International Journal of Pharmaceutics, 48 (1988) 179–188
Elsevier

IJP 01630

Degradation of the antineoplastic drug, clomesone (2-chloroethyl(methylsulfonyl)methanesulfonate, NSC-338947). A kinetic and mechanistic study

P.E. Kennedy, C.M. Riley and V.J. Stella

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

(Received 5 January 1988)
(Modified version received 22 March 1988)
(Accepted 2 June 1988)

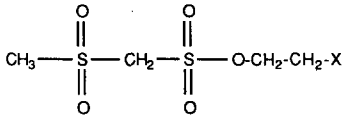
Key words: Clomesone; Antineoplastic drug; Stability; Kinetics, Mechanism; pH–rate profile; Nucleophile effect; Product analysis; Solvent isotope effect

Summary

Clomesone (2-chloroethyl(methylsulfonyl)methanesulfonate) is a chloroethylating agent with known antineoplastic activity. The effects of pH, buffers (concentration and type), ionic strength, solvent isotopes and various nucleophiles on the kinetics of clomesone degradation have been investigated using a stability-indicating, high-performance liquid chromatographic assay. To probe the mechanism of degradation further, the kinetic experiments were supplemented by analysis of the products of degradation using gas chromatography, a chloride-specific electrode and mass spectrometry, as well as by solvent isotope studies. The pH–rate profile of clomesone was sigmoidal with a significant enhancement of the rate of degradation being observed at higher pH values. The change in rate observed with increasing pH was paralleled by a change in the nature of the reaction products. At low pH, hydrolysis of the sulfonate ester via cleavage of the C–O bond and production of chloroethanol, appears to be the dominant pathway of degradation. At higher pH values, a mechanism involving intramolecular alkylation appears to operate, resulting in the production of a compound whose mass spectra is consistent with a cyclic sulfonate (sultone). The change in mechanism with changing pH was consistent with the acidic pK_a value of 10.62 for clomesone which has been determined previously by spectrophotometry to be 10.71. The degradation of clomesone was found to be accelerated by added halide ions due to nucleophilic catalysis. Finally, the pH–rate profile of ethyl(methylsulfonyl)methanesulfonate, a structurally related compound, was determined and compared with that of clomesone.

Introduction

Clomesone (2-chloroethyl(methylsulfonyl) methanesulfonate, NSC-338947, Fig. 1) is a chloroethylating agent which is currently being in-



I, X = Cl
II, X = H

Fig. 1. The structures of clomesone (**I**) and ethyl(methylsulfonyl)methane sulfonate (**II**).

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

vestigated in the clinic as a potential anticancer drug. Clomesone entered clinical investigations after in vitro studies by Shealy et al. (1983, 1984) had shown it to be superior to other drugs in this class against P388 leukemia cells. The antineoplastic activity of the chloroethylating agents is believed to be due to their ability to induce intra- and inter-strand crosslinking of DNA. Previously, we have reported (Umprayn et al., 1987) on a stability-indicating, high-performance liquid chromatography (HPLC) assay for clomesone which utilizes a polystyrene-divinylbenzene column (PRP-1) and an alkaline mobile phase (pH 11). The latter was required to promote dissociation of the acidic methylene group and thereby allow detection of the conjugate base of clomesone at 214 nm. In the present study, this assay procedure (Umprayn et al., 1987) was used, along with other supplementary analytical techniques, to quantify the degradation of clomesone in aqueous solution and to identify the mechanism and the degradation products. A structurally related agent, ethyl(methylsulfonyl)methanesulfonate (**II**, Fig. 1) was also studied to provide further insight into the mechanism and routes of clomesone degradation.

Experimental

Chemicals and reagents

Clomesone (**I**, NSC-338947) was obtained from the National Cancer Institute, Bethesda, MD. A sample of **II** was kindly provided by Dr. Y. Fulmer Shealy of the Southern Research Institute, Birmingham, AL. All the organic solvents were HPLC grade and all the other chemicals were ACS grade from various sources. The chloroethanol (**III**), ethylene glycol (**IV**) and methylsulfonylmethanesulfonic acid (**V**) were obtained from Aldrich Chemical Co. The water was deionized and distilled using the Mega-Pure system (model MP-1, Corning). The $H^{18}O$ (97.2%) was obtained from MSD Isotopes, Montreal, Canada. Deuterium oxide (99.8%) was obtained from Stohler Isotope Chemicals, Waltham, MA.

Liquid chromatography

The concentrations of **I** and **II** in aqueous solutions was determined using the HPLC assay described previously by Umprayn et al. (1987). Very briefly, a PRP-1 column (10 μm particles, 150 × 4.6 mm, i.d.) was eluted with a mobile phase of 22% v/v acetonitrile in a phosphate buffer (pH 11, 0.1 M). Detection was at 214 nm and the quantifications of **I** and **II** were made on the basis of peak height measurements. A flow rate of 1 ml/min was used for the assay of **I** and a flow rate of 1.5 ml/min was used for the assay of **II**. The retention volumes were 5 ml and 6.8 ml for **I** and **II**, respectively. The remaining conditions were as described previously (Umprayn et al., 1987). The assay was stability indicating for both **I** and **II**.

Gas liquid chromatography (GLC)

The production of chloroethanol (**III**) and ethylene glycol (**IV**) was monitored by gas-liquid chromatography (GLC) on a Varian 3700 using a 10% carbowax 20M stationary phase on 80–100 mesh Chromasorb Q. Isothermal conditions of 130 °C and 150 °C were used for the analysis of **III** and **IV**, respectively. The temperatures of the injector and the flame ionization detector were 160 °C and 220 °C, respectively. Standard curves of **III** and **IV** were prepared in water and quantification was based on peak height measurements.

Gas chromatography-mass spectrometry (GC-MS)

These techniques were also used to monitor the production of **III**, **IV** and **V** arising from the degradation of **I**. Low and high resolution spectra were obtained using a Nermag R10-10 quadrapole mass spectrometer which was interfaced with a Girdel model 31 gas chromatograph. The chromatography was performed on an SE 30/Chromosorb column (1.52 m × 4.6 mm, i.d.). A temperature gradient of 80–200 °C was used with a ramp rate of 5 °C/min. Chemical ionization (CI, NH_3) and electron ionization (EI, 70 mV) were performed by direct probe insertion.

Chloride Specific Electrode

An Iotrode Chloride Selective Electrode (Model AB110, Newport Beach, CA) was used in conjunction with an Orion digital pH meter (model 701). Calibration curves of potential (E) versus log[NaCl] were linear over the range 10^{-4}–10^{-1} M Cl^- were obtained, as reported by the manufacturer of the electrode. The ionic strength of the

calibration solutions was adjusted to 0.5 with sodium perchlorate.

Stability studies

The stability of clomesone (I) and (II) (initially 0.2 mg/ml) was determined over the pH range of 2–13. The temperature of the solutions was maintained at $25.0 \pm 0.1°C$, unless otherwise stated. The degradation of **I** and **II** was followed for at least 3 half-lives and each experiment was conducted in duplicate. The logarithm of the peak heights of **I** and **II** were plotted against time and the pseudo-first-order rate constants (k_{obs}) obtained from the slopes, using linear regression analysis. Reproducibility between runs was better than $\pm 5\%$. Unless stated otherwise, the ionic strength of the solutions was adjusted to 0.5 with sodium perchlorate. The following buffers were used to control the pH of the solutions: perchloric acid (pH 2), acetate (4–5), phosphate (6–8, 11–12), and carbonate (9–10). Three buffer concentrations (25, 40 and 60 mM) were used at each pH and the "buffer corrected" first-order rate constants were obtained from the plots of k_{obs} against buffer concentration. Sodium hydroxide was used for solutions whose pH was greater than 12. After initiation of the reaction, 20 μl samples were removed periodically and injected directly into the liquid chromatograph. The data for the pH–rate profiles were fitted to the appropriate equations using the non-linear regression analysis program, MULTI.

The generation of III was studied by GLC by allowing an aqueous solution of clomesone (26.1 mM) to degrade for 72 h. The pH of this unbuffered solution decreased from an initial value of 5 to 2.5. However, this decrease in pH was of no consequence since it had been previously determined that the rate of degradation of **I** was independent of pH over this range. *The production of IV* was studied in a similar fashion by allowing clomesone to degrade in an aqueous solution buffered at pH 11.5 with 0.06 M phosphate for 6 h. Similar experiments were also conducted in which the production of **III**, **IV** and **V** was monitored by MS or GC-MS. To monitor the production of **III** in acidic solutions, 1 mg of clomesone was dissolved in 250 μl of $H_2{}^{18}O$ and analyzed when fully degraded based on a control H_2O experiment. An aliquot of this reaction medium was then injected into the GC-MS to test for the presence of **III**. *To test for the presence of V*, the same reaction medium was inserted directly onto the probe and the water and chloroethanol (**III**) allowed to evaporate for several hours, prior to ionization. To monitor the production of **IV** in alkaline solutions, 1 mg of clomesone was dissolved in 250 μl of $H_2{}^{18}O$ in a glass ampule. The pH of this solution was adjusted to 11.5 by the addition of 3 mg of NaOH and the reaction allowed to proceed at room temperature. The solution was then analyzed by MS and GC-MS as previously described.

The production of Cl^- arising from the degradation of **I** in acidic and alkaline solutions was determined by specific ion electrode as previously described. For the acidic degradation, a 4.2 mM solution of **I** in 60 mM acetate buffer (pH 4.0, $\mu = 0.5$) was used and the chloride concentration was determined directly. For the alkaline degradation, a 4.2 mM solution of **I** in dilute NaOH (pH 11.5, $\mu = 0.5$) was used. A 10-ml aliquot was taken and the pH adjusted to 6.8 by the addition of 250 μl of sulfuric acid. This was necessary to prevent interference by the conjugate base of **I** with the potential reading of the chloride electrode. No interference by sulfate was observed as has been reported previously by Brown and Parker (1982). The rate constant for the production of chloride was calculated from the slope of $\log([Cl^-]_\infty - [Cl^-])$ vs time.

The solvent isotope effect was determined by measuring the degradation of clomesone in deuterium and protium oxides at high and low pH values. These experiments were performed in triplicate. An acidic pH of 4 was maintained with an acetate buffer (60 mM) and an alkaline pH of 11.8 was maintained with a phosphate buffer (60 mM). The pD values were calculated according to the expression $pD = pH + 0.45$ as described by Glascoe and Long (1960).

Results and Discussion

pH–rate profile of clomesone and II

The degradation of clomesone in aqueous solutions at $25.0 \pm 0.1°C$ obeyed pseudo-first-order

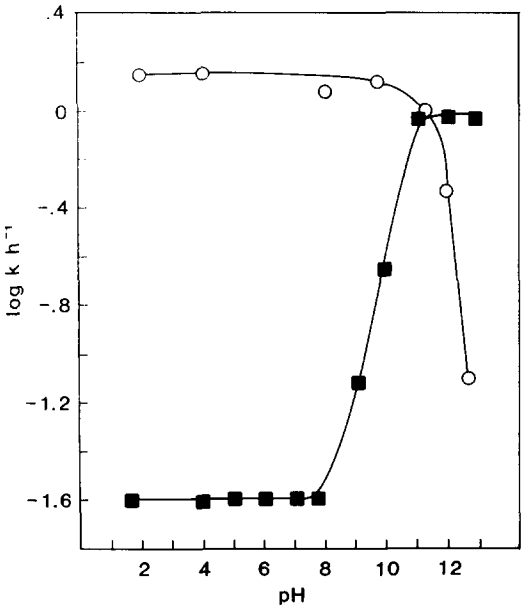
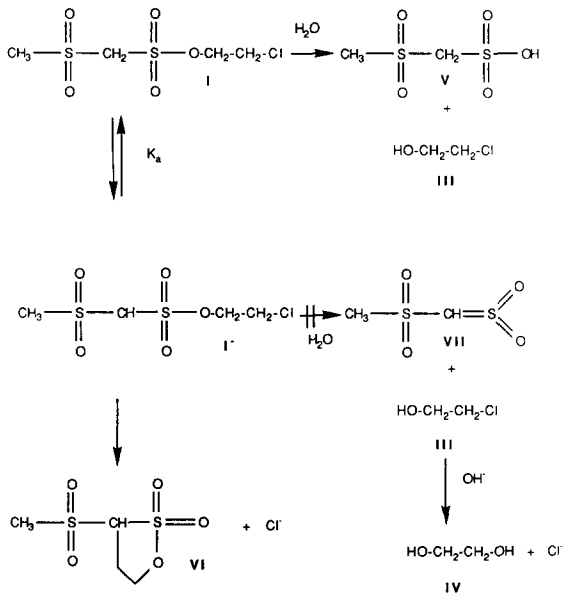


Fig. 2. pH–rate profiles for the degradation of clomesone (■) and II (○) at 25.0±0.1. (Data was obtained after extrapolation to zero buffer concentration, $\mu = 0.5$; the data points are experimental and the line has been drawn according to Eqn. 1, using the constants given in the text.)



Scheme 1. Possible reaction pathways for the degradation of clomesone in aqueous solutions.

kinetics for at least 3 half-lives over the pH region of 1.95–13.0. The pH–rate profile (Fig. 2, Table 1) was constructed from the pseudo-first-order rate constants (k_{obs}) extrapolated to zero buffer concentration. It should be noted, however, that negligible buffer catalysis was observed in the buffer systems used. The pH–rate profile (Fig. 2) was sigmoidal with pH-independent regions above pH 11 and below pH 8. The data were fitted to Eqn. 1 using the non-linear regression analysis program, MULTI, to obtain the following values: $k_1 = 2.22 \times 10^{-2}$ h^{-1}, $k_2 = 1.20$ h^{-1} and $K_a = 2.40 \times 10^{-11}$ ($pK_a = 10.62$).

TABLE 1

Pseudo-first-order rate constants for the degradation of clomesone in aqueous solution at 25.0 ± 0.1 °C, μ = 0.5, after correction for buffer effects

Buffer	pH	$k_{obs} \times 10^2$ [a] (h^{-1})	$t_{1/2}$ (h)
Perchlorate	1.95	2.51	27.6
Acetate	4.00	2.25	30.8
	5.00	2.27	30.5
Phosphate	6.00	2.45	28.3
	7.00	2.50	27.7
	7.70	2.52	27.5
Carbonate	9.00	5.90	11.8
	10.00	23.7	2.92
Phosphate	11.00	94.4	0.73
	11.80	120	0.58
Sodium hydroxide	13.00	120	0.58

[a] Mean of two determinations.

$$k_{obs} = (k_1[H^+] + k_2K_a)/(K_a + [H^+]) \quad (1)$$

As can be seen from Fig. 2, there is a sharp increase in the rate of degradation of clomesone above pH 8 which can be attributed to dissociation of the methylene group at higher pH values (see Scheme 1). The pK_a of 10.62 determined from the kinetic data is in excellent agreement with the value of 10.71 determined previously by spectrophotometry (Umprayn et al., 1987). The shape of the pH–rate profile suggested that the degradation of clomesone follows a different mechanism at high and low pH. A number of potential mechanisms were postulated (Scheme 1) and a series of experiments were then conducted

TABLE 2

Pseudo-first-order rate constants for the degradation of ***II*** *in aqueous solution, after correction for buffer effects (25.0 ± 0.1 °C, μ = 0.5)*

Buffer	pH	k_{obs} [a] (h^{-1})	$t_{1/2}$ (h)
Perchlorate	2.00	1.49	0.47
Acetate	4.00	1.52	0.46
Phosphate	7.80	1.28	0.54
Carbonate	10.00	1.42	0.49
Phosphate	11.00	0.83	0.83
	11.50	0.45	1.54
Sodium hydroxide	12.80	0.094	7.34

[a] Mean of two determinations.

to elucidate the actual pathways for the degradation of clomesone.

In addition, the pH–rate profile for the structural analogue of clomesone, **II**, was determined at 25.0 ± 0.1° C (Table 2, Fig. 2) over the pH range of 2–13. The analysis of the pH–rate profile for **II** gave the following values, when fitted to Eqn. 1, at 25.0 ± 0.1° C and an ionic strength of 0.5: $k_1 = 1.49\ h^{-1}$, $k_2 = 9.42 \times 10^{-2}\ h^{-1}$ and $K_a = 10^{-11}$ ($pK_a = 11.0$).

The effect of pH on the degradation of both clomesone and **II** may be described by an equation of the same form (Eqn. 1). However, the relative contributions of k_1 and k_2 to the overall observed rate constant are quite different. In the case of clomesone, $k_2 \gg k_1$, whereas the converse is true of **II**. The pK_a values on the other hand are quite similar for the two compounds. These results indicate that ionization of clomesone at higher pH values facilitates degradation probably by allowing the formation of the cyclic sultone (**VI**, Scheme 1; discussion to follow). This reaction is not possible in the case of **II** since it lacks the requisite chlorine atom and in this case the rate of reaction decreases with increasing pH. However, it is interesting to note that the protonated form of **II** is more than two orders of magnitude more reactive than the neutral form of clomesone. These points will be discussed later.

Based on the work of Paborji et al. (1987) and Mori et al. (1971), and the kinetic data presented so far (Table 1, Fig. 2), the degradation of clomesone at low pH is consistent with a mechanism involving nucleophilic attack by water at the sulfonate group resulting in breakage of either a C–O or S–O bond to give compound **V** (Scheme 1). At higher pH, the ionized form of clomesone is the degrading species and the rate of degradation reaches a maximum with increasing pH until the extent of ionization approaches 100%. To account for k_2 being greater than k_1, a number of mechanisms for the degradation for the ionized form of clomesone are possible. Two possible mechanisms are shown in Scheme 1. The first would involve an E_1CB type of mechanism with the generation of **VII** (Scheme 1). The alternative mechanism would involve an intramolecular displacement of chloride to give a 5-membered cyclic sultone (**VI**). A third mechanism, which is kinetically equivalent to the spontaneous hydrolysis of clomesone anion, is the displacement of chloride by hydroxide to produce hydroxyethyl(methylsulfonyl)methanesulfonate. Hydroxide ion attack on clomesone to produce chloroethanol and **V** and can be ruled out by the fact that **II**, which in theory should be capable of undergoing a similar reaction, was not subject to base-catalyzed hydrolysis. To probe both the low and high pH reaction pathways in detail, a series of experiments were performed using a variety of analytical techniques including HPLC, GLC, GC-MS and chloride ion-specific electrodes.

Product analysis

Clomesone was allowed to degrade in aqueous solution and the amount of chloroethanol produced was determined by GLC. In this experiment, the solution was unbuffered and the pH decreased from 5 to about 2.5 due to the production of an acidic species, later confirmed as **V** (Scheme 1). This decrease in pH was not expected to have an effect on the mechanism or kinetics of degradation since the rate is independent of pH in this range (Fig. 2). The initial concentration of clomesone was 26.1 mM by HPLC and the GLC analysis revealed a final concentration of 21.1 mM chloroethanol, representing at least 80.8% conversion. The combination of analytical error and further hydrolysis of the chloroethanol (**III**) to ethylene glycol (**IV**) and chloride ion probably accounts for the ≈ 20% difference.

No chloroethanol was detected in a solution of clomesone (initially 26.1 mM) allowed to degrade completely at pH 11.5. The presence of ethylene glycol was also determined, since it is produced rapidly from the hydrolysis of chloroethanol in alkaline solutions (Brundrett, 1980). Only 2.42 mM ethylene glycol was detected, representing about 10% of the original concentration of clomesone. Since the pH of these experiments was about one unit above the pK_a of clomesone, one would expect about 10% of the drug to degrade via hydrolysis to **V** plus chloroethanol which would then be rapidly converted to ethylene glycol (Brundrett, 1980). These results tend to suggest that the ionized form of clomesone does not degrade in alkaline solutions to generate chloroethanol and/or ethylene glycol, and compound **V** (Scheme 1). These observations rule out the E_1CB mechanism that produces **III** and/or **IV** via **VII**.

The mechanism of alkaline hydrolysis of clomesone in Scheme 1 that produces **VI** and the mechanism of direct chloride displacement by hydroxide ion should produce one equivalent of chloride at a comparable rate to clomesone loss. On the other hand, the hydrolysis of clomesone below pH 8 would not result in the production of chloride, assuming that chloroethanol is stable under these conditions (Brundrett, 1980). The production of chloride was followed initially by determining the amount generated after complete degradation of clomesone in acidic and basic media. The initial concentration of clomesone was 4.24 mM in both cases. Negligible (0.048 mM) chloride was detected in the acidic solution. However, at pH 11.5, the complete degradation of clomesone was paralleled by the production of 3.3 mM chloride which is equivalent to about 80% of the initial concentration of drug. This result, however, does not differentiate between a mechanism involving direct production of chloride or one which involves the indirect production of chloride due to the hydrolysis of chloroethanol. To eliminate one of these two possibilities, the production of chloride from the degradation of clomesone and chloroethanol was followed as a function of time at pH 11.5 and $25.0 \pm 0.1°C$ and the results are shown in Fig. 3. The pseudo-first-order rate constant for the production of chloride from chloroethanol was 0.22

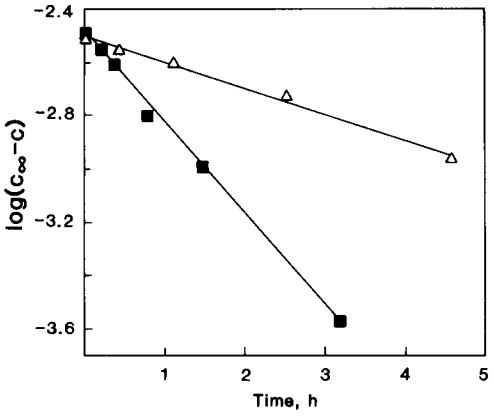


Fig. 3. Graph of $\log([Cl^-]_\infty - [Cl^-])$ vs time, describing the production of chloride from clomesone (■) and chloroethanol (Δ) at pH 11.5. ($\mu = 0.5$, $25.0 \pm 0.1°C$).

h^{-1} which is substantially less than the rate constant for the degradation of clomesone itself under these conditions ($k_{obs} = 1.06\ h^{-1}$). In contrast, the apparent rate constant for the production of chloride from clomesone at pH 11.5 was $0.77\ h^{-1}$ and approaches that for the hydrolysis of clomesone. The difference in the rate constants ($0.77\ h^{-1}$ compared with $1.06\ h^{-1}$) probably reflects other minor routes of degradation which do not involve the production of chloride. These results suggest that the chloride produced from clomesone under alkaline conditions is not produced from chloroethanol.

Mass spectrometry and GC-MS were used to further identify the products of the degradation of clomesone and to add weight to the possible pathways and mechanisms of hydrolysis of clomesone. Chloroethanol was detected in solutions of clomesone which had been allowed to degrade under acidic conditions. A molecular ion cluster (m/e 81 and 83) was detected, characteristic of the naturally occurring isotopes of chlorine present in chloroethanol (Fig. 4). When this experiment was conducted in $H_2{}^{18}O$, the molecular ion cluster was shifted by two mass units (m/e 83 and 85). This is consistent with incorporation of the labeled oxygen into the chloroethanol and suggests that clomesone degrades in acidic media via cleavage of a C–O and not an S–O bond consistent with the observation of Paborji et al., (1987) who studied the hydrolysis of a sulfamic acid ester. It should

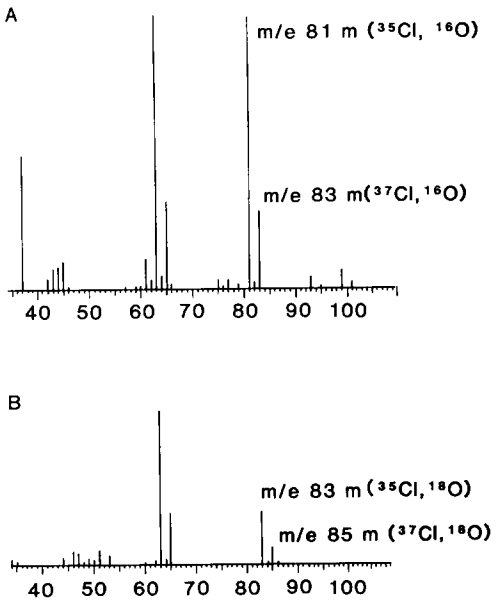


Fig. 4. Mass spectra of chloroethanol (**III**) generated from the degradation of clomesone at low pH in H_2O (A) and $H_2{}^{18}O$ (b).

be noted that oxygens attached to sulfur do not undergo solvent exchange (Mori et al., 1971; Paborji et al., 1987). This result was supported by the lack of incorporation of ^{18}O into (methylsulfonyl)methane sulfonate, **V**, which was detected by MS.

No chloroethanol and ethylene glycol were detected by GC/MS in solutions of clomesone allowed to degrade at pH 11.5, confirming the previous analyses conducted by GLC. However, a molecular ion of m/e = 201(M + 1) was detected in these solutions, consistent with the formation of the cyclic sulfonate (sultone), **VI** (Scheme 1). When run in $H_2{}^{18}O$, the molecular ion of m/e = 201 was again observed. This is consistent with no ^{18}O incorporation into this product. No signal corresponding to hydroxyethyl(methylsulfonyl)methanesulfate was observed. The fragmentation pattern of the parent ion was consistent with the structure of **VI** given in Scheme 1.

TABLE 3

Effect of halogenated nucleophiles on the stability of clomesone. (25.0 ± 0.1 °C)

Nucleophile [a]	$k_{obs} \times 10^2$ [b] (h^{-1})	$k_2 \times 10^2$ [c] ($M^{-1} \cdot h^{-1}$)	n [d]
Water	2.73	0.049	0
Perchlorate	2.47	na	na
Fluoride	3.76	2.06	2.00
Chloride	5.60	5.74	2.70
Bromide	8.70	11.9	3.50

[a] Nucleophile concentration was 0.5 M except for water.
[b] Mean of two determinations.
[c] $k_n = (k'_{obs})/[Nu]$, where [Nu] = 0.5 M except for water which is 55.51 M.
[d] Nucleophilicity index (Wells, 1963).

TABLE 4

Stability of clomesone at 25.0 ± 0.1 °C in sterile water for injection (WFI), 5% dextrose injection (D5W) and 0.9% sodium chloride injection (NS)

Diluent	$k_{obs} \times 10^2$ [a] (h^{-1})	$t_{90\%}$ (h)	$t_{1/2}$ (h)
WFI	2.59	4.07	26.8
D5W	2.55	4.14	27.2
NS	3.16	3.34	21.9

[a] Mean of two determinations.

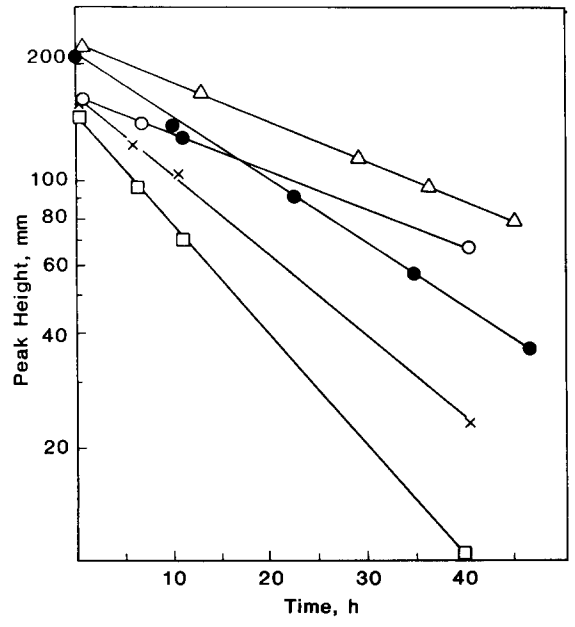


Fig. 5. First-order degradation of clomesone at 25.0 ± 0.1 °C in water (Δ) and in the presence of 0.5 M sodium perchlorate (○), sodium fluoride (●), sodium chloride (×) and sodium bromide (□).

Mechanistic studies

In preliminary experiments, sodium chloride was used to adjust the ionic strength of the solutions. However, it was observed that chloride ions were participating in the degradation of clomesone (Tables 3, 4) and subsequent experiments were conducted using sodium perchlorate to adjust the ionic strength. It should be noted that ionic strength had little effect on the degradation of clomesone and that the addition of sodium perchlorate also had little influence (Table 3, Fig. 5). The effects of sodium fluoride, chloride and bromide (all 0.5 M) on the degradation of clomesone was investigated at $25.0 \pm 0.1°C$. In these studies, the pH was not adjusted. However, this was of no consequence (Fig. 2) since the solutions were initially slightly acidic and the pH decreased during the course of the experiment.

The rate of degradation of clomesome was accelerated by the presence of the 3 halides studied (Table 3, Fig. 5) and the effect was related directly to their nucleophilicity (Wells, 1963; Swain and Scott, 1953). Swain and Scott (1953) showed that the effect of added halide nucleophiles may be described by the two-term rate law. Applying this rate law to clomesone

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

where k_{obs} is the first-order rate constant which describes the reaction of clomesone with the solvent in the absence of the nucleophile and k_n is the second-order rate constant for the reaction of clomesone with the nucleophile, Nu. The values of the k_n for each nucleophile were obtained by the appropriate analysis of the data according to Eqn. 2 and are given in Table 3 (see footnote c). The reactivity of the substrate was analyzed quantitatively (Fig. 6) according to Eqn. 3

$$\log(k_n/k_o) = ns \quad (3)$$

where n is the nucleophilicity (Wells, 1963), k_o is the second-order rate constant for the reactivity of the reference compound (generally an alkyl halide) with the reference solvent (generally water) and s is the sensitivity factor. Fig. 6 shows that log k_n was linearly related to n ($r = 0.997$) with a slope

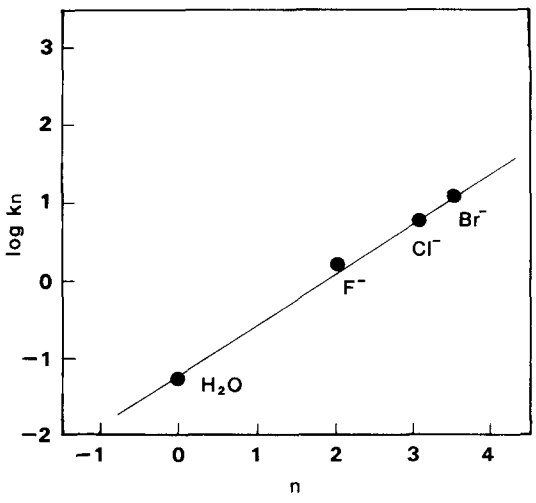


Fig. 6. Relationship between the values of k_n and the nucleophilicity (n) of various halide ions and water.

of 0.64. This value of 0.64 for s is very similar to the value of 0.66 reported by Swain and Scott (1953) for the one-step nucleophilic substitution (Sn2 mechanism) of ethyltosylate by halide ions, indicating a commonality of reaction mechanisms within the two substrates.

These results have important practical implications for the formulation of clomesone in intravenous injections. Table 4 shows that clomesone is somewhat less stable in 0.9% sodium chloride injection than that it is in sterile water or 5% dextrose injection. Assuming a value of 0.025 h^{-1} for k_{obs} in the absence of added chloride and a value of 0.056 M^{-1} h^{-1} for k_n (Table 4) for chloride catalysis, Eqn. 2 may be used to predict the stability of clomesone in any solution containing sodium chloride. Using this approach, a value of 0.033 h^{-1} for k_{obs} is obtained for the degradation of clomesone in 0.9% sodium chloride injection which agrees very well with the observed value of 0.032 h^{-1} (Table 4).

The effect of ionic strength on the degradation of clomesone was studied at pH 4.5 and 11.0 (Table 5). At low pH the effect of ionic strength was negligible, presumably reflecting the lack of charge build-up in the transition state. In alkaline solution (pH 11.0) there was an increase in the rate of degradation with increasing ionic strength (Table 5). This, and the fact that the increase in

TABLE 5

Pseudo-first-order rate constants for the degradation of clomesone in aqueous solution, as a function of pH and ionic strength (25.0 ± 0.1 °C)

pH	μ [a]	$k_{obs} \times 10^2$ [b] (h^{-1})
4.5 [c]	0.05	3.08
	0.10	3.19
	0.25	3.09
	0.50	3.09
11.0 [d]	0.15	78.0
	0.30	78.0
	0.50	101
	0.75	107

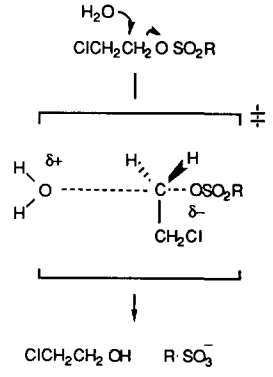
[a] Ionic strength adjusted with sodium perchlorate.
[b] Mean of two determinations.
[c] pH 4.5 maintained with a 60 mM acetate buffer.
[d] pH 11.0 maintained with a 60 mM phosphate buffer.

the rate is relatively small with increasing ionic strength, is consistent with the cyclization reaction depicted in Scheme 1.

The kinetic solvent isotope effect on clomesone hydrolysis was investigated by studying the degradation of clomesone in H_2O and D_2O. These experiments were conducted at pH 4.0 (60 mM acetate, $\mu = 0.5$) and pH 11.8 (60 mM phosphate, $\mu = 0.5$). The ratios for the rate constants of degradation in D_2O to those in H_2O were 1.12 ± 0.07 and 1.02 ± 0.04 at pH 4.0 and pH 11.8, respectively. In alkaline solutions, the lack of a significant kinetic solvent isotope effect is consistent with the unimolecular reaction (**I** to **VI**) in which water does not participate in the formation of the transition state.

The absence of a large kinetic solvent isotope effect at low pH is somewhat unexpected if the reaction proceeds by an Sn2 mechanism as opposed to an Sn1 mechanism. Two explanations of this observation are possible. One is that the cleavage of the C–O bond in the sulfonate group has substantial Sn1 character involving formation of a primary carbocation. This primary carbocation, though generally disfavoured energetically, could be stabilized by the anchimeric assistance of the neighboring chlorine atom to give a chloronium ion (Smolina et al., 1974). The involvement of a chloronium intermediate during the degradation of these types of compounds has been implicated previously by Shealy et al. (1983) and Hehre and Hiberty (1974). With this mechanism, the generation of the carbonium ion would be rate-limiting and would not require the involvement of water, consistent with the solvent isotope results. Alternatively, it is possible that water participates in an Sn2 mechanism involving an early transition state (see Scheme 2). In this early, reactant-like, transition state there is little appreciable bond development between the incoming nucleophile, water, and the carbon atom of clomesone. This mechanism is similar to that postulated by Paborji et al. (1987) to explain an identical observation for the hydrolytic degradation of NSC-329680, a sulfamic acid ester which also involved C–O bond cleavage. Consistent with the early transition state Sn2 mechanism is the good adherence of the clomesone degradation kinetics to the Swain–Scott equation and the results of the ionic strength experiments. That is, an Sn1 mechanism would be more sensitive to ionic strength effects and would be insensitive to the presence of other halide nucleophiles.

The more rapid degradation of **II** compared to clomesone at acidic pH values is consistent with the electron donating effect of the terminal methyl group of **II** ($-CH_2CH_3$) stabilizing the build up of positive charge on the methylene group in the transition state relative to the electron withdrawing terminal chloromethyl ($-CH_2CH_2Cl$) group of



Scheme 2. Mechanism for the cleavage of the C–O bond of clomesone at low pH.

clomesone. The slower degradation of **II** under alkaline relative to acidic pH conditions can be explained as follows; by either the Sn1 mechanism, which cannot be totally ruled out, or the more likely Sn2 mechanism, resulting in C–O bond cleavage, the sulfonic acid oxygen would develop a partial negative charge (Scheme 2) in the transition state. Such a negative charge build-up would not be favorable if **II** or clomesone were ionized. Thus **II**, as expected, degraded slower at pH values above its pK_a. On the other hand, for clomesone, ionization results in a change in the reaction pathway, i.e. **VI** is formed by the displacement by the methylene anion of the terminal chlorine.

In conclusion, a variety of analytical techniques was used to elucidate the kinetics, reaction pathways and mechanism of degradation of clomesone as a function of pH, ionic strength, buffer concentration and solvent isotopes at 25.0 $\pm$ 0.1°C. The pH–rate profile for clomesone was sigmoidal with $k_1 = 2.22 \times 10^{-2}$ h^{-1}, $k_2 = 1.20$ h^{-1} and $pK_a = 10.62$. The kinetic pK_a of 10.62 was in good agreement with a value of 10.71 determined previously by spectrophotometry. At pH values below the pK_a of clomesone, the main products of degradation were chloroethanol and (methylsulfonyl)methanesulfonate produced by cleavage of a C–O rather than a S–O bond of clomesone. Halide ions were found to catalyze the degradation of clomesone and their reactivities were directly related to their nucleophilicity. Based on various other mechanistic probes, the most probable mechanism for the reaction appeared to be an early transition state, Sn2 mechanism. 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. At pH values above the pK_a of clomesone, the degradation of clomesone was found to result in the production of a cyclic sulfonate (a sultone) and chloride ion. The most probable mechanism being an intramolecular nucleophilic displacement reaction.

Acknowledgements

The authors are grateful to Dr. M. Paborji for his helpful comments and suggestions, to Mrs. W. Waugh for her technical assistance and to Dr. C. Judson for the mass spectral work. This work was presented in part at the 39th national meeting of the American Pharmaceutical Association and was supported by the National Cancer Institute, Contracts NO1-CM-37562 and NO1-CM-67912.

References

Brown K.D. and Parker, G.A., Determination of chloride ions in concentrated sulphuric acid with a chloride ion-selective electrode. *Analyst*, 107 (1982) 1510–1512.

Brundrett, R.B., Chemistry of nitrosoureas. Intermediacy of 4,5-dihydro-1,2,3-oxadiazole in 1,3-bis(2-chloroethyl)-1-nitrosourea decomposition. *J. Med. Chem.*, 23 (1980) 1245–1247.

Glascoe, P.K. and Long, F.A., Use of glass electrodes to measure acidities in deuterium oxide. *J. Phys. Chem.*, 64 (1960) 188–190.

Hehre, W.J. and Hiberty, P.C., Theoretical approaches to rearrangements in carbocations. 1. The haloethyl system. *J. Am. Chem. Soc.*,, 96 (1974) 2665–2677.

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, a experimental cytotoxic agent, in water and ^{18}oxygen-enriched water. *J. Pharm. Sci.*, 76 (1987) 161–165.

Shealy Y.F., Krauth, C.A., Struck, R.F. and Montgomery, J.A., 2-Haloethylating agents for cancer chemotherapy. 2-Haloethyl sulfonates. *J. Med. Chem.*, 26 (1983) 1168–1173.

Shealy Y.F., Krauth, C.A. and Laster W.R., 2-Chloroethyl (methylsulfonyl)methane sulfonate and related (methylsulfonyl)methanesulfonates. Antineoplastic activity in vivo. *J. Med. Chem.*, 27 (1984) 664–670.

Smolina, T.A., Gopius, E.D. and Reutov, O.A., Solvolysis of 2-chloroethyl arenesulfonates, *Zh. Org. Khim.*, 10 (1974) 1121.

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) 141–147.

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.

Wells, P.R., Linear free energy relationships. *Chem. Rev.*, 63 (1963) 171–219.