value for k_2—the rate coefficient for the acid-catalysed addition of water—has been determined for several epoxides by measuring the rate or glycol formation in solutions of different hydrogen-ion concentration; namely, in water alone (pH = 7), in 0·05N-acetic acid (pH = 3), and in 0·1N-hydrochloric acid (pH = 1). The rate coefficient (k_2) is obtained from the expression $k_2 = (k^x - k_1)/[\text{H}^+]$, where k^x is the rate coefficient for the formation of glycol calculated from equation (vii) at pH = x (Brønsted notation). The amount of ester formed in the reaction with dilute acetic acid was neglible. The results are formulated in Table VI.

Discussion.—As Branch and Calvin have pointed out ("The Theory of Organic Chemistry," New York, 1941, p. 403), the reactions of epoxides in aqueous solutions are examples of the Lowry mechanism, that is, the processes involve the combined action of an acid and a base. The effects of structural modifications on the reactivity of the oxiran ring towards water and anions

TABLE III.

Reaction of epoxides in aqueous solutions containing 0·1N-*acetic acid and various concentrations of sodium acetate.* (Concn. of oxide, 0·1M. for monoepoxides and 0·05M. for diepoxides. Temp., 37°. Time, 24 hours.)

NaOAc, M.	% ester formed.	% glycol formed.	% epoxide reacting.	k_1.	k' (Ac).	k_3 (Ac).
Epichlorohydrin.						
0	—	36·5	36·5	0·019	—	—
0·1	20·6	31·4	52·0	0·0185	0·012	0·12
0·2	34·5	28·0	62·5	0·0185	0·023	0·115
0·5	59·8	21·7	81·5	0·019	0·052	0·104
1·0	77·3	16·9	94·2	0·021	0·098	0·098
Glycidol.						
0	—	15·6	15·6	0·0071	—	—
0·1	7·8	15·0	22·8	0·0078	0·0036	0·036
0·2	13·5	15·3	28·8	0·0076	0·0066	0·033
0·5	27·3	14·7	42·0	0·0080	0·0150	0·030
1·0	44·8	14·2	59·0	0·0089	0·028	0·028
1 : 2-3 : 4-*Diepoxybutane.*						
0	—	15·5	15·5	0·0070	—	—
0·1	11·1	15·2	26·3	0·0074	0·0055	0·055
0·2	19·7	14·7	34·4	0·0075	0·0101	0·050
0·5	38·4	12·2	50·6	0·0071	0·0225	0·045
1·0	58·5	10·5	69·0	0·0074	0·041	0·041
1 : 2-5 : 6-*Diepoxyhexane.*						
0	—	42·0	42·0	0·023	—	—
0·1	6·05	64·95	71·0	0·047	0·0044	0·044
0·2	11·0	57·8	68·8	0·041	0·0078	0·039
0·5	20·6	50·0	70·6	0·036	0·0150	0·030
1·0	33·4	44·2	77·6	0·036	0·027	0·027
Di-(2 : 3-*epoxypropyl*) *ether.*						
0	—	15·5	15·5	0·0071	—	—
0·1	6·6	15·2	21·8	0·0071	0·0031	0·031
0·2	12·6	15·9	28·5	0·0078	0·0087	0·031
0·5	28·8	16·2	45·5	0·0090	0·0161	0·032
1·0	47·6	14·4	62·0	0·0094	0·031	0·031

TABLE IV.

Reaction of 1 : 2-3 : 4-*diepoxybutane with various anions.* (Concn. of epoxide, 0·025M. Temp., 37° Time, 24 hours.)

Anion.	% ester formed.	% epoxide reacting.	% conversion into ester.
1. 0·25M-Thiosulphate, 0·05N-acetic acid	100	100	100
1*a*. 0·025M-Thiosulphate, 0·05N-acetic acid	92·5	98	94·5
2. 0·5M-Iodide, 0·05N-acetic acid	97	100	97
3. 0·166M-Citrate, 0·05N-citric acid	47·2	57·7	82·5
4. 0·5M-Chloride, 0·05N-acetic acid	53	66	80·3
5. 0·5M-Acetate, 0·05N-acetic acid	38·4	50·9	75·5
6. 0·5M-Benzoate, 0·05N-benzoic acid	30·1	45·8	65·6
7. 0·25M-Tartrate, 0·05N-tartaric acid	23·7	40·7	58·1
8. 0·25M-Oxalate, 0·05N-oxalic acid	31	54·7	56·6
9. 0·5M-Formate, 0·05N-formic acid	20·2	64·85	31·2
10. 0·5M-Nitrate, 0·05N-acetic acid	2·5	32	7·6
11. 0·5M-Acetate	3·6	50·7	
12. 0·5M-Benzoate	6·0	44·5	
13. 0·5M-Chloride	2·5	27·5	
14. 0·25M-Thiosulphate	21·8	68	
15. 0·00125N-Sodium hydroxide	—	15·7	
16. 0·1N-Sodium hydroxide	—	85	
17. 0·5N-Sodium hydroxide	—	100	
18. Water alone	—	15·5	
19. 0·05N-Acetic acid	nil	30·0	

TABLE V.

Compound.	k_1.[a]	k_3 (Ac).[a]	k' (thio).[b]	k' (thio).[c]
Glycidol	0·0069	0·036	1·15	0·90
Epichlorohydrin	0·0185	0·12	1·30	1·12
1 : 2-3 : 4-Diepoxybutane	0·0075—0·0050	0·055—0·033	0·85	0·67
Di-(2 : 3-epoxypropyl) ether	0·0068	0·033	0·69	0·55
1 : 2-5 : 6-Diepoxyhexane	0·023	0·043	0·40	0·34

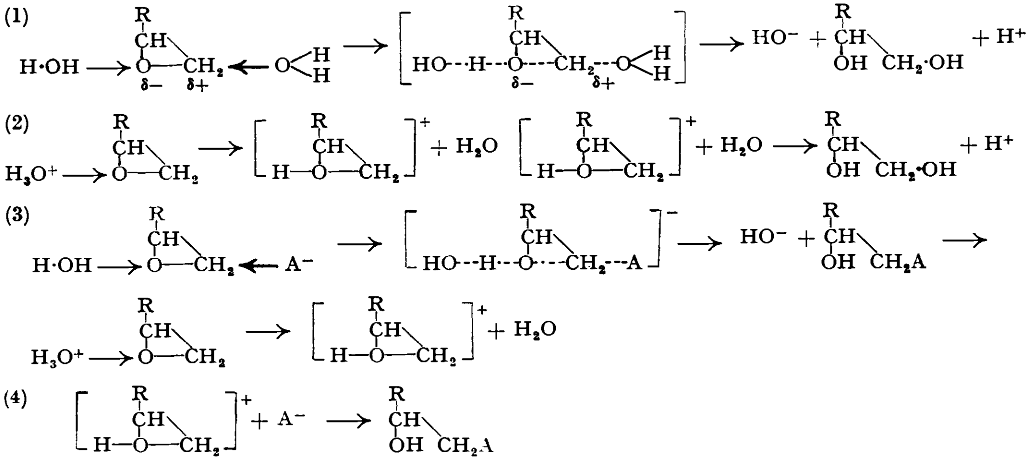
From Table II. [b] From Table I. [c] The conditions were exactly as described for results in Table I except that 0·2M-sodium acetate was also present.

TABLE VI.

Rate coefficient (k_2) *for the acid-catalysed addition of water* (for conditions, see p. 2271).

Compound.	k_1 (at pH = 7).	k^3 (at pH = 3).	k^1 (at pH = 1).	Average value of k_2.
Propylene oxide	0·0091	0·495	40·5	—
$k_2 = k^x - k_1/[H^+]$	—	484	405	445
Glycidol	0·0071	0·050	4·75	—
k_2	—	43	47	45
Epichlorohydrin	0·019	0·026	0·71	—
k_2	—	7·0	6·9	6·95
1 : 2-3 : 4-Diepoxybutane	0·0070	0·0143	0·68	—
k_2	—	7·3	6·7	7·0
1 : 2-5 : 6-Diepoxyhexane	0·023	0·66	64·0	—
k_2	—	637	640	639
Di-(2 : 3-epoxypropyl) ether	0·0070	0·316	2·17	—
k_2	—	24·6	21·6	23·1
Methiodide of *NN*-di-(2 : 3-epoxypropyl)-*p*-anisidine	0·0073	—	0·0304	—
k_2	—	—	0·23	0·23
2 : 4 : 6-Tri(ethyleneimino)-1 : 3 : 5-triazine	0·024	30·0	—	—
k_2	—	$2·98 \times 10^4$	—	$2·98 \times 10^4$

under conditions where " spontaneous " and acid-catalysed reaction can occur are best explained by considering that the following mechanisms operate for the reactions (1)—(4) on p. 2258.



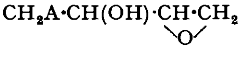
The unstable oxiran ring will be partly polarised with a fractional negative charge on the oxygen atom and a similar positive charge on the terminal carbon atom. The direction of ring fission shown in this scheme applies to those compounds where R is an electron-attracting group (see below). Spontaneous addition of water (1) is regarded as an attack on the positively-charged carbon atom by a water molecule acting as a base by reason of the lone pair of electrons on its oxygen atom, followed by solvation of the charged oxygen atom and subsequent elimination of hydrogen and hydroxyl ions in equivalent amounts so that the solution remains neutral. Similarly, the reaction with an anion (3) involves the attack of the carbon atom by the anion followed by solvation of the charged oxygen atom. In this instance hydroxyl ions alone are eliminated and the solution becomes alkaline. In the acid-catalysed reactions (2) and (4) the two stages are considered to be the attack of the hydrogen—or more likely the oxonium—

ion on the oxygen atom yielding an acid complex followed by the attack of the base (anion or water molecule) on the carbon atom.

It is now suggested that the initial step in the spontaneous reactions is the attack of the base on the terminal carbon atom but in the acid-catalysed reaction it is the attack of the oxygen atom by the oxonium ion. The dependence of the rate of the spontaneous reaction upon the concentration of base (Table III) and its independence of hydrogen-ion concentration over a wide range, and the dependence of the rate of the acid-catalysed reaction upon the hydrogen-ion concentration (Table VI) seem to support this view.

If these mechanisms are correct it would be expected that substituents in R which are electron-attracting and which tend to increase the positive charge on the terminal carbon atom and decrease the negative charge on the oxygen atom would favour reactions (1) and (3) and retard reactions (2) and (4).

The effect of electron-attracting groups such as $\geqslant\overset{+}{N}\cdot CH_2-$, $Cl\cdot CH_2-$, and $HO\cdot CH_2-$ on the velocity of reaction with the thiosulphate anion, shown in Table I, support the proposed mechanism. As would be expected, the positively-charged nitrogen atom in the quaternary compound causes by far the greatest increase in reaction rate. Electron-repelling groups such as CH_3-, $CH_2{:}CH-$, and $R_2N\cdot CH_2-$ decrease the reactivity towards anions in neutral solutions. If both carbon atoms of the epoxide ring are substituted by such groups a large diminution of reactivity results as in the case of the oxides of *cyclo*hexene, limonene, and dihydromyrcene. The high reactivity of 1 : 2-3 : 4-diepoxybutane is probably due to the fact that when one epoxide ring has reacted the product has a glycidol-like structure (inset), and the second ring will react more readily, leading to a higher overall reaction rate.



$CH_2A\cdot CH(OH)\cdot \underbrace{CH\cdot CH_2}_{O}$

Conversely, electron-attracting groups should decrease the rate of acid-catalysed reactions by lowering the effective negative charge on the oxygen atom ; in fact, substituents should have an opposite effect on the two types of reaction. That this is so is clearly shown by the results of Brønsted *et al.* (*loc. cit.*), who found the following rate coefficients for ethylene oxide and epichlorohydrin at 20° :

	Ethylene oxide.	Epichlorohydrin.
k_1	$3{\cdot}6 \times 10^{-7}$	$9{\cdot}7 \times 10^{-7}$
k_2	$5{\cdot}33 \times 10^{-3}$	$0{\cdot}41 \times 10^{-3}$

The relative values for the rate of reaction with the thiosulphate ion (Table I) and the acid-catalysed addition of water (Table VI) for epichlorohydrin, di-(2 : 3-epoxypropyl) ether, glycidol, and 1 : 2-3 : 4-diepoxybutane on the one hand and propylene oxide and 1 : 2-5 : 6-diepoxyhexane on the other support these results. It is noteworthy that these workers found a very high value for k_2 (1·92 at 20°) for *cyclo*hexene, and the rate of reaction of this compound with the thiosulphate ion is now found to be of a low order. Another example of the decrease in the effectiveness of acid catalysts brought about by electron-attracting substituents is the work of Watson and Yates (*J.*, 1932, 1207) on the bromination of halogenated acetones.

The rate coefficients for the reaction of several typical epoxides, including three radiomimetic diepoxides, with water and with the acetate ion under conditions where the acid-catalysed reactions can be disregarded (see, however, below for a discussion of 1 : 2-5 : 6-diepoxyhexane) are given in Table II. Except in the case of 1 : 2-3 : 4-diepoxybutane the rate coefficients are constant throughout the course of the reaction, indicating that in the case of the two diepoxides the oxiran rings are reacting independently. In the case of the butane derivative the coefficient for the addition of water and also that for the reaction with anion decrease as the reaction proceeds. The slowing down of the rate of addition of water cannot be ascribed to the formation of a glycidol-like compound since glycidol reacts at the same rate as the initial value for the diepoxide, but it is interesting to note that the coefficient for the reaction of the butane compound with acetate ions gradually falls to the value for glycidol.

The rate of addition of water to 1 : 2-5 : 6-diepoxyhexane in 50% acetone is much less than in water alone, but there is not a proportional drop in the rate of reaction with the acetate ion, with the result that considerably more ester is formed when the reaction proceeds in aqueous acetone.

The value of the rate coefficient for the addition of water (k_1) with increasing salt concentration (Table III) is practically constant in the case of epichlorohydrin and 1 : 2-3 : 4-diepoxybutane, but rises in the case of glycidol and di-(2 : 3-epoxypropyl) ether. Brønsted *et al.* (*loc. cit.*) described this positive salt effect: our own experiments were only accurate enough to detect large changes. The values of the coefficient k_3 are tolerably constant—the decrease in most cases can probably be ascribed to incomplete ionisation of the sodium acetate at higher concentrations. The values for 1 : 2-5 : 6-diepoxyhexane are again abnormal, but these can be accounted for (see below).

The various coefficients determined in this study are collected in Table V. The rate coefficients for reaction with acetate and thiosulphate anions are in the same order for epichlorohydrin, 1 : 2-3 : 4-diepoxybutane, and di-(2 : 3-epoxypropyl) ether, but there is a divergence in the case of glycidol and 1 : 2-5 : 6-diepoxyhexane. Close correlation in the coefficients could hardly be expected since the values for acetate were determined in aqueous solution, and although an excess of acetic acid was always present, the buffering action of the acetate ions meant that the pH never fell below about 4·5; the thiosulphate values —k'(thio)— were determined in 50% acetone and the pH of the solution was probably lower, for although only small additions of acetic acid were made, the thiosulphate ion has no buffering action. The effect of this acidity would be expected to be most marked in the case of the more rapidly reacting oxides for with these it is necessary to follow the reaction by adding an excess of acetic acid and timing the reappearance of the phenolphthalein colour after each addition : the reaction is too rapid to follow by a continous titration as can be done with the less reactive compounds. Any appreciable change in pH can be prevented by the addition of sodium acetate to the thiosulphate solution—the rate coefficient for the reaction with the acetate ions alone was negligible [$k'(\mathrm{Ac}) < 0{\cdot}002$ in boiling 50% acetone]. Some values for the rate coefficients in buffered thiosulphate are given in Table V. It will be seen that the rate of reaction is always lowered to some extent if the pH is kept nearer neutrality, but the reactivity of glycidol towards thiosulphate is still high as compared with its reactivity to acetate ions.

The reactions of 1 : 2-5 : 6-diepoxyhexane in aqueous solution are known to be abnormal. Wood and Wiggins (*Nature*, 1949, **164**, 402) have shown that when the compound is boiled with water, 2 : 5-di(hydroxymethyl)furan is formed in 65% yield. This furan derivative has now been obtained in good yield by allowing the diepoxide to react in dilute hydrochloric acid. Another unusual feature is the low yield of ester when 1 : 2-5 : 6-diepoxyhexane reacts in 0·1M-sodium acetate solution. Only about 8% of the reacting oxide is converted into ester, whereas the yields of ester in other cases are : epichlorohydrin, 40% ; glycidol, 34% ; 1 : 2-3 : 4-diepoxybutane, 38% ; di-(2 : 3-epoxypropyl) ether, 33%. Despite this low yield of ester in dilute acetate solution, this diepoxide reacts quantitatively with 0·2M-thiosulphate.

There is an abnormally large increase in the value of k_1 (Table II) when reaction takes place in 0·1M-sodium acetate containing 0·1N-acetic acid—from 0·023 in water alone to 0·046 hr.$^{-1}$ in acetate–acetic acid solution. This increase is not due to a large salt effect, but has been traced to a high value for the rate coefficient of the acid-catalysed reaction (k_2) (Table VI).

As a consequence of the high value of k_2 for 1 : 2-5 : 6-diepoxyhexane, a small increase in acidity will cause a much larger increase in the rate of glycol formation than in the case of the other oxides studied, which have a lower value of k_2. In particular, at the pH of the sodium acetate–acetic acid mixture—found to be 4·6 by direct measurement with a glass electrode system—the coefficient for the rate of formation of glycol by acid catalysis, $k^{4{\cdot}6}$, will be $640 \times 10^{-4{\cdot}6}$ or 0·016. The overall rate coefficient for the production of glycol will be $k_1 + k^{4{\cdot}6}$ or 0·39; thus it can be seen that the increase in the rate of acid-catalysed addition of water accounts for most of the increase observed in Table II.

It was noted that in those cases where the amount of ester formed in dilute acetate solution was between 30 and 40%, and where the value of k_2 was low, the epoxide contained a system which tended to attract electrons away from the terminal carbon atom. In the case of 1 : 2-5 : 6-diepoxyhexane the mutual effect of the two epoxide groups is likely to be small since they are separated by two carbon atoms, in contrast with the system in the butane derivative (see above), and the system approximates to that found in propylene oxide where the tendency would be for electrons to be repelled towards the terminal carbon atom. In fact when the reactions of propylene oxide were studied they were found to have much in common with those of the hexane derivative. The value of k_1 for propylene oxide was 0·008, k_1 in 0·1M-acetate–0·1M-acetic acid was 0·024, k_3(Ac) was 0·040, k_2 was 445, and the values in Table I are of the same order. Only 16% of the epoxide reacting in 0·2M-sodium acetate solution was converted into ester.

Kadesch (*J. Amer. Chem. Soc.*, 1946, **68**, 41) and Swern, Billen, and Knight (*ibid.*, 1949, **71**, 1152) have discussed the direction of ring fission during the reactions of epoxides under conditions of acid and alkali catalysis. They conclude that, when the group R is electron-attracting, cleavage in the conjugate acid formed during the acid-catalysed reaction will occur almost exclusively as in (I), whereas if the group is electron-repelling, a considerable proportion of the ring fission will be as in (II) :

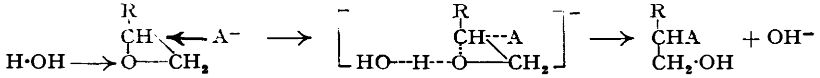
(I.) (Ia.) (II.) (IIa.)

The carbonium ions (I*a*) and (II*a*) can be regarded as intermediates in the acid-catalysed reactions. It was shown by Everett and Ross (*J.*, 1949, 1972) that, of the carbonium ions (III) and (IV) derived from halogenoalkylamines, the ion (III) reacted with anions to give about four

(III.) $R_2N{\cdot}CH_2{\cdot}CH_2^+$ $R_2N{\cdot}CH_2{\cdot}\overset{+}{C}H{\cdot}CH_3$ (IV.)

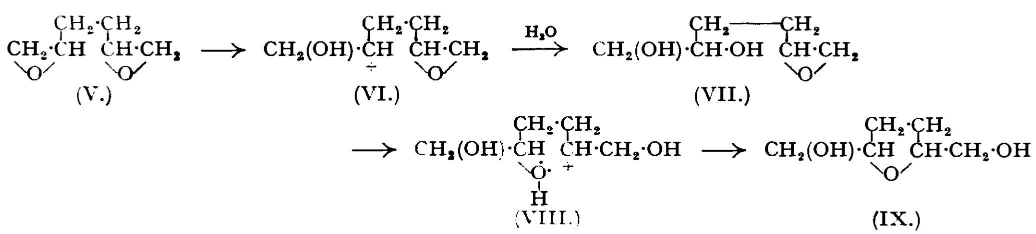
times the yield of ester afforded by the ion (IV). The yields of ester in the reaction of various epoxides with 0·1N-hydrochloric acid (under the conditions given in Table VI) are as follows : epichlorohydrin, 35·5% ; 1 : 2-3 : 4-diepoxybutane, 32% ; di-(2 : 3-epoxypropyl) ether, 27·6% ; glycidol, 25·4% ; propylene oxide, 17% ; 1 : 2-5 : 6-diepoxyhexane, 9%. Should the abilities of the ions (I*a*) and (II*a*) to react with anions be similar to those of (III) and (IV), respectively, these figures would indicate that very approximately one-half of the propylene oxide molecules undergo ring fission to give an ion of type (II*a*) whilst the remainder give (I*a*). This ratio of products is found in the reaction of propylene oxide with ethanol in the presence of 1·3% sulphuric acid (Chitwood and Freure, *J. Amer. Chem. Soc.*, 1946, **68**, 680; see also Reeve and Sadle, *ibid.*, 1950, **72**, 125).

The yields of ester when the various epoxides react in almost neutral solutions of acetate ions (p. 2265) are of the same order as for the acid-catalysed reaction above; this suggests that the initial step in the spontaneous reactions of epoxides where R is electron-repelling involves an attack by the anion on the non-terminal carbon atom, thus :



Chitwood and Freure (*loc. cit.*) have shown that when propylene oxide reacts with ethanol in the absence of a catalyst, ring fission still occurs in both directions, though a higher proportion opens as in (I). In the case of 1 : 2-epoxy-2-methylpropane, which has two electron-repelling methyl groups attached to the same carbon atom, the uncatalysed reaction with methanol and propan-1-ol gives equal yields of primary and secondary ethers, while the reaction with ethanol gives twice as much primary as secondary ether (Sparks and Nelson, *J. Amer. Chem. Soc.*, 1936, **58**, 671).

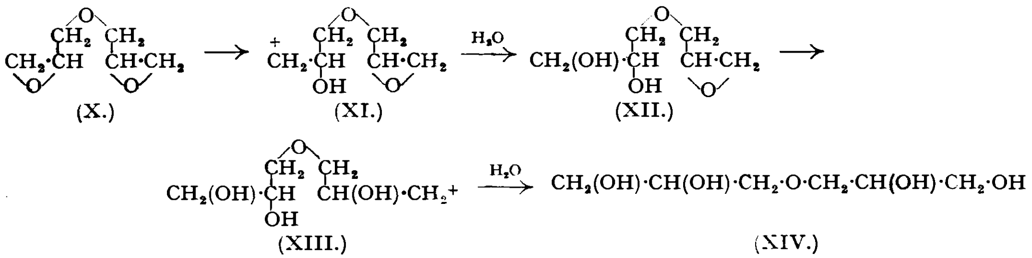
The formation of a furan derivative from 1 : 2-5 : 6-diepoxyhexane and the non-formation of a dioxan derivative from di-(2 : 3-epoxypropyl) ether in their reactions in aqueous solution can also be explained as follows : the first stage in the reaction of the hexane derivative (V) in acid solution will be the formation of the monoglycol (VII) by way of the ion (VI). The conjugate

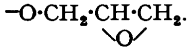


(V.) (VI.) (VII.) (VIII.) (IX.)

acid from (VII) will yield the ion (VIII) which can readily react internally to form the stable five-membered furan ring system (IX). By analogy with the reaction of propylene oxide, about 16% of the ion (VI) should react to form ester in 0·1M-acetate solution but the ion corresponding to (VIII) would probably have a greater tendency to react internally; this would account for the low overall yield of ester. If the ion (VIII) only reacts internally, the overall yield of ester will be about 8%—this is the amount actually formed. When the reaction is carried out in the presence of the highly competing thiosulphate ion, the reaction of the two carbonium ions (VI) and (VIII) with water or the hydroxyl group will be suppressed and no furan will be formed—the reaction with this anion is quantitative.

Di-(2 : 3-epoxypropyl) ether (X) will yield an ion of type (I*a*), that is (XI), which gives a monoglycol (XII). This will give an ion (XIII) which cannot react to give a dioxan derivative but affords the tetrol (XIV). Confirmation of the suggested direction of ring opening in the case of this ether is afforded by the high yield of ester in dilute acetate solution, comparable with that of epichlorohydrin and glycidol.


(X.) (XI.) (XII.) (XIII.) (XIV.)

The pure 1 : 2-5 : 6-diepoxyhexane (b. p. 187—188°) used in this work was prepared by Professor G. A. R. Kon and Mr. J. L. Everett by the oxidation of diallyl with perbenzoic acid (compare Wood and Wiggins, *loc. cit.*). The material obtained by Przybytek (*Ber.*, 1885, **18**, 1352) from the corresponding dichlorohydrin doubtless consists mainly of this diepoxide, although its b. p. is somewhat lower (179—180°). The product of b. p. 153° obtained by the action of sodium on epichlorohydrin (Bigot, *Ann. Chim. Phys.*, 1891, **22**, 433) has also been described as 1 : 2-5 : 6-diepoxyhexane. When this compound (kindly supplied by Professor J. B. Speakman) was examined for reactivity towards the thiosulphate ion under the standard conditions of Table I, it was found to contain only one epoxide group—as might have been expected since it only reacts with one molecule of water and one molecule of hydrogen halide (Bigot, *loc. cit.*)—and this oxiran ring had a higher reactivity towards thiosulphate ions than those of the genuine compound. The value of *k'*(thio) was close to that of compounds containing the system  $-O{\cdot}CH_2{\cdot}CH{\cdot}CH_2$.

It has been suggested above that in the reaction with anions the opening of the oxiran ring in the case of compounds in which R is electron-attracting occurs exclusively in a direction resulting in the formation of an ester of a primary alcohol. That this is true for 1 : 2-3 : 4-diepoxybutane has been verified as follows: the diepoxide was allowed to react with a concentrated solution of sodium benzoate to which benzoic acid was added as the solution became alkaline to phenolphthalein. The product was resolved by fractional crystallisation into a dibenzoate, m. p. 148°, and a residue of lower melting point which had the same composition. The higher-melting ester was shown to be identical with *meso*-erythritol 1 : 4-dibenzoate (XVI) (Ohle and Melkonian, *Ber.*, 1941, **74**, 292) by conversion into the known tetrabenzoate and by oxidation with lead tetra-acetate to the benzoate of glycollaldehyde—identified as its 2 : 4-dinitrophenylhydrazone.

$$CH_2(OBz){\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_2{\cdot}OBz \quad \text{(XVI.)}$$

When the low-melting residue was similarly oxidised, practically the same yield of 2 : 4-dinitrophenylhydrazone was obtained; this indicates that the reaction of the dioxide yields almost exclusively a product with the benzoate groups on the terminal carbon atoms—the mixture must contain D-, L-, and *meso*-erythritol 1 : 4-dibenzoates.

The direction of ring opening in the reaction of propylene oxide with benzoate ions has also been examined. The product obtained was treated with toluene-*p*-sulphonyl chloride, giving a mixture similar to that obtained by Chapman and Owen (*J.*, 1950, 579) by benzoylation of the product of reaction between propylene oxide and toluene-*p*-sulphonic acid. Fractional crystallisation of this mixture gave 2-toluene-*p*-sulphonyloxypropyl 1-benzoate—also obtained after treating the mixture with potassium thiolacetate (Chapman and Owen, *loc. cit.*)—and 2-benzoyloxypropyl 1-toluene-*p*-sulphonate.* Thus, contrary to the statement of Fraenkel-Conrat and Olcott (*J. Amer. Chem. Soc.*, 1944, **66**, 1420), when propylene oxide reacts with anions ring fission occurs with the formation of both isomeric hydroxy-esters. An attempt to estimate the relative proportions of the two esters formed in the reaction with sodium iodide

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* The author is indebted to Dr. L. N. Owen and Mr. G. A. Haggis for advice on the separation of these esters and for a specimen of 2-benzoyloxypropyl 1-toluene-*p*-sulphonate (Haggis and Owen, *J.*, 1950, 2250).

in acetone at 100° (Oldham and Rutherford, *J. Amer. Chem. Soc.*, 1932, **54**, 366) was unsuccessful since both isomers reacted quantitatively under these conditions.

Table IV shows the extent of the reaction of the physiologically active 1 : 2-3 : 4-diepoxybutane with various anions under comparable conditions. The figures given in the final column represent the percentage of the reacting epoxide which has been converted into ester. This method of expressing the results has been adopted because the amount of epoxide reacting will depend on the pH of the solution and the different organic anions will buffer the solution to different extents, whilst the inorganic anions will have little buffering action. Since Brønsted *et al.* (*loc. cit.*) have shown that the acid-catalysed reactions with water and with anions are increased proportionally with increasing acidity, the fraction of the reacting epoxide which is converted into ester is a measure of the ability of the particular anion to compete with water for reaction with the epoxide. The order of reactivity of the anions towards the oxiran ring is similar to that found for their reactivity towards the carbonium ions derived from halogeno-alkylamines (Ross, *J.*, 1949, 2589). The nitrate ion competes only feebly, and the formate ion is again the least reactive of the organic anions. The high reactivities of the thiosulphate and iodide ions is also common to both types of compound.

If no acid is present (11—14, Table IV) the solution soon becomes alkaline owing to reaction (3) (p. 2258); under these conditions considerably less ester is formed in the presence of acetate, benzoate, and chloride ions and appreciably less in the case of the thiosulphate ion. Although less ester is formed in the reaction with acetate and benzoate ions in the absence of added acid, the total amount of epoxide reacting is practically the same (compare 5 and 11, and 6 and 12, Table IV). The increase in alkalinity in the case of the reaction with the acetate ion results in the solution becoming 0·00125N. with respect to hydroxyl ions. That this increase in pH—to 11·7—is not responsible for the increased amount of epoxide reacting is shown in the table (compare 15 and 18). Since the amount of epoxide reacting is dependent on the anion concentration, although little ester is formed, and as it has been shown that there is no significant increase in the rate of reaction with water with increasing anion concentration (Table III), it appears that the reaction proceeds by the initial formation of an ester which is subsequently hydrolysed.

In the reactions with chloride and thiosulphate ions less epoxide is transformed than when acid is present, though more reacts than in the absence of the anion. It seems likely that esters are again the primary products, but this time the increase in pH probably causes a reversal of the reaction and an equilibrium state is reached. The conversion of 1-chloro-2-hydroxypropane into propylene oxide in 76—84% yield by treatment with N/40-sodium hydroxide at 37° shows that the reaction is reversible under the mild conditions used to obtain the results given in Table IV.

When the concentration of hydroxyl ions is increased to 0·10M. there is a considerable increase in the amount of epoxide reacting, and when the concentration is 0·5M.—comparable to the concentration of the other anions in Table IV—all the epoxide is transformed. Thus, contrary to the statement of Brønsted *et al.* (*loc. cit.*) which is discussed by Hammett ("Physical Organic Chemistry," New York, 1940, p. 301), it appears that the hydroxyl ion does react with an epoxide ring system, the rate of reaction being faster than with chloride, acetate, and nitrate ions. This increase in the rate of conversion of epoxides into glycols in the presence of 0·1M-hydroxyl ions is quite general (see Table VII). The reaction of epichlorohydrin with hydroxyl ions could not be studied because the chlorine atom is rapidly hydrolysed in 0·1N-sodium hydroxide. Ogston (*loc. cit.*) also found that hydroxyl ions were able to react more readily than chloride and acetate ions with the carbonium ions derived from di(chloroethyl) sulphide.

TABLE VII.

Time of reaction, 24 hours. Temp., 37°. Concn. of monoepoxide, 0·1M.; of diepoxide, 0·05M.

Compound.	% reacting in water.	% reacting in 0·1N-NaOH.
Ethylene oxide	18·4	94·5
Propylene oxide	16·3	92·5
Glycidol	15·6	88
1 : 2-3 : 4-Diepoxybutane	15·5	85
Di-(2 : 3-epoxypropyl) ether	15·5	98

A first-order rate function being assumed with respect to each reactant the unimolecular constant for the reaction of an epoxide in mixed aqueous solution can be represented as

$$k = k_1 + k_2[\mathrm{H}^+] + k_{\mathrm{OH}}[\mathrm{OH}^-] + k_3[\mathrm{A}^-]$$

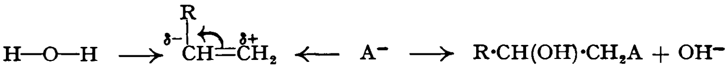
This is of the same form as the equation for an acid–base-catalysed reaction. Approximate values for the various constants on the right-hand side of this equation can be deduced for several epoxides from the figures in Tables V, VI, and VII; they are collected together in Table VIII.

TABLE VIII.

Rate coefficients for the reactions of epoxides in aqueous solution.

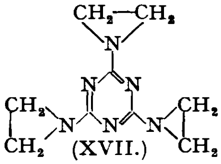
Compound.	$k_1 \times 10^3$.	k_2.	k_{OH}.	k_3(Ac) $\times 10^3$.
Glycidol	6·9	45	0·82	3·6
Propylene oxide	8	445	0·99	5
Epichlorohydrin	18·5	7	—	12
Di-(2 : 3-epoxypropyl) ether	6·8	23	*ca.* 1·6	3·3

Unsaturated compounds containing an electron-attracting group adjacent to the ethylenic linkage would also be expected to react with nucleophilic reagents owing to the polarisation induced by the displacement of the π-electrons. When divinyl sulphone, acraldehyde, and acrylonitrile are heated with aqueous acetone solutions of thiosulphate under the conditions described in Table I, alkalinity is developed at a measurable rate. The reaction of divinyl sulphone with thiosulphate was previously observed by Stahman, Golumbic, Stein, and Fruton (*J. Org. Chem.*, 1946, **11**, 719). This reaction can also be regarded as proceeding by the Lowry mechanism, the attack on the polarised double bond being as follows :



$$H{-}O{-}H \longrightarrow \overset{\delta-}{\underset{|}{\overset{R}{}}}CH{=}\overset{\delta+}{C}H_2 \longleftarrow A^- \longrightarrow R{\cdot}CH(OH){\cdot}CH_2A + OH^-$$

The rate of reaction of divinyl sulphone is comparable with that of the biologically active diepoxides and, although it has proved to be too toxic to be of value as a tumour-growth inhibitor, it does produce chromosome abnormalities in the growing root tips of *Vicia faba* similar to those obtained with other difunctional electrophilic reagents (personal communication from Mr. A. Loveless).



(XVII.)

Another type of compound examined which reacts in a manner very like that of the diepoxides is the 2 : 4 : 6-triethyleneimino-derivative of 1 : 3 : 5-triazine (XVII). Like several diepoxides, this substance has been used as a cross-linking agent in the textile industry (Preston, "Fibre Science," Manchester, 1949, p. 266). Since it reacts with wool fibres under quite mild conditions—the treatment involves steeping the material for a short time in a 5—10% solution at 60°—the compound was of interest as a potential radiomimetic agent. The rate of reaction of the triazine with water at 37° was of the same order as the effective diepoxides (k_1 for a 24-hour period being 0·023 hr.$^{-1}$) and the rate of reaction with thiosulphate was also comparable (Table I).

The yield of ester in acetate solutions under the conditions described in Table II was 26% (compare p. 2264) and the yield of chloride in the reaction with 0·1N-hydrochloric acid was 42%—slightly higher than for most epoxides. The only significant respect in which the triazine differs from the epoxides is in the very high value of the coefficient for the rate of the acid-catalysed reactions (Table VI). In view of these close analogies, it was hardly surprising to find that triethyleneiminotriazine was effective as a tumour-growth inhibitor and in the production of chromosomal derangements (unpublished work by Professor A. Haddow and Mr. A. Loveless).

Table I shows the results of the biological testing of some of the compounds now examined for chemical reactivity. An outstanding feature is the finding that no monofunctional epoxide is effective as a tumour-growth inhibitor. In general, the toxicity of the compounds is proportional to the rate at which the oxiran ring will react with an anion except that a difunctional compound is more toxic than a monofunctional compound of comparable chemical reactivity [compare epichlorohydrin and glycidol with 1 : 2-3 : 4-diepoxybutane and di-(2 : 3-epoxypropyl) ether; and also propylene oxide with 1 : 2-5 : 6-diepoxyhexane]. This is paralleled in the mustard-gas types of compound; for example, ethyl 2-chloroethyl sulphide and dimethyl-2-chloroethylamine are much less toxic than di-2-chloroethyl sulphide and methyldi-2-chloroethylamine respectively. Even amongst the diepoxides it would appear that a certain minimum reactivity—as measured by the rate of reaction with the thiosulphate ion—must be exceeded before the compound is growth-inhibitory, though this level of reactivity is not, in itself, sufficient for activity. The methiodide of *NN*-di-(2 : 3-epoxypropyl)-*p*-anisidine is not active, nor is the ester of di-(2 : 3-epoxypropyl)acetic acid—the latter is probably hydrolysed in the tissues to give a carboxylate anion in which the epoxide groups would be less reactive on account of the negative charge. These two exceptions yield ions, and since these compounds probably have to diffuse into the cell to exert their effect, they may be inactive because of an inability to penetrate the cell membrane (compare Seligman, Friedman, and Rutenburg, *Cancer*, 1950, **3**, 342). Grove and MacGowen (*Chem. and Ind.*, 1949, 647) give an account of a series of fungicides in which ions are inactive because of an inability to penetrate cells. The inactivity of the epoxypropyl derivatives of quinol and resorcinol is unexpected, but since these compounds have a much lower water and lipoid solubility than the other simple diepoxides, it may be that a sufficient concentration of the agents cannot be attained at the required site of action. The high-melting isomer of *p*-di-(2 : 3-epoxypropoxy)benzene only exerts a transient effect upon the growth of the transplanted Walker tumour; it is not as active as the simpler diepoxides.

The substitution of both carbon atoms of the oxiran rings reduces the reactivity below that required and hence it would not seem worth investigating other compounds of this type. There are, however, indications that, although two reactive centres are required in the molecule, these centres need not be equally reactive; for example, the effective 1 : 2-3 : 4-diepoxy-2-methylbutane must contain centres of unequal reactivity. Another example is *N*-2-chloroethyl-*N*-3-chloropropylaniline (Kon and Roberts, *J.*, 1950, 978) which is a growth-inhibitor though the corresponding di-(3-chloropropyl) derivative is ineffective. These results suggest that only one group of high chemical reactivity is required—this would anchor the molecule to a receptor and the second group would then have more time to combine with another receptor (compare Goldacre, Loveless, and Ross, *Nature*, 1949, **163**, 667).

The high rate coefficient for the acid-catalysed reactions of certain diepoxides, such as 1 : 2-5 : 6-diepoxyhexane is of considerable interest, for it explains why this compound is ineffective when administered by mouth. It can be calculated that, at the pH of the stomach, over 90% of this oxide is decomposed within two minutes. It would be expected that this type of compound would react more readily with nucleophilic centres (see p. 2268) in regions where the acidity is relatively higher; this might be significant from the standpoint of selective action towards different tissues.

Fraenkel-Conrat (*J. Biol. Chem.*, 1944, **154**, 227) has shown, and the present study confirms, that epoxides react readily with ionised acid groups, but not with undissociated acids, and it is also known that they react with free amino-groups. These characteristics are to be expected if the epoxides react through a carbonium-ion mechanism. An important difference between the epoxides and the mustard-gas type of compound is that, whereas the latter react by an S_N1 mechanism and the extent of reaction is largely independent of the concentration of reacting centres, the former react by a bimolecular mechanism and the extent of reaction will depend on the availability of reacting centres. This is probably of some importance in the reactions of epoxides with biological systems where the concentration of reacting groups will vary from site to site. Nucleic acids contain a high proportion of ionised phosphate groups and so reaction with epoxides would be specially favoured.

It has been pointed out (Ross, *J.*, 1950, 815) that agents reacting by a carbonium-ion mechanism will be capable of reacting with free amino-groups and with ionised thiol groups as well as with anions. Since compounds, such as di-acid chlorides, di*iso*cyanates, and di-iodoacetyl derivatives of diamines * and of diphenols, which would be expected to react with amino- and thiol groups but not with acidic groups, do not inhibit the growth of the Walker tumour (unpublished work by Professor A. Haddow), nor do they produce chromosomal abnormalities in the growing root tip of *Vicia faba* (Mr. A. Loveless, in the press), one is led to the conclusion that the various radiomimetic compounds owe their especial properties to an ability to react with ionised acidic groupings under mild conditions.

EXPERIMENTAL.

Determination of Epoxides with Thiosulphate.—When an epoxide is heated in a solution of sodium thiosulphate in 50% acetone, the solution rapidly becomes alkaline. It has been shown that the reaction producing hydroxyl ions (reaction 3, p. 2258) is quantitative, provided that the solution is kept almost neutral by titration with a weak acid. Satisfactory results were obtained with the more rapidly reacting epoxides if acetic acid was added before the heating and the excess was titrated after reaction was

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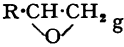
* The production of chromosome aberrations in the epithelial cells of the newt, *Triturus palmatus*, by the action of *NN*-di(iodoacetyl)-*o*-phenylenediamine reported previously (*J.*, 1949, 1972) has not been confirmed by later work—the original results remain unexplained (private communication, Mr. A. Loveless).

complete, but this method was unsuitable for the less reactive compounds since the more prolonged heating resulted in the decomposition of the unstable thiosulphuric acid with deposition of sulphur. This decomposition does not occur at 37° in completely aqueous solution. The method finally adopted for the determination of epoxides was as follows: the monoepoxide (1 millimol.) or the diepoxide (0·5 millimol.) was added to a neutralised 0·2M-solution of sodium thiosulphate in 50% acetone containing phenolphthalein, and the mixture was heated in a flask carrying a reflux condenser and a stopper holding a 10-ml. burette. As the solution was heated, the pink colour which developed was continuously discharged by adding 0·2N-acetic acid from the burette. When no further alkalinity was produced—generally after 10—20 minutes—the solution was cooled and any excess of acetic acid titrated with 0·1N-sodium hydroxide (1 ml. of 0·1N-acid = 0·1 milliequiv. of oxiran oxygen). All epoxides of structure  $R{\cdot}CH{\cdot}CH_2$ (with O bridging) gave a quantitative liberation of hydroxyl ions under these conditions.

Determination of k′ (*thio*) *for Various Epoxides* (Table I).—The conditions were exactly the same as for the determination of epoxides except that the thiosulphate solution was heated to boiling before the epoxide was added in a small sample tube. The solution was continuously titrated with 0·2N-acetic acid and the burette readings were recorded at regular intervals. With the more rapidly reacting epoxides it was found necessary to add a known small amount of acid and then to time the return of the pink colour and so on. A typical experiment was that with 1 : 2-3 : 4-diepoxybutane:

Time, secs.	14	22	30	39	52	67	76	87
% epoxide reacted...	17·9	26·8	35·7	44·6	53·6	62·5	67	71·5
100 k' (thio), min.$^{-1}$	85	85	87	89	87	85	87	87

Time, secs.	104	117	135	160	205	236	∞
% epoxide reacted...	76	80·4	85	89·5	94	96	100
100 k' (thio), min.$^{-1}$	85	83	85	85	83	83	—

Average value: k' (thio) $= 85 \times 10^{-2}$ min.$^{-1}$.

Reaction of Epoxides in Water and Aqueous Acetate Solutions (Tables II and III).—The monoepoxide (10 millimols.) or diepoxide (5 millimols.) was dissolved in (*a*) water (100 ml.) or (*b*) 0·1N-acetic acid (100 ml.) containing sodium acetate (1·36 g.) and kept at 37°. Immediately after the mixing, an aliquot (10 ml.) was removed and in (*a*) after addition of an equal volume of acetone and then sodium thiosulphate—to give a 0·2M-solution—the sample was heated and the epoxide content determined as described above; in (*b*) the solution was first titrated with 0·1N-sodium hydroxide, and then the epoxide content was determined as before. The addition of acetone is not essential but its presence prevents the stem of the burette from emptying and the addition of acid is much easier. At suitable intervals similar aliquots of the solution were removed and titrated for epoxide content and acidity. The percentage of ester formed was calculated from the amount of acid neutralised.

The figures given in Table III were obtained in a similar manner by varying the amount of sodium acetate in the solution.

Reaction of 1 : 2-3 : 4-*Diepoxybutane with Different Anions* (Table IV).—1 : 2-3 : 4-Diepoxybutane (86 mg., 1 millimol.) was dissolved in a solution of the appropriate acid (40 ml., 0·05N.) containing the sodium salt (sufficient to give the concentration shown in the table). After the solution had been kept at 37° for 24 hours, the acidity and epoxide content were determined and the amounts of ester formed and epoxide reacting were calculated in the usual manner.

Determination of the Rate Coefficient for the Acid-catalysed Addition of Water (k_2) (Table VI).—The rates of decomposition of an epoxide in water and in 0·05N-acetic acid were determined as above, but for the reaction in 0·1N-hydrochloric acid it was necessary to stop the reaction by rapidly adding sodium hydroxide equivalent to the amount of acid originally present. The excess of alkali was then titrated and the amount of ester formed was calculated; the epoxide remaining was determined on the same aliquot of solution.

Conversion of 1 : 2-5 : 6-*Diepoxyhexane into* 2 : 5-*Di*(*hydroxymethyl*)*furan*.—A solution of 1 : 2-5 : 6-diepoxyhexane (2 g.) in 0·1N-hydrochloric acid (50 ml.) was slowly evaporated on a steam-bath. The last traces of water were removed by repeated evaporation with methanol and finally with benzene. The residue was dissolved in pyridine (5 ml.) and, after cooling to 0°, toluene-*p*-sulphonyl chloride (4 g.) was gradually added. Next day the mixture was poured on ice and left for 2 hours. The solid product crystallised from methanol in the form of flattened needles, m. p. 114—117°, not depressed by admixture with material of m. p. 116—118° obtained by the reaction with water alone. Wood and Wiggins (*loc. cit.*) obtained a mixture of di-toluene-*p*-sulphonates of m. p. 114—118° by the action of water.

Methiodide of NN-*Di*-(2 : 3-*epoxypropyl*)-p-*anisidine*.—The anisidine (5 g.), dissolved in methyl iodide (10 ml.), was kept at 37° for 24 hours. The sticky solid obtained after rubbing the product with benzene was collected and digested with hot acetone. The resultant colourless solid was crystallised by dissolving it in a small quantity of hot methanol, adding hot acetone, and then cooling slowly. In this way thick plates of the *methiodide*, m. p. 138—139°, were obtained (Found: C, 46·1; H, 5·2; oxiran-O by thiosulphate method, 8·6; I′, 34·9. $C_{14}H_{20}O_3NI$ requires C, 46·3; H, 5·6; oxiran-O, 8·8; I′, 35·0%). The methiodide is soluble in water (20 mg. per ml. at 37°).

Conversion of 1-*Chloropropan*-2-*ol into Propylene Oxide*.—(*a*) The chlorohydrin (131 mg.) was dissolved in 0·025N-sodium hydroxide (40 ml.) and kept at 37° for 17 hours. All the alkali had then been neutralised, showing that 72% of the chlorohydrin had reacted. Titration of the solution by the thiosulphate method indicated the presence of propylene oxide (49 mg.); thus 84% of the chlorohydrin which had reacted was converted into epoxide. (*b*) The chlorohydrin (94·5 mg., 1 millimol.) was similarly heated with 0·025N-sodium hydroxide (40 ml., 1 millimol.) for 24 hours. All the alkali was neutralised and the solution contained propylene oxide (44 mg.); thus, the whole of the chlorohydrin had reacted and 76% was converted into epoxide.

Reaction of Epoxides in Dilute Sodium Hydroxide Solution.—The monoepoxide (2 millimols.) or diepoxide (1 millimol.) was dissolved in 0·1N-sodium hydroxide (20 ml.) and kept at 37° for 24 hours. After titration of the alkali with 0·2N-acetic acid the epoxide content of the solution was determined in the usual manner. No consumption of alkali was observed in any experiment.

Reaction of 1 : 2-3 : 4-*Diepoxybutane with Benzoate Ions.*—1 : 2-3 : 4-Diepoxybutane (8·6 g.) was dissolved in a solution of sodium benzoate (50 ml., 2M.) containing phenolphthalein. Solid benzoic acid was added to the heated solution at such a rate that the pink colour was continuously discharged. When 20 g. of acid had been added, no further alkalinity developed and the cooled solution was poured into dilute aqueous ammonia. The oil which separated was dried and shaken with benzene, and the solid (6 g.) thus obtained was crystallised from benzene and finally from methanol, giving the dibenzoate as prisms (1·5 g.), m. p. 145—146° (Found: C, 65·6; H, 5·3. Calc. for $C_{18}H_{18}O_6$: C, 65·4; H, 5·5%). Ohle and Melkonian (*loc. cit.*) give m. p. 148° for the 1 : 4-dibenzoate of *meso*-erythritol. Benzoylation of this dibenzoate with an excess of benzoyl chloride in pyridine afforded the tetrabenzoate, needles (from benzene), m. p. 187°; Ohle and Melkonian give m. p. 188—188·5° for *meso*-erythritol tetrabenzoate. The mother-liquors from the original benzene solution were evaporated to dryness, giving a solid (6 g.), m. p. 124—136° (Found: C, 65·7; H, 6·0%).

Oxidation of the Dibenzoate with Lead Tetra-acetate.—The dibenzoate (1 g.) [(i), m. p. 145—146° or (ii), m. p. 124—136°] and lead tetra-acetate (1·35 g.), dissolved in dry benzene (50 ml.), were heated under reflux for 2 hours. The filtered solution was then washed with sodium hydrogen carbonate solution (50 ml.; 2N.) and water, and finally dried (Na_2SO_4) and evaporated. The residue was dissolved in a saturated solution of 2 : 4-dinitrophenylhydrazine in alcohol (70 ml.) and acetic acid (10 ml.). Next day the precipitated dinitrophenylhydrazone was collected: (i) yielded 300 mg. and (ii) 280 mg.—both products melting at 184—185° (Ohle and Melkonian give m. p. 185°). It is realised that the yield is not quantitative but since the mixture (ii) gives the same yield under identical conditions as the purified dibenzoate (i) of known structure it is reasonable to conclude that (ii) contains mainly 1 : 4-dibenzoate.

Reaction of Propylene Oxide with Benzoate Ions.—Propylene oxide was allowed to react in sodium benzoate solution exactly as described above. A solution of the oily product (28 g.) in dry pyridine (100 ml.) was cooled to 0° and treated with toluene-*p*-sulphonyl chloride (40 g.), the temperature being kept below 5°. After 24 hours at room temperature the mixture was poured on ice and the solid product was dried and repeatedly crystallised from light petroleum (b. p. 60—80°). The less soluble fraction—also obtained after treating the mixture with potassium thiolacetate (Chapman and Owen, *loc. cit.*)—formed flattened needles, m. p. 105° (Found: C, 61·1; H, 5·7. Calc. for $C_{17}H_{18}O_5S$: C, 61·1; H, 5·4%), and was identical with the 2-toluene-*p*-sulphonyloxypropyl 1-benzoate prepared by Chapman and Owen. The more soluble fraction, m. p. 77—80°, was extracted several times with ether, and the residue was repeatedly crystallised from light petroleum (b. p. 60—80°), affording fine needles, m. p. 91—92° undepressed by admixture with an authentic specimen of 2-benzoyloxypropyl 1-toluene-*p*-sulphonate, m. p. 93°, kindly supplied by Dr. L. N. Owen and Mr. G. A. Haggis (Found: C, 61·0; H, 5·2%).

Action of Sodium Iodide on the Isomeric Toluene-p-*sulphonate–Benzoates of Propane*-1 : 2-*diol.*—The ester (2 g.) was heated in a sealed tube at 95—100° for 2 hours with a solution of sodium iodide (2 g.) in acetone (20 ml.). During this time plates of sodium toluene-*p*-sulphonate separated from the solution. The salt was collected, washed with a little acetone, and dried. Yield: from ester, m. p. 105°, 1·17 g.; from ester, m. p. 92°, 1·18 g.; theory, 1·16 g.

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