International Journal of Pharmaceutics, 66 (1990) 253-262
Elsevier

IJP 02282

Mechanism of degradation of the investigational cytotoxic agent, cyclodisone (NSC-348948)

K. Umprayn, W. Waugh and V.J. Stella

Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045 (U.S.A.)

(Received 7 March 1990)
(Modified version received 1 June 1990)
(Accepted 22 August 1990)

Key words: Degradation; Cytotoxicity; Cyclodisone; Kinetics

Summary

Cyclodisone (1,5,2,4-dioxadithiepane-2,2,4,4,-tetraoxide, NSC 348948) is a chemically unstable, poorly water soluble investigational cytotoxic agent. The aqueous degradation of cyclodisone at 25°C (ionic strength of 0.5, $NaClO_4$ and NaCl) as a function of pH, was investigated. In the presence of NaCl, the pH-rate profile could be described by, $k_{obs} = k_1[H^+]/(K_a+[H^+]) + k_2K_a/(K_a+[H^+])$, where k_1 equals 5.27×10^{-4} min^{-1}, k_2 equals 2.7×10^{-5} min^{-1} and K_a was 1×10^{-10} (pK_a of 10). In the presence of $NaClO_4$, k_2 was negligible and the data could be fit with a value of 3.10×10^{-4} min^{-1} for k_1 and 8.56×10^{-11} for K_a (pK_a of 10.06). The pK_a in this case refers to the ionization of the methylene group between the two sulfur atoms. At both high and low pH values, the major degradation step appeared to be hydrolysis, via an early transition state Sn2 mechanism, of the ethylene-oxygen bond to produce the monohydroxyethyl ester of methane disulfonic acid. The primary decomposition product undergoes further hydrolysis to give the final products, ethylene glycol and methane disulfonic acid. The results from ^{18}O-enriched water experiments clearly demonstrated the C-O bond cleavage occurred in both steps of the degradation pathway. Buffer studies, effect of halide nucleophiles and deuterium solvent isotope studies were used to further characterize the mechanism and products.

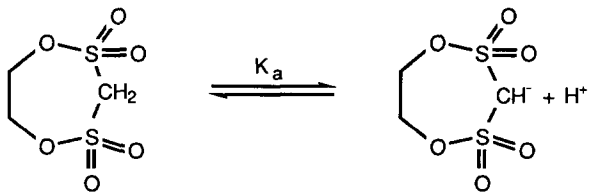
Introduction

Cyclodisone (1,5,2,4-dioxadithiepane-2,2,4,4,-tetraoxide, NSC 348948, **1**) is a chemically unstable, poorly water soluble investigational cytotoxic agent (Gibson, 1988, 1989). In vivo evaluations from the National Cancer Institute indicated that **1** is active against P388 and L1210 leukemia, M5076 sarcoma and MX-1 mammary carcinoma in animal testing (J. Plowman, personal communication).

The hydrolysis of aliphatic sulfonic acid esters and sulfamates have been studied in the past (Hartman et al., 1960; Barnard et al., 1961; Konasiewicz et al., 1968; Mori et al., 1971; Feit et al., 1973; Williams et al., 1974; Thea et al., 1985a,b; Hassan et al., 1986; Paborji et al., 1987; Kennedy et al., 1988), although very little is known about the hydrolysis of cyclic alkyl esters of sulfonic acids such as **1**.

Umprayn et al. (1987) published a stability indicating, high performance liquid chromatography (HPLC) assay for cyclodisone which utilizes a polystyrene-divinylbenzene column (PRP-1) and an alkaline mobile phase (pH 9.5). The latter was required to promote dissociation of the acidic methylene group and thereby allow detection of the

Correspondence: V.J. Stella, Dept. of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, U.S.A.



1 CYCLODISONE

Scheme 1.

conjugate base of cyclodisone at 214 nm. In the present study, this assay procedure was used along with other supplementary analytical techniques, GC-mass spectrometry (with EIMS and CIMS-NH_3 probe) and titration to confirm the decomposition products, and deuterium solvent isotope effects and ^{18}O enriched water studies to characterize the mechanism of degradation of cyclodisone.

Experimental

Materials

Cyclodisone was obtained from the NCI (National Cancer Institute, Bethesda, MD). All the chemicals and buffer reagents used were A.C.S. reagent grade and were used as received. All organic solvents used were HPLC grade. Water used for kinetics studies was deionized and distilled (Mega-Pure™ system, model MP-1, Corning N.Y.).

Kinetics measurements

All kinetic measurements were carried out at 25 ± 0.1 °C in a temperature controlled water bath (Bath & Circulator model 2095, Forma Scientific, Ohio). The pH range studied was 2.66–11.6; the ionic strength was adjusted to 0.5 M by the addition of sodium perchlorate (or sodium chloride). Initial studies used sodium chloride to adjust the ionic strength. Subsequent experiments showed the reaction to be catalyzed by halides. All pH measurements were made at 25°C, with a digital pH meter (Corning model 155, Medfield, MA). The accuracy of pH measurements were found to be not more than ± 0.01 units. Buffer concentrations used to study cyclodisone degradation were in the range of 0.04–0.08 M which were needed to maintain pH since the degradation of cyclodisone produces various sulfonic acids which tend to lower solution pH if poorly buffered. Buffers used to maintain adequate pH control were as follows: pH 5.0 (acetate); pH 7.45, 11.1 and 11.5 (phosphate); pH 10 and 10.5 (carbonate). At pH 2.66, HCl was used. Buffer concentration was varied to allow an analysis of the effects of buffers on the degradation rate and to allow extrapolation to zero buffer concentration. Cyclodisone was introduced into an appropriate buffer solution from a standard acetonitrile solution. Final acetonitrile concentration was 1.3–1.5%. The reaction mixtures were assayed at time zero and at the predetermined time intervals by the HPLC method described elsewhere (Umprayn et al., 1987). The pH values of the reaction mixtures were monitored and were found to vary less than 0.1 units over the course of the reaction. All reactions followed apparent first order kinetics over 3–4 half-lives. The accuracy of rate constant measurements was found to be better than ± 5%.

Effect of halide nucleophiles

The rates of degradation of **1** in water and in the presence of 0.5 M NaF, NaCl, NaBr and NaI were compared. Solutions of 9.9×10^{-4} M of **1** in water and in various halide solutions were maintained at 25 ± 0.1 °C (pH not controlled). The reaction mixtures were run in duplicate and assayed for intact cyclodisone by HPLC. The observed pseudo first order rate constants were analyzed according to a modification of the Swain-Scott equation (1953):

$$\log (k_{obs} - k_{o,obs})/k_{o,obs} = sn \qquad (1)$$

where k_{obs} and $k_{o,obs}$ are the observed rate constants of cyclodisone in the presence of halide nucleophiles and in water, respectively; n is the nucleophilicity of halide in study; s is the constant characteristic of substrate. From Eqn 1, the term, $k_{obs} - k_{o,obs}$ therefore represents the net catalytic effect of the halide nucleophile.

Product analysis

The possible route of degradation of **1** is given in Scheme 2. The stability indicating assay for **1**

was not capable of detecting the proposed initial hydrolysis product of **1**, the monohydroxyethylester of methane disulfonic acid, **2**. It was also incapable of detecting ethylene glycol, **3**, and methane disulfonic acid, **4**, the proposed secondary hydrolysis products. Ethylene glycol production, however, was determined by gas chromatography using a Varian 3700 (Sunnyville, CA) chromatograph equipped with a flame ionization detector. The chromatographic column (4.5 mm i.d. × 1.5 m) was a 10% carbowax, 20 mesh, on 80–100 mesh chromosorb Q column (Supelco, Houston, TX). The oven temperature was 120°C (isothermal) while the detector and injector port temperatures were 230 and 190°C, respectively. Nitrogen was the carrier gas, hydrogen and compressed air were used as the detector gases. The gas flow rates were 30, 5 and 300 ml/min, respectively. Samples were prepared by accurately weighing **1** and dissolving in water (≈20 min by sonication) to obtain the final concentration of 4.93×10^{-3} M. The solutions were stored at ambient temperature and assayed for ethylene glycol production by direct injection of the reaction mixture as a function of time until both the primary and secondary degradation steps appeared to be complete.

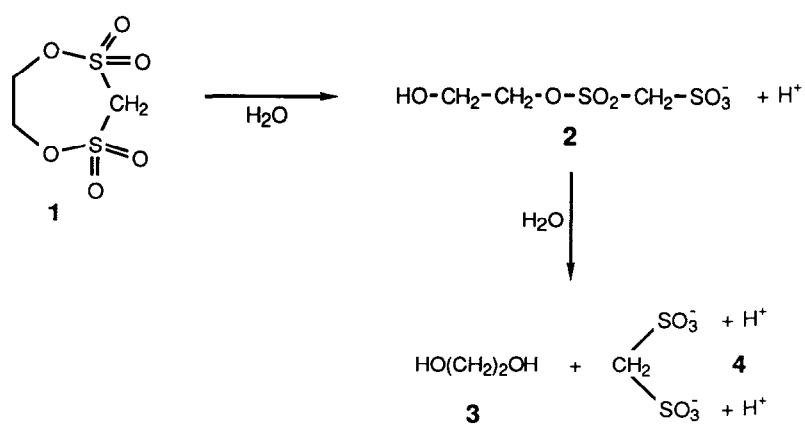
Since it was proposed that **1** degraded to **2**, then **4**, a titration method was used to further confirm the results obtained from the GC studies. That is, quantitative degradation of **1** to **2** would titrate for one mole equivalent of acid while **1** to **4** would titrate for two mole equivalents of acid. The volume of titer (pre-standardized NaOH 0.0023 M) necessary to bring pH of the reaction mixture at time t, to neutrality on hydrolysis of a unbuffered solution of **1**, was subtracted from the volume of titer necessary to bring the pH of the reaction mixture at time zero to neutrality, since a solution of **1** was acidic (pH ≈ 3.3–3.4). The reaction mixtures were prepared by the same procedure as in GC studies, but 5 ml samples were removed and assayed by titration at each predetermined time.

Deuterium solvent isotope effect studies

Degradation kinetics of **1** in water and in deuterium oxide were compared for further characterization of the hydrolytic mechanism. Solutions of 1.67×10^{-3} M **1** in water and in deuterated water (Bio-Rad Lab., CA) were maintained at 25 ± 0.1°C with and without ionic strength control (pH not controlled). Where controlled, the ionic strength was maintained by 0.5 M $NaClO_4$ or NaCl. In addition, the degradation of **1** at acidic pH and pD (corrected for meter readings) values was also investigated. In D_2O, the solutions were acidified by the addition of a standard DCl solution (Bio-Rad Lab., CA).

Water-enriched ^{18}O studies

The hydrolysis of **1** was also carried out in water and ^{18}O enriched water, 97.2 atom % (MSD Isotopes, Montreal, Canada). A solution of 4.93×10^{-3} M **1** in water and ^{18}O enriched water were kept at an ambient temperature until the degra-



Scheme 2.

dation was completed. The reaction mixtures were then analyzed by mass spectrometry (MS). Compound **4** was detected by the electron impacted mass spectrometry, EIMS (Quadrapole Mass Spectrometry model R10-10, Nermag, France) while compound **3** was detected by GC-mass spectrometry (GC model 31, Girdel France with EIMS and CIMS-NH3 detector probe).

Results and Discussion

The kinetics of degradation of **1** at various pH values in the presence of buffers at various concentrations were found to be pseudo-first order for 3–4 half-lives. A representative example was presented earlier (Umprayn et al., 1987).

pH and buffer dependency

The observed pseudo-first order rate constants, k_{obs}, when plotted against total buffer concentration, $[B_T]$, according to Eqn 2 at any given pH were reasonably linear.

$$k_{obs} = k'_{obs} + k_{cat}[B_T] \quad (2)$$

The intercepts, k'_{obs} give the values of the observed buffer independent rate constants for cyclodisone which were then used to generate the pH-rate profiles. All the values for k_{obs} are summarized in Table 1 for the $NaClO_4$ data. The NaCl data is not reported in tabular form.

A summary of the calculated k'_{obs} values at all pH values is given in Table 2.

The pH-rate profiles for **1** in $NaClO_4$ and NaCl

TABLE 1

Pseudo-first order rate constants for the degradation of cyclodisone at various pH values and various buffer concentrations and ionic strength 0.5 M ($NaClO_4$) at 25 ± 0.1°C

Buffer	Concentration (M)	pH	$10^4 k_{obs}$ (min^{-1})[a]	Half-life (h)
		2.66 (HCl)	3.11	37.13
Acetate	0.04	5.00	3.80	30.39
	0.06	5.00	4.40	26.25
	0.08	5.00	4.47	25.67
Phosphate	0.04	7.45	5.40	21.39
	0.06	7.45	6.70	17.24
	0.08	7.45	8.02	14.40
Carbonate	0.04	9.60	6.53	17.69
	0.06	9.60	8.74	13.20
	0.08	9.60	10.96	10.50
Carbonate	0.04	10.00	5.08	22.74
	0.06	10.00	6.10	18.93
	0.08	10.00	8.34	13.84
Carbonate	0.04	10.50	2.32	49.78
	0.06	10.50	2.90	39.83
	0.08	10.50	3.55	32.54
Phosphate	0.04	11.10	0.69	167.39
	0.06	11.10	0.80	144.38
	0.08	11.10	1.14	101.32
Phosphate	0.04	11.50	0.24	481.25
	0.06	11.50	0.46	251.09
	0.08	11.50	0.62	186.29

[a]Average of duplicate experiments.

TABLE 2

The observed rate constants at zero buffer concentration, k'_{obs}, catalytic rate constants, k_{cat}, and the catalytic rate constants corrected for the fraction of the unionized cyclodisone, k'_{cat}, at 25°C, μ=0.5 with $NaClO_4$

Buffer	pH	$10^4 k'_{obs}$ (min^{-1})	$10^4 k_{cat}$ ($M^{-1}\ min^{-1}$)	$10^2 k'_{cat}$ ($M^{-1}\ min^{-1}$)
HCl	2.66	3.1	–	–
Acetate	5.00	3.2	16.8	0.17
Phosphate	7.45	2.8	65.5	0.66
Carbonate	9.60	2.1	110.8	2.14
Carbonate	10.00	1.6	81.5	2.73
Carbonate	10.50	1.1	30.8	2.59
Phosphate	11.10	0.21	11.3	3.42
Phosphate	11.50	a	9.45	7.11

[a]Plot of k_{obs} versus buffer concentration gave a small negative intercept.

are shown in Fig. 1.

The pH-rate profile shows a pH-independent region followed by a pH-dependent decrease in degradation at pH values greater than the pK_a. The pH-rate profile indicated no specific acid and base catalysis during the degradation of cyclodisone. The solid lines in Fig. 1 were generated by the non- linear least squares fit (Yamaoka et al., 1981) of k'_{obs} as a function of hydrogen ion concentration to Eqn 3:

$$k'_{obs} = k_1[H^+]/([H^+] + K_a) + k_2K_a/([H^+] + K_a) \quad (3)$$

where k'_{obs} is the observed rate constant at zero buffer concentration; k_1 and k_2 are the rate constants for the unionized and ionized cyclodisone, respectively; and K_a is the dissociation constant for cyclodisone. Values for k_1 of $5.27 \times 10^{-4}\ min^{-1}$, k_2 of $2.7 \times 10^{-5}\ min^{-1}$ and K_a of 1×10^{-10} (pK_a, 10) were found to adequately describe the profile for the NaCl solutions, while values of k_1 of 3.10×10^{-4} min^{-1}, k_2 of ≈ 0 and K_a of 8.56×10^{-11} (pK_a, 10.06) adequately described the profile for the $NaClO_4$. The pK_a values of 10 and 10.06 from the kinetic fits are in reasonable agreement with that obtained spectrophotometrically, 9.62–9.64 (Umprayn et al., 1987). The rate of degradation of the unionized species is at least 19.5 times faster (k_1/k_2) than of the ionized species. For the $NaClO_4$ solutions, k_2 was statistically insignificant.

The rate of **1** degradation significantly increased with increasing carbonate and phosphate buffer concentration at constant pH. Acetate, however, showed a weak catalytic effect. The slopes of k_{obs} versus total buffer concentration plots represent the observed second order catalytic rate constants, k_{cat} (Eqn 2). The k_{cat} values obtained from the plots decreased as the fraction of **1** in its unionized form decreased (Table 2). This suggested that the catalytic effect of the buffer was only operating on unionized **1**, i.e., k_{cat} equals $k'_{cat}f_1$, where f_1 is the fraction of **1** in its unionized state at any given pH and k'_{cat} is the buffer catalytic constant for the buff-

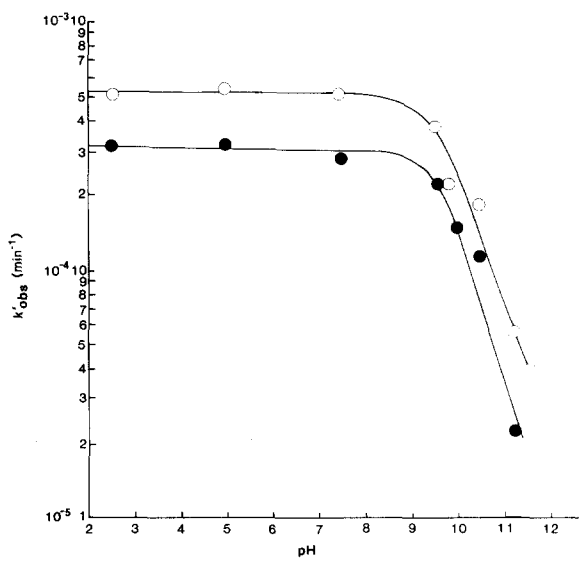


Fig. 1. pH-rate profile for the degradation of cyclodisone at 25.0 ± 0.1°C extrapolation to zero buffer concentration (μ = 0.5 with either NaCl(O) or $NaClO_4$ (•)). The data points are experimental and the line drawn according to Eqn 3, using the constants given in the text.

er operating on unionized **1**. k'_{cat} values were determined from Eqn 4 by assuming a K_a value of 2.34×10^{-10} (pK_a 9.63; determined spectrophotometrically; Umprayn et al. (1987)). Different

$$k'_{cat} = k_{cat}/f_1 = k_{cat}([H^+] + K_a)/[H^+] \quad (4)$$

numerical values for k'_{cat} would be calculated if a K_a value of 8.56×10^{-11} (pK_a 10.06, from the fit of the kinetic data) was used.

The values for k'_{cat} were then used to analyze the data for the presence of the catalytic effect for each buffer species (Table 2). For carbonate, the mono-anion had a catalytic effect on the reaction while the major catalytic species appeared to be the carbonate di-anion. In the case of phosphate buffer, both the mono- and di-anionic species are present at pH 7.45, while at pH 11.1 and 11.5, both the di- and tri-anionic species are present. A plot of k'_{cat} versus the fraction of phosphate present in its tri-anionic form was tried. Tri-anionic phosphate was found to be a better catalytic specie than the di-anion of phosphate, see Table 2. Both carbonate and phosphate probably act as nucleophilic catalysts by attacking the ethylene bridge of cyclodisone, see later discussion, rather than general base catalysts, however, this was not confirmed by a product analysis under these conditions.

Product analysis

The most probable routes of degradation of **1** are given in Scheme 2. After complete hydrolysis of **1** in water, a mass spectral analysis by EIMS of the reaction mixture showed a peak corresponding to the mass peak of **4**. The mass spectrum was comparable to that of an authentic sample of **4** in water.

Ethylene glycol formation. The appearance of ethylene glycol, **3**, was assayed by GC from a 4.93×10^{-3} M solution of **1** in water maintained at ≈ 25°C. The results are given in Fig. 2. A lag time of approximately 65 h was observed suggesting that hydrolysis of **1** to **2** was faster than its subsequent hydrolysis to **3** and **4**. After complete hydrolysis of **1**, the total amount of **3** formed was 5.3×10^{-3} M which is comparable to the theoretical value expected if all of **1** eventually degraded to **3** and **4**.

Titration studies. The intermediate product **2** and the final product **4** (see Scheme 2) have mono- and di-acidic functionalities, respectively. The results from the titration study after partial-to-full hydrolysis of a 2.6×10^{-2} M solution of **1** at 25 °C, in water (pH not controlled) is given in Table 3.

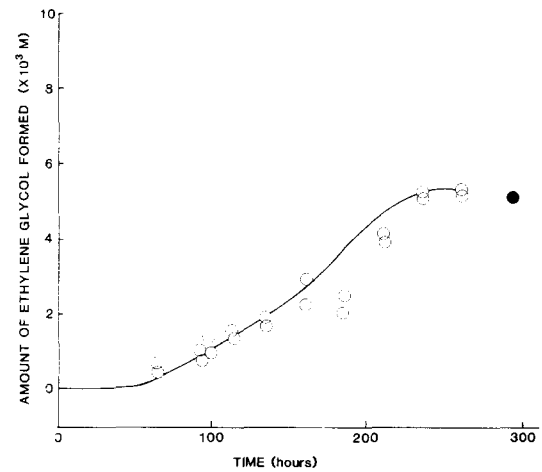


Fig. 2. Plot of the amount of ethylene glycol formed (open symbol), as determined by GC, from cyclodisone (4.93×10^{-3} M) in water at 25°C (pH not controlled) as a function of time. The closed symbol refers to the theoretical amount of ethylene glycol produced if cyclodisone was completely converted to ethylene glycol.

The results indicate that the intermediate product **2** has a mono acidic functional group. At 65 h (≈ 2.6 half-lives) > 80% of **2** should be formed which is in agreement with the lag time results obtained from the GC studies for ethylene glycol formation and the titration data in Table 3. The final product, **4**, having two acidic functions should consume two mole equivalents of base which is what was observed when the cyclodisone solution was allowed to stand for 5 weeks.

^{18}O enriched water studies. The site of bond cleavage, i.e., C-O versus S-O cannot be deduced from the data presented to date. Therefore, the ^{18}O enriched water studies were undertaken to determine the site of cleavage and to furnish further insight into the mechanism of degradation of **1** in water. Because of the difficulty in isolating **2**, the degree of ^{18}O incorporation was determined only in the final products, **3** and **4**, by GC-mass spectrometry (EIMS and CIMS-NH_3 detector probes) from a reaction mixture maintained at 25°C for >

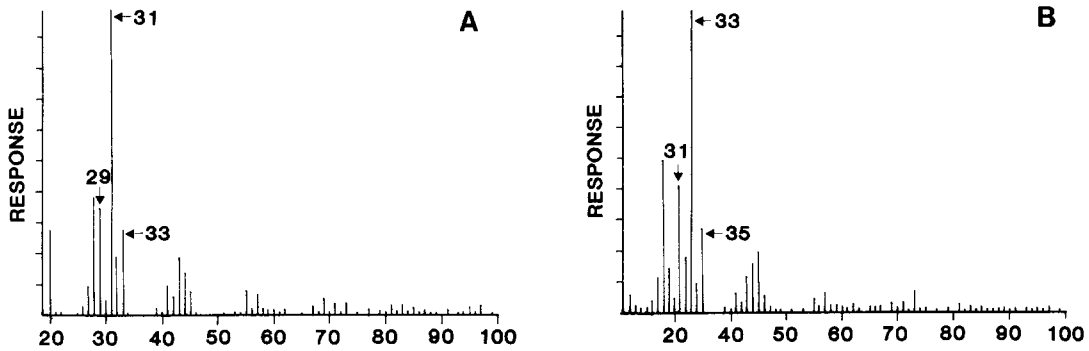


Fig. 3. Mass spectra fragmentation pattern (EIMS) of ethylene glycol generated from the degradation of cyclodisone (4.93×10^{-3} M) in water (A), and ^{18}O enriched water (B).

10 half lives of **1**. The results of GC mass spectral analyses of **3** formed after complete hydrolysis of **1** in water and in ^{18}O enriched water are given in Fig. 3A and B, respectively.

The mass spectrum of **3** from water (^{16}O) was found to be identical to the GC mass spectrum of authentic ethylene glycol. The major fragment ions were at *m/z* 29($CH^{16}O$), *m/z* 31 ($CH_2^{16}OH$, 100% relative abundance) and *m/z* 33 (presumably $CH_3^{16}OH_2^+$). The molecular peak (*m/z* = 62) had a small relative abundance of approximately 1% and could not be used for comparison purposes. In the ^{18}O enriched water study, the major fragments were *m/z* 31 ($CH^{18}O$), *m/z* 33 ($CH_2^{18}OH$) and *m/z* 35 (presumably $CH_3^{18}OH_2^+$). The relative abundance of the peaks suggest that two ^{18}O atoms were incorporated into the final product, **3**. To more clearly demonstrate the site of bond cleavage, the detector probe of the GCMS was changed to a CIMS-NH_3 probe. Using this detector probe, the molecular peak (M + 18) of **3** in both water and ^{18}O enriched water was detected. The results are given in Fig. 4A and B respectively. The results indicate that the molecular peak (M + 18) of **3** in ^{18}O enriched water was 4 mass units higher than that in water and clearly demonstrated that two ^{18}O atoms were incorporated into the final product, **3**. Control experiments with ethylene glycol maintained in ^{18}O enriched water under identical conditions to those used for the hydrolysis of **1** showed no incorporation of ^{18}O.

EIMS analysis of the reaction mixtures showed a fragmentation pattern for the final product **4**. There were no differences in the fragmentation pattern between water and ^{18}O enriched water which suggested no oxygen isotope incorporation into **4**. If the hydrolysis of **1** occurs via S-O bond cleavage as opposed to a C-O bond cleavage, one

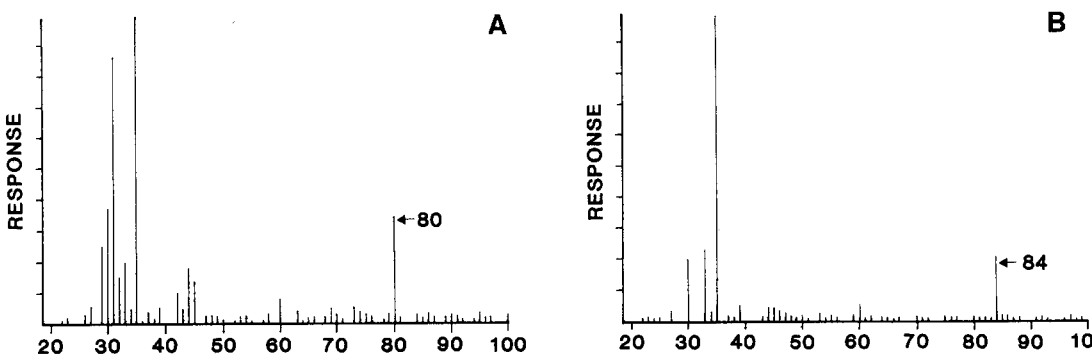


Fig. 4. Mass spectra (CIMS-NH_3) of ethylene glycol generated from the degradation of cyclodisone (4.93×10^{-3} M) in water (A) and ^{18}O enriched water (B).

would expect to see the isotope peaks of the final product **4** in the EIMS of the ^{18}O enriched water at m/z = 98 (presumably $CH_3SO_2^{18}OH$) and 181 ($CH_2(SO_2^{18}OH)_2$), respectively.

Carbon-oxygen bond cleavage of **1** in water leading to **2** might involve either a unimolecular reaction (Sn1 mechanism) involving formation of a primary carbocation or a bimolecular displacement reaction (Sn2 mechanism). A mechanism leading to a primary carbocation, generally unfavorable energetically, is unlikely.

Cleavage of the C-O bond in **1** by an Sn2 mechanism (or an Sn1 mechanism) would result in the development of a partial to full negative charge on the leaving oxygen group. Such a negative charge build-up would not be favorable if **1** was ionized. This would explain the increase in stability of **1** at pH values greater than the pK_a.

Mechanistic studies

Various probes were used in an attempt to understand the mechanism of degradation of **1**. These included the effects of halide nucleophiles and a solvent isotope study.

Effect of halide nucleophiles. The rate constants for cyclodisone degradation in the pH range of study were found to be accelerated by the presence of NaCl (see Fig. 1) that could not be accounted for on the basis of simple ionic strength effects. This led to a study of the effects of other halide nucleophiles on the degradation of **1**. The rate degradation of **1** in water and in the presence of 0.5 M NaF, NaCl, NaBr and NaI were compared with the rate constant in water, $k_{o,obs}$ and evaluated using a modification of the Swain-Scott equation (Eqn 1). The plot of log $(k_{obs}-k_{o,obs})/k_{o,obs}$ versus n according to Eqn 1 is given in Fig. 5. The results indicate that the stability of cyclodisone decreases with an increase in nucleophilic strength of the halide ($I^- > Br^- > Cl^- > F^-$) as predicted by the Swain-Scott equation. This observation suggests that the mechanism of degradation of **1** was similar to that proposed for clomesone, another sulfonic acid ester (Kennedy et al., 1988) and is consistent with a one-step nucleophilic substitution reaction (Sn2 mechanism).

Solvent isotope study. A kinetic solvent isotope study was initiated to provide information

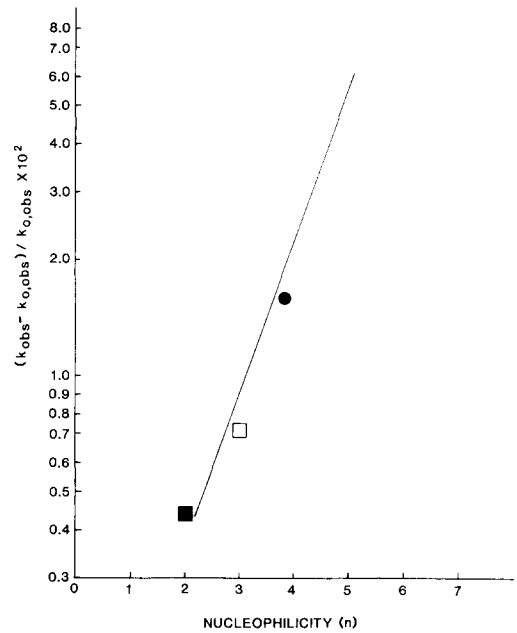


Fig. 5. A plot of $\log(k_{obs}-k_{o,obs})/k_{o,obs}$ versus n, the Swain-Scott nucleophilicity number, for the hydrolysis of cyclodisone in the presence of various halide ions (I^-, ○; Br^-, ●; Cl^-, □; F^-, ■).

about the nature of transition states along with the reaction pathway. Changing the solvent from water to deuterium oxide will alter the zero point energy of the overall reactant state on going to the transition state as well as affect solute-solvent interactions (solvent effect) on the degradation of **1**. The kinetic solvent isotope results for the hydrolysis of **1** under a variety of conditions are summarized in Table 4.

The results indicate that the hydrolysis rate of **1** in water was comparable to that in deuterium oxide. The absence of a large kinetic solvent isotope effect is somewhat unexpected if the reaction proceeds by an Sn2 mechanism, as indicated by the halide data, as opposed to an Sn1 mechanism. This probably indicates that the water molecule participates in an early transition state Sn2 mechanism (Paborji et al., 1987). In this early, reactant-like, transition state there is little appreciable bond development between the incoming nucleophile, water, and the carbon atom of cyclodisone.

The combination of the isotope and buffer studies and the effect of halide nucleophiles tends to

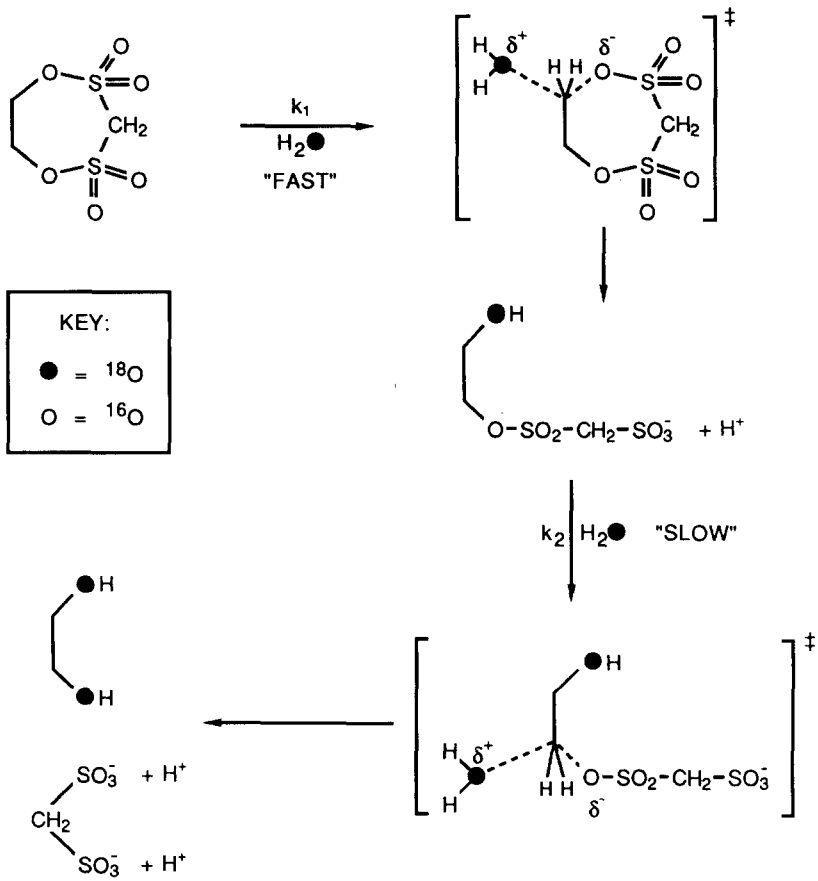
TABLE 3

Titration of hydrogen ions after partial-full hydrolysis of cyclodisone (2.6×10^{-3} M) at 25°C, in water (pH not controlled)

Time	Number of half-lives	Expected compound forming	$[H^+]$ Theoretical yield (M)	$[H^+]$ Experimental yield (M)[a]
41 h	1.6	**2** + H^+	1.6×10^{-2}	1.3×10^{-2}
65 h	2.6	**2** + H^+	2.6×10^{-2}	2.5×10^{-2}
2.3 weeks	15.5	**4** + $2H^+$	5.2×10^{-2}	4.8×10^{-2}
5 weeks	33.7	**4** + $2H^+$	5.2×10^{-2}	4.8×10^{-2}

Half-life of cyclodisone degradation followed by HPLC under these experimental conditions was 24.9 h.
[a]Average of two runs.

support the Sn2 mechanism with a water molecule involved in an early transition state (reactant like transition state). Similar conclusions were obtained for the hydrolysis of clomesone (Kennedy et al., 1988) and sulfamic acid 1,7-heptanediyl ester (Paborji et al., 1987). A proposed mechanism of degradation of **1** in water is given in Scheme 3.



Scheme 3.

TABLE 4

Results of solvent isotope study for cyclodisone hydrolysis

Solvent	k_{H2O}/k_{D2O}[a]
H_2O/D_2O (HCl, pH 2.66; DCl, pD 2.70)	0.94
H_2O/D_2O (pH not controlled)	1.16
H_2O/D_2O (μ=0.5 with NaCl)	1.02
H_2O/D_2O (μ=0.5 with $NaClO_4$)	1.07

[a]Average of two runs.

Conclusion

Based on various mechanistic probes, the degradation of **1** appears to be an early transition state, nucleophilic displacement reaction by water at one of the ethylene carbons resulting in C-O bond fission to produce the monohydroxyethylester of methane disulfonic acid, **2**. Although an Sn1 mechanism was unlikely, based both on the experimental observations and the need to postulate the formation of a primary carbocation, this mechanism could not be totally ruled out. The primary decomposition product undergoes further hydrolysis via C-O bond fission to give the final products, ethylene glycol and methane disulfonic acid, **4**.

Acknowledgements

The authors are grateful to Dr C. Judson for the mass spectral work. This work was supported by the National Cancer Institute, Contracts N01-CM-37562, N01-CM-67912 and N01-CM-97576

References

Barnard, P.W.C. and Robertson, R.E., The hydrolysis of a series of straight-chain alkyl methanesulphonic esters in water. *Can. J. Chem.*, 39 (1961) 881–888.

Feit, P.W. and Anderson, N.R., 4-Methanesulfonyloxybutanol: Hydrolysis of Busulfan. *J. Pharm. Sci.*, 62 (1973) 1007–1008.

Gibson, N.W., Comparison of the in vitro cytoxicity of cyclodisone with a series of structurally related analogues. *Anti-Cancer Drug Dis.*, 3 (1988) 199–204.

Gibson, N.W., Characterization of DNA damage and cytotoxicity induced in human colon carcinoma cell lines by cyclodisone. *Cancer Res.*, 49 (1989) 154–157.

Hartman, S. and Robertson, R.E., Nucleophilic displacement of methyl sulphonic esters by hydroxide ion in water. *Can. J. Chem.*, 38 (1960) 2033–2038.

Hassan, M. and Ehrsson, H., Degradation of busulfan in aqueous solution. *J. Pharm. Biomed. Anal.*, 4 (1986) 95–101.

Kennedy, P.E., Riley, C.M. and Stella, V.J., Degradation of the antineoplastic drug, clomesone (2-chloroethyl(methylsulfonyl)methanesulfonate, NSC-338947). A kinetic and mechanistic study. *Int. J. Pharm.*, 48 (1988) 179–188.

Konasiewicz, A.K., Sammy, G.M. and Maccoll, A., The methanolysis of nitrophenyl esters. Part III. The kinetics of the base-catalyzed methanolysis and pyridinolysis of 2,4-dintrophenyl toluene-p-sulphonate. *J. Chem. Soc. B.*, (1968) 1364–1369.

Mori, A., Nagayama, M. and Mandai, H., Mechanism and reactivity of hydrolysis of aliphatic sulfonate esters. *Bull. Chem. Soc. Jap.*, 44 (1971) 1669–1672.

Paborji, M., Waugh, W.N. and Stella, V.J., Mechanistic investigation of the degradation of sulfamic acid 1,7-heptanediyl ester, an experimental cytotoxic agent, in water and ^{18}oxygen-enriched water. *J. Pharm. Sci.*, 76 (1987) 161–165.

Swain, C.G. and Scott, C.B., Quantitative correlation of relative rates. Comparison of hydroxide ion with other nucleophilic reagents toward alkyl halides, esters, epoxides and acyl halides. *J. Am. Chem. Soc.*, 75 (1953) 141–7.

Thea, S., Guanti, G., Hopkins, A.R. and Williams, A., Catalysis of sulfonate ester hydrolysis by intramolecular amide group assistance. *J. Org. Chem.*, 50 (1985a) 3336–3341.

Thea, S., Guanti, G., Hopkins, A.R. and Williams, A.J., Evidence for an anionic sulfene intermediate in the alkaline hydrolysis of aryl(methylsulfonyl)methane sulfonate esters. *J. Org. Chem.*, 50 (1985b) 5592–5597.

Umprayn, K., Kennedy, P.E., Lee, J.C., Waugh, W., Riley, C.M. and Stella, V.J., Development of reversed phase HPLC assays for cyclodisone and clomesone. *J. Pharm. Biomed. Anal.*, 5 (1987) 625–633.

Williams, A. and Douglas, K.T., Hydrolysis of aryl *N*-methylaminosulphonates: Evidence consistent with an E_1cB mechanism. *J. Chem. Soc. Perkin Trans.*, II (1974) 1727–1732.

Yamaoka, K., Tanigawara, Y., Nakagawa, T. and Uno, T., A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharm. Dyn.*, 4 (1981) 879–885.