The formation of epoxides from cytotoxic polyol methanesulphonates under physiological conditions

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In connection with the design of agents that might be toxic towards those neoplastic cells which are at a lower pH than normal cells, the effect of hydrogen ion concentration on the reactivity of various cytotoxic methanesulphonates has been studied. The rate of hydrolysis of ethyl methanesulphonate (Table 1) is not significantly affected by changes in pH in the range 5–9 whereas that of 1,6-dimethanesulphonyl-D-mannitol is considerably increased as the pH of the solution is raised from 7·5 to 9·5 (Table 2).

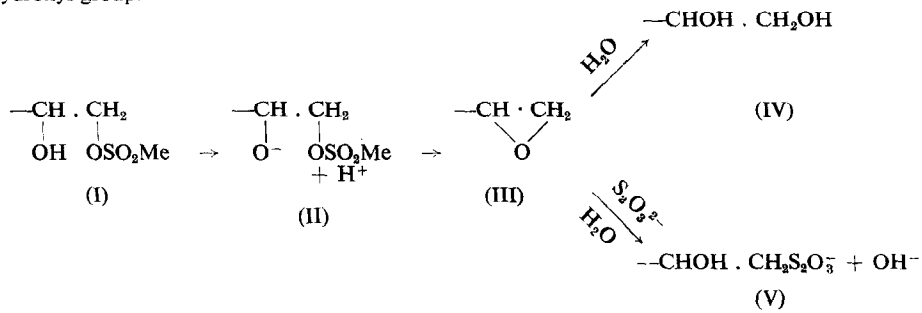
Table 1. Rate coefficient for hydrolysis of ethyl methanesulphonate at 37°

pH	Rate coefficient $\times 10^{-7}$ sec^{-1}
5	162
6	162
7	186
8	179
9	184

Table 2. Rate coefficient for hydrolysis of 1,6-dimethanesulphonyl-D-mannitol at 60°

pH	Rate coefficient $\times 10^{-5}$ sec^{-1}
7·5	41
8	101
8·5	225
9	887
9·25	1295
9·5	2210

A feature of the reaction of β-hydroxymethanesulphonates (I) is the possibility of epoxide (III) formation by an internal displacement of the methanesulphonate group by the anion (II) of the vicinal hydroxyl group.



This displacement reaction will be enhanced at higher pH since this condition increases the proportion of hydroxyl groups in the anionic form. If the rate of formation of epoxides at a given pH and temperature exceeds the rate of hydrolysis to the glycol (IV) then buffered solutions of polyol methanesulphonates should contain appreciable amounts of these epoxides.

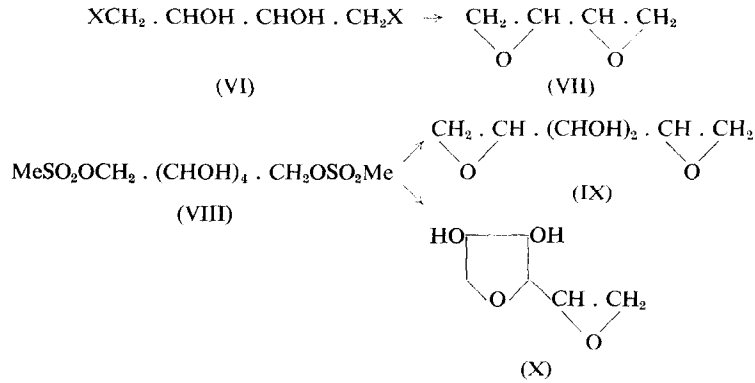
To obtain information about the extent of formation of epoxides under physiological conditions 1,6-dimethanesulphonyl-D-mannitol and the related 1,4-dimethanesulphonyl-L-threitol and 1,4-dibromo-1,4-dideoxy-DL-threitol were kept in aqueous solution, the pH of which was maintained at 7·5 by the addition of aqueous sodium hydroxide. After reaction, as measured by the amount of alkali required to maintain the pH, had proceeded to about 30 per cent thiosulphate was added and

the alkalinity produced in the reaction leading to the formation of the Bunte salt (V) was titrated with standard acid. The amount of acid required is a measure of the epoxide formed.[1] In the case of the two threitol derivatives practically all the reaction resulted in epoxide formation whereas the mannitol derivative yielded about 70 per cent of epoxide (Table 3).

TABLE 3. EXTENT OF EPOXIDE FORMATION FROM POLYOL DERIVATIVES IN AQUEOUS SOLUTION AT pH 7·5, 37°

Compound	% conversion to epoxide
1,4-Dimethanesulphonyl-L-threitol	100
1,4-Dibromo-1,4-dideoxy-DL-threitol	98
1,6-Dimethanesulphonyl-D-mannitol	73, 66

Thus, if one makes the reasonable assumption that the terminal groups react independently, under physiological conditions 1,4-dimethanesulphonyl-L-threitol (VI, X = $MeSO_2O$) and 1,4-dibromo-1,4-dideoxy-DL-threitol (VI, X = Br) are converted almost quantitatively into butadiene diepoxide (VII) whilst at least 40 per cent of 1,6-dimethanesulphonyl-D-mannitol (VIII) is converted into 1,2;5,6-diepoxy-3,4-dihydroxyhexane (IX). The figure of 40 per cent is deduced from the fact that the formation of an epoxyethylfurane, such as (X), could account for 50 per cent conversion to epoxide but a conversion of 70 per cent must imply diepoxide formation to the extent of 2 × (70–50) or 40 per cent.



It is therefore probable that diepoxides would be formed *in vivo* after administration of the polyol methanesulphonates (VI, X = $MeSO_2O$) and (VIII) and it may be that the cytotoxic effects of these polyol derivatives is due to the derived epoxides.[2] Some support for this view comes from the observation of certain cytotoxic effects in rats. The interval between the administration of a toxic dose and the ensuing death and the relative effects on peripheral blood components produced by the polyol methanesulphonates more closely resemble those produced by various cytotoxic epoxides than by the simpler methanesulphonates, e.g. myleran.[3] The possibility that β-hydroxymethanesulphonates produce their biological effects by way of epoxide intermediates must therefore be borne in mind although direct reaction with a powerful nucleophile, such as an ionised thiol group, cannot be excluded.

EXPERIMENTAL

Determination of rate coefficients for hydrolysis of methanesulphonates

Hydrolyses were carried out at constant pH using a "Radiometer" automatic recording titrator. Since the pH was constant throughout the hydrolysis the reaction should exhibit first order kinetics, that is, a plot of log $a/a - x$ against t (where the symbols have their usual significance) should give a straight line. In practice the plot was linear for the first 50 per cent of reaction and the rate coefficients were calculated from the slopes so obtained. The total amount of acidity developed did not reach the theoretical values and the coefficients were calculated from the observed values. The difference may be due to the partial formation of more stable chlorohydrins.

In a typical run 4 mg 1,6-dimethanesulphonyl-D-mannitol were introduced into the thermostatted vessel and dissolved in 10 ml 0·2 M aqueous KCl and the apparatus was set to maintain the required pH by the continuous addition of 0·1 N NaOH. The use of 0·2 M KCl was necessary to provide a solution of sufficient ionic strength to ensure accurate pH control by the instrument.

Percentage conversion of polyol derivatives into epoxides

A typical run was carried out as follows: 430 mg 1,6-dimethanesulphonyl-D-mannitol were dissolved in 10 ml water and kept at 37°. 9·5 ml 0·1 N NaOH were required to keep the pH at 7·5 during a 130 min period. After this time the addition of about 5 g sodium thiosulphate caused a rapid development of alkalinity and this was titrated with 0·1 N HCl keeping the pH at 7·5. In 10 min 7·0 ml acid had been added and no further alkalinity was being developed. Thus the extent of hydrolysis was 37·4 per cent and of epoxide formation was 27·6 per cent, that is, 73 per cent of the reaction led to epoxide formation.

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Inhibition of glutamate decarboxylase by salicylate congeners

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SALICYLATE has been reported to inhibit *E. coli* glutamate decarboxylase activity *in vitro*. The present report shows that this inhibitory action is shared by a number of related compounds. The estimation of the enzyme activity and the effects of the salicylate congeners were studied by the techniques described previously[1] and the results are given in Table 1.

TABLE 1. INHIBITION OF GLUTAMATE DECARBOXYLASE ACTIVITY BY SALICYLATE CONGENERS AT A CONCENTRATION OF 20 mM

(The results, which are expressed as percentage inhibitions, represent the means of six different observations Analysis of the results by the t-test showed that P wa less than 0·05 in each case).

Congener	% Inhibition
Salicylic[1] (2-Hydroxybenzoic)	22
Benzoic	14
Hexahydrobenzoic	40
3-Hydroxybenzoic	11
4-Hydroxybenzoic	20
3-Hydroxysalicylic	11
3-Methylsalicylic	40
3-*Cyclo*hexylsalicylic (sat. soln.)	98
4-Hydroxysalicylic	16
5-Hydroxysalicylic	7
5-Nitrosalicylic	61
6-Hydroxysalicylic	42