Studies on Epoxide Formation from (2S,3S)-Threitol 1,4-Bismethanesulfonate. The Preparation and Biological Activity of (2S,3S)-1,2-Epoxy-3,4-butanediol 4-Methanesulfonate

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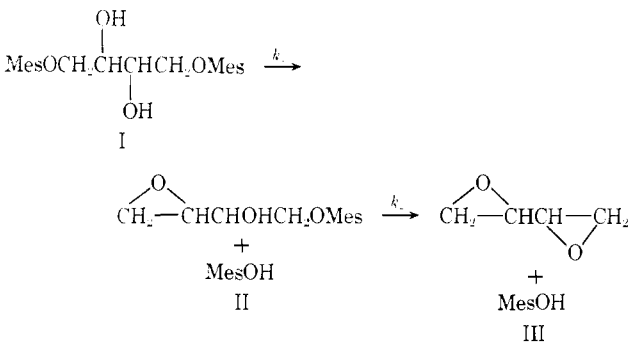
The *in vitro* transformation of (2S,3S)-threitol 1,4-bismethanesulfonate (I) to (2S,3S)-diepoxybutane (III) at constant pH is reinvestigated. By means of nmr spectroscopy the intermediate accumulation of (2S,3S)-1,2-epoxy-3,4-butanediol 4-methanesulfonate (II) is proved. Kinetic considerations allow the calculation of the concentration of II during the reaction from I to III. II can be isolated after the transformation reaction is allowed to proceed to 50%. The reaction rates k_1 (II from I) and k_2 (III from II) at 25° and pH 8.5 are determined. Induction of chromosomal aberrations by II is studied in onion root-tip cells and the compound is found only to have $^1/_{40}$ of the activity of III.

(2S,3S)-Threitol 1,4-bismethanesulfonate (I)[1], a bifunctional alkylating agent of the group of mesylated polyalcohols, is known to possess antineoplastic activity in the animal tumor screen[2] and in clinical trials.[3]

Reported biological studies on plant materials[4] have revealed that activity for induction of chromosome breakage and mutation can be observed only under conditions where I is pretransformed or transformed into epoxide compounds. Earlier, Davis and Ross[5] had proved, by chemical methods, the formation of epoxides when I was kept in aqueous solution at pH 7.5 and 37° while titrating liberated $MeSO_3H$, and suggested that diepoxides would be formed *in vivo* after administration of polyol bismethanesulfonates. Using the hypothesis that the intermediate (2S,3S)-1,2-epoxy-3.4-butanediol 4-methanesulfonate (II) would never accumulate during the transformation of I into (2S,3S)-diepoxybutane (III),[6] Matagne[4a] had determined a rate coefficient "k" for the overall reaction from I to III (Scheme I).

Scheme I



Inasmuch as the biological activity of I is apparently dependent upon transformation to epoxides we felt it to be of interest to reinvestigate the *in vitro* transformation of I into III in order to prove whether or not the monoepoxide II accumulates. Furthermore, we were interested in investigating the biological activity of II.

Chemical Results and Discussion.—Titration of liberated $MeSO_3H$ during reaction of I to III at constant pH and temperature does not allow one, at any given time of reaction, to calculate the concentration of either I, the reactive intermediate II, or the diepoxide III, but gives the concentration of intact mesyloxy groups and consequently epoxide ring formed (Scheme I). Within the limits of the experimental determinations, the log of the concentration of mesyloxy groups plotted against time resulted in a straight line. A mathematical calculation revealed that the transformation of I over II into III has therefore to be one of two possible special cases on consecutive first-order reactions. Either the intermediate II is consumed as soon as it is formed—its net formation being zero—or the rate of reaction from I to II is twice that from II to III, giving the correlations of the rate coefficients $k_1 \ll k_2$ or $k_1 = 2k_2$. In the first case the monoepoxide II would never be present in detectable amounts during transformation while in the latter case the concentration of II is calculated to reach a maximum after 1 mole of base is consumed corresponding to 1 mole of $MeSO_3H$ liberated (Figure 1). In order to decide between these two possibilities we attempted to follow the transformation reaction performed in D_2O with NaOD as base by means of nmr spectroscopy. Nmr investigation has been used earlier to prove the end product of the *in vitro* transformation of 1,4-dibromo-1,4-dideoxy-*meso*-erythritol to be the meso isomer of III.[7]

A solution of I in D_2O was treated stepwise with NaOD and nmr spectra were taken as soon as the added base had been consumed. The expected signals of III could be observed only after nearly the theoretical amount of 2 moles of NaOD necessary for the transformation to III had been added. During the addition however, a rise and, later, decay of signals not belonging to either I or III were seen. In particular, integration

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(1) P. W. Feit, *J. Med. Chem.*, **7**, 14 (1964).

(2) (a) R. Jones, W. B. Kessler, H. E. Lessner, and L. Rane, *Cancer Chemother. Rep.*, **10**, 99 (1960); (b) F. R. White, *ibid.*, **24**, 95 (1962); L. H. Schmidt, R. Fradkin, R. Sullivan, and A. Flowers, *ibid.*, *Suppl.*, **2** (1965).

(3) (a) V. Loeb, Jr., *Cancer Chemother. Rep.*, **42**, 39 (1964); (b) F. S. Dietrich, *ibid.*, **52**, 603 (1968); F. Lundvall, Vth World Congress of Gynaecologists and Obstetricians, Australia, 507 (1967).

(4) (a) R. Matagne, *Mutat. Res.*, **4**, 621 (1967); (b) R. Matagne, *Radiat. Bot.*, **8**, 489 (1968); (c) R. Matagne, *Mutat. Res.*, **7**, 241 (1969).

(5) W. Davis and W. C. J. Ross, *Biochem. Pharmacol.*, **12**, 915 (1963).

(6) A stereospecific reaction can be suggested as no attack at the asymmetric carbon atoms 2 and 3 occurs, and III has been isolated after treatof I with KOH.[1]

(7) A. Dávid, G. Horváth, I. P. Horváth, L. Institóris, A. Neszmelyi, and L. Radics, *Experientia*, **27**, 512 (1967).

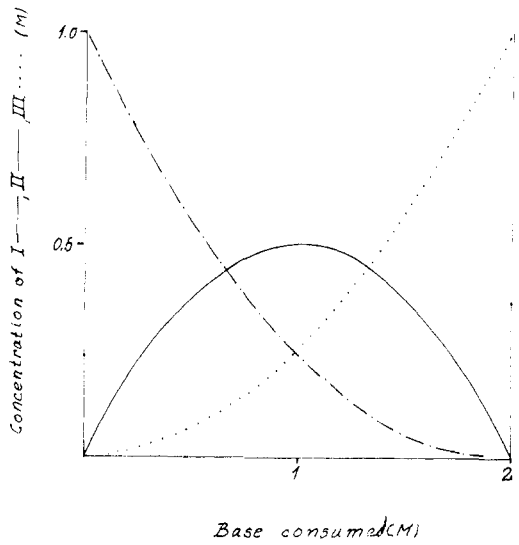

Figure 1.—Calculated dependence of concentration of I, II, and III on base consumption during transformation of I into III.

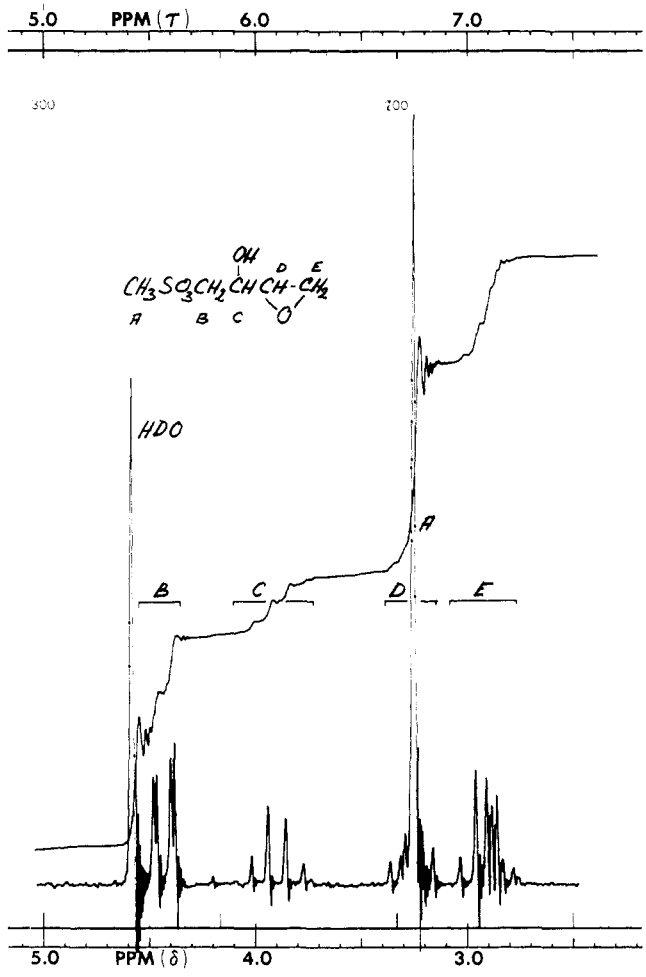

Figure 2.—Nmr spectrum of II in D_2O.

of a "quartet" at 3.9 ppm had raised maximum value between 0.8 and 1.1 moles of base consumption. The nmr spectra of mixtures of II and II showed the signals of the components only. When the addition of NaOD was performed at constant pD of 7.5 nmr signals of samples taken during transformation corresponded well to those observed in the stepwise experiment proving the formation of an intermediate assumed to be II.

Furthermore we succeeded in isolating II after treatment of I with 1 mole of NaOH in aq solution. The structure of II was established by its nmr spectrum (Figure 2). Absorptions within the range expected for epoxide protons, CH_2 protons geminal to O, and a CH proton geminal to OH were exhibited[8] and correlated well with the spectra I and III. The expected complexity of the strongly coupled 6-spin system was observed, but detailed assignment and recalculation of the theoretical nmr spectrum exceeds the scope of this paper and will not be given in this journal.

Total hydrolysis of II at pH 10.5 consumed the theoretical amount of base (1 mole). For the transformation of II into III the rate coefficient $k_{2\,(pH\,8.5;\ 25°)}$ was determined to be $5.1\ 10^{-3}$ min^{-1}. The equation $k_1 = 2\,k_2$ results in $k_{1\,(pH\,8.5;\ 25°)} = 10.2 \times 10^{-3}$ min^{-1}.

For the overall formation of III from I a hypothetical "k"$_{(pH\,8.5;\ 25°)}$ based on $k_1 \ll k_2$ was determined to be $5.2 \pm 0.6 \times 10^{-3}$ min^{-1},[9] and $k_{1\,(pH\,8.5;\ 25°)}$ calculated to be $10.4 \pm 1.2 \times 10^{-3}$ min^{-1} from the approximative correlation $k_1 = 2$ "k" for $k_1 = 2k_2$.

Biological Studies and Discussion.—The induction of chromosomal aberrations by the monoepoxide II was studied in onion root-tip cells. At concentrations 10 mM or higher II induced a strong mitotic inhibition. A 4 mM solution reduced the mitotic index especially in cells observed 24 and 32 hr after treatment. Solutions (2 mM or lower) had no appreciable effect on mitotic process. The percentages of anaphases with chromosomal aberrations at different times after treatment are given in Figure 3 (results from two independent experiments).

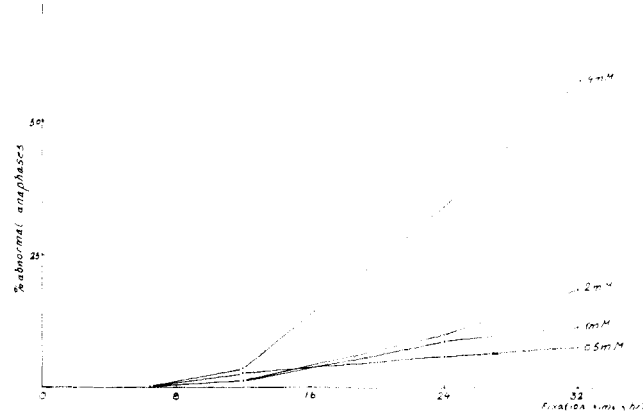

Figure 3.—Percentages of anaphases with chromosomal aberrations induced after treatment with compound II.

The results (Figure 3) indicate that for the 4 analyzed concentrations, the first aberrations were observed about 12 hr after treatment and their percentages increased during the sampling period. There is a delayed effect as usual after treatment with alkylating agents.

As the rate of conversion reaction from II into III is pH dependent,[4a] we have tested the influence of pH of the solution during the treatment (1 hr) of roots. The roots were treated with 2 mM solutions of II buffered

(8) For lit. see J. W. Emsley, J. Feeney, and L. H. Sutcliff, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. II, Chapter 10, Pergamon Press, Oxford, 1966.

(9) Reported[4a] value for "k" $= 10.2 \times 10^{-3}$ min^{-1}.

(0.02 *M* Sørensen buffer) at pH 5.5, 6, 6.5, 7, and 7.5. Anaphases were analyzed 30 hr after treatment. The percentages of abnormal anaphases were 16.7, 15, and 14 at pH 5.5, 6, and 6.5, respectively. At pH 7 and 7.5 the treatment induced complete mitotic inhibition. These experiments were repeated for a concentration of 0.5 m*M*. In the same way, no significant difference between the percentages (1 to 3%) of abnormal anaphases was observed at pH lower than 7 and a strong mitotic inhibition could still be observed at pH 7.5.

These series of experiments confirmed that the monoepoxide II is not transformed into a significant amount of the diepoxide III, during treatment in acid conditions. At pH higher than 7, however, conversion of II into III probably causes inhibition of mitosis in the root tips. It was previously shown[4b] that a 0.1 m*M* solution of III induced a maximum of 45% of abnormal anaphases in the same biological material. Concentrations higher than 0.2 m*M* inhibited the mitotic process.

The results obtained here for II and previously described for the alkylating agents I and III in experiments on onion root-tips[4b] are summarized in Table I.

Table I

Comparison of Cytological Effects Induced by Compounds I, II, and III after Treatment of Onion Root Tips (1 Hr). Fixation Time: 32 Hr after Treatment

Compd	Concentrations (m*M*) of treatment solutions	% of anaphases with aberrations
I	20	1.7
	50	3
II	0.5	6
	2	24.5
	4	58
III	0.05	21
	0.1	45
Control (H_2O)		1

The diepoxide III is about 40 times more effective than the monoepoxide II, and the latter is at least 100 times more active than I, which does not induce a significant amount of chromosome abnormalities or depression of the mitotic index at 50 m*M* concentration.

It can be considered that the biological effect described in Figure 3 is actually due to II itself. Contamination by III, if any, must be very low since a 0.2 m*M* solution of III induced complete mitotic inhibition. Another fact favors this hypothesis. A related compound, the DL-1,2-epoxy-4-butanolmethanesulfonate, which cannot be transformed into a diepoxide, induced chromosome breakage in the same range of concentrations than II.[10] Nevertheless, it cannot be excluded that for the criteria chosen (chromosome breakage and mitotic inhibition) the effect of II is due to formation of the second epoxide ring inside the cells, and the difference in activity between I, II, and III depends mainly on their ability to penetrate the cells.

Experimental Section[11]

(2*S*,3*S*)-1,2-Epoxy-3,4-butanediol 4-Methanesulfonate (II).—1 *N* NaOH (40 ml) was added to a stirred soln of I (11.2 g) in H_2O (180 ml) and after 10 min the reaction mixture was evapd under reduced pressure in a water bath not exceeding 25°. After the residue had been dried by addition of CH_2Cl_2 (50 ml) and evapd *in vacuo*, additional CH_2Cl_2 (100 ml) was added, the insol mixture of starting material and $NaOSO_2Me$ was removed by filtration and washed with CH_2Cl_2 (50 ml). The combined filtrates were dried (Na_2SO_4) and evapd under reduced pressure. The residue (3.6 g) was triturated with Et_2O (70 ml) and left standing in a refrigerator for 16 hr affording crystallization. The mother liquor was decanted and the solution redissd in Et_2O (200 ml). Insoluble material was removed by filtration, and the filtrate coned to 40 ml. After standing in a refrigerator the crystallized II (1.8 g) was isolated and dried *in vacuo:* mp 34–35°; $[\alpha]^{20}D$ +14.6° (*c* 2, $CHCl_3$); nmr spectrum Figure 2; *Anal.* ($C_5H_{10}O_6S$) C, H, S.[12]

Kinetic Studies.—The compound was titrated in aq soln at pH 8.5 and 25° with 3 *N* NaOH in a radiometer Titrator Type TTT la equipped with a Radiometer Titregraph SBR 2b. The log *A* was plotted against time and from the resulting straight line *k* was determined using $k = 2.303 (\log A_1 - \log A_2)$. *A* is defined as the concentration of the reactant at time *t* and was calcd from the NaOH consumption.

Nmr Studies.—The spectra were taken in concentrations of 5 to 10% in D_2O using the Na salt of 3-(trimethylsilyl)propanesulfonic acid as internal reference on a Varian A60 A spectrometer. The stepwise experiment was performed in the nmr tube itself using 2 *N* NaOD. In the experiment at constant pD an automatical titrator and 1.07 *N* NaOD were used. The test samples were taken during the titration.

Biological Experiments.—Onion bulbs (*Allium cepa* var. Stuttgart) were cultivated in aerated tap H_2O at 21°. After 3 days of culture, the roots were treated for 1 hr with fresh solutions of II in acidified distd H_2O (pH 5). In other experiments, the compound was dissolved in Sørensen buffer (0.02 *M*) at various pH (see Biological Studies).

After treatment, the roots were carefully washed and transferred to tap H_2O. Roots were fixed (2 hr in EtOH–AcOH, 3:1 v/v) at various time elapsing after treatment. The root tips were stained (1 hr) according to the Feulgen method and spread on a microscope slide (squash technique). Anaphases were scored for chromosomal aberrations (dicentric bridges and/or acentric fragments).

Acknowledgment.—The authors are indebted to H. Engberg-Pedersen for mathematical calculations.

(10) R. Matagne, to be published.

(11) Technical assistance by H. Dannacher and K. Dehn; analyses by G. Cornali and W. Egger.

(12) Analytical results were within 0.35% of the theoretical values.