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Stability and solubilization of oxathiin carboxanilide, a novel anti-HIV agent

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Summary

Oxathiin carboxanilide (benzoic acid, 2-chloro-5-[[(5,6-dihydro-2-methyl-1,4-oxathiin-3-yl)carbonyl]amino]isopropyl ester (NSC 615985; **I**)) is a novel inhibitor of HIV which is currently undergoing preclinical evaluation as an anti-AIDS agent. The purpose of this study was to generate preliminary information on the stability, degradation products, and solubility of oxathiin carboxanilide in order to develop prototype parenteral dosage forms to be used in the early stages of preclinical and clinical evaluation of this candidate. A stability-indicating HPLC assay was developed for use in monitoring drug solubility and stability. The rate of degradation of **I** was determined in aqueous buffers as a function of pH and temperature. An analysis of the pH-rate profile indicates that oxathiin carboxanilide undergoes specific acid and specific base catalyzed hydrolysis in addition to a pH-independent reaction between pH 5 and 7. The maximum shelf-life of **I** in aqueous solution is $\approx$ 16 days at 25°C. In acid, the primary route of decomposition of **I** involves water addition to the oxathiin ring and subsequent ring-opening. In alkaline solution, ester hydrolysis predominates. The water solubility of **I** was found to be extremely low (1.3 μg/ml) necessitating a search for cosolvent systems or complexing agents which would provide solubilities > 5 mg/ml for toxicological studies. The desired solubility was achieved in 70% dimethylacetamide/water and 70–80% dimethylsulfoxide/water cosolvents. A more physiologically compatible extemporaneous lipid emulsion was also prepared containing 0.75 mg/ml of **I** in Liposyn II 20%. The stability of **I** in the 20% lipid emulsion was established over a period of 48 h at 25°C.

Introduction

Oxathiin carboxanilide (benzoic acid, 2-chloro-5-[[(5,6-dihydro-2-methyl-1,4-oxathiin-3-yl)carbonyl]amino]isopropyl ester (NSC 615985, **I**)) has recently been identified as a highly potent inhibitor of HIV induced cytopathicity in vitro with a point of action within the virus reproductive cycle which differs from that of active nucleosides (Bader et al., 1990; Schultz et al., 1990). This compound has therefore been selected by the NCI for preclinical development and as a potential candidate for clinical evaluation. Before **I** could be further evaluated in vivo, a suitable intravenous formula-

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tion had to be developed. Thus, preformulation studies were undertaken to characterize the compound's solubility and other physical properties, solution stability, and degradation products.

Materials and Methods

Chemicals

Oxathiin carboxanilide (**I**) and 2-chloro-5-([(5,6-dihydro-2-methyl-1,4-oxathiin-3-yl)carbonyl]amino]benzoic acid (**II**) were supplied by the National Cancer Institute (Bethesda, MD). 5-Amino-2-chlorobenzoic acid was purchased from Aldrich Chemical Co. (Milwaukee, WI). Liposyn II 20% was purchased from Abbott Laboratories (North Chicago, IL). Hydroxypropyl-β-cyclodextrin (Molecusol^{TM}) was a gift from Pharmatec, Inc. (Alachua, FL). All other compounds were reagent grade obtained from commercial sources and were used as received without purification. Aqueous solutions were prepared using deionized water.

Analytical instrumentation

All pH measurements were performed using a PHM82 standard pH meter (Radiometer America, Cleveland, OH) and a Ross® combination pH electrode (Orion Research, Boston, MA). HPLC analyses were conducted using one of two modular systems. One system employed a model M-45 pump, a model 441 detector operated at 254 nm, and a model 740 Data Module (Waters Associates, Milford, MA). The other system consisted of two model 110B pumps linked to a model 421A Controller (Beckman Instruments, San Ramon, CA), a Waters model 480 variable-wavelength detector and a Waters model 730 Data Module integrator. The HPLC columns employed were a Beckman Ultrasphere-ODS-5 μm, 4.6 × 150 mm column or Supelco LC-18-S 5 μm, 4.6 × 250 mm column with acetonitrile/water mobile phases at pH 4. LC/MS determinations were conducted using a Vestec Model 201 instrument (Vestec Corp., Houston, TX) under discharge assisted thermospray ionization conditions. ^{1}H-NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 using tetramethylsilane as an internal standard on a Model NR/200 FTNMR spectrometer (IBM Instruments Inc.). Emulsion particle size determinations were conducted by photon correlation spectroscopy on a Brookhaven Model BI-90 90° fixed-angle spectrometer (Brookhaven Instruments Corporation, Holtsville, NY) equipped with an He-Ne laser light source of 632.8 nm wavelength. The instrument was operated in the standard mode, which assumes a log-normal particle size distribution in the sample. Measurements were made at 25°C and at least ten readings were taken for each sample.

Solubility studies

The solubility of **I** in various solvents was determined by adding an amount of drug well in excess of its expected saturation solubility in the solvent system under investigation to glass vials containing solvent. Samples ($n = 1$–4) were rotated in a 25 or 37°C water bath for at least 1 day (2 days for emulsion solubilities). Aliquots of the samples were filtered with 0.45 μm syringe filters (Gelman, Acro LC 3A), diluted and analyzed by HPLC. Successive aliquots of filtrate were analyzed until a constant solution concentration was attained, as **I** was found to adsorb to filter membranes from aqueous solutions. Solubilities were determined in propylene glycol/ethanol/water, dimethylacetamide (DMA)/water, and dimethylsulfoxide (DMSO)/water cosolvents as well as in water and Liposyn II 10% and 20% emulsions. Solubilities of **I** were also determined in aqueous solutions containing various concentrations of hydroxypropyl-β-cyclodextrin. Typically, the coefficient of variation in solubility determined for replicates in a given solvent was $\approx$ 10% and was independent of the magnitude of the solubility.

Partition coefficient determinations

Octanol/water and soybean oil/water partition coefficients were determined by combining saturated solutions of drug in either octanol or soybean oil (5 ml) with deionized water (5 ml) in 15 ml centrifuge tubes. The tubes were placed in a 25°C water bath, vortexed, centrifuged at 3000 rpm for 5 min, and then kept in the water bath for 1 day at 25°C. The separated phases were removed with a disposable pipette. n-Octanol was evaporated to dryness under nitrogen and the

residue was reconstituted with mobile phase and analyzed by HPLC. The soybean oil solution was diluted with ethanol followed by subsequent dilution in HPLC mobile phase. The aqueous solutions were analyzed directly by HPLC.

Kinetic methods

Kinetic studies in dilute aqueous solutions were conducted over a temperature range of 25°–70°C (±0.1°C) in 0.01 μ buffers (Perrin and Dempsey, 1974) varying in pH. Additional studies were conducted in dilute (0.1 and 0.01 N) HCl and NaOH solutions. Due to the extremely low aqueous solubility of **I**, the concentrations used for these studies were ≤ 1 μg/ml. **I** was first dissolved in acetonitrile and diluted 1000-fold with the appropriate buffer to make 1 l. 2-ml aliquots of these solutions were transferred to glass ampules which were sealed and stored in water baths or controlled temperature chambers. Ampules were removed at appropriate intervals and the solutions were adjusted to pH 4 then diluted in 20% CH_3CN/H_2O for analysis by HPLC. At least three standard solutions were used in the HPLC analyses covering the expected sample concentration range (50–500 ng/ml). Peak heights were linear with concentration over this range with coefficients of variation in response factors (peak ht/concentration) typically < 5% and always < 10%. First-order rate constants were calculated from the decline in drug concentration with time using nonlinear least squares regression analysis.

The stability of **I** in dimethylacetamide was also determined at an initial drug concentration of 65 mg/ml and over a temperature range of 25–70°C. Solutions were stored in ampules and monitored over a 6 month period. Additional initial rate kinetic studies were conducted to identify and quantify the degradation products formed from **I** in 40% THF/water at pH 1 and pH 12 and 50°C. Solutions for these studies contained 175–350 μg/ml of drug.

Preparation and stability of extemporaneous emulsion formulations

Emulsions containing **I** at a concentration of 0.75 mg/ml were prepared by slowly adding 1 ml of a stock solution of drug in either DMSO or DMA (75 mg/ml) to 100 ml of Liposyn II 20% with continuous shaking or stirring. These preparations were stored at 25°C and aliquots were taken at various times over a 48 h period to determine drug concentration by HPLC and mean particle size by photon correlation spectroscopy. Emulsions supersaturated with respect to drug were also prepared by slowly adding 150 μl of a 350 mg/ml solution of **I** in DMA to 5 ml of Liposyn II 10% or 20% with vigorous mixing.

*Analysis of **I** in emulsion formulations*

A solid-phase extraction technique was developed to separate the drug from the lipid components of emulsions prior to HPLC analysis. A portion of emulsion containing drug was filtered through a 5 μm membrane filter (SM type, Millipore Corp., Bedford, MA) or, in solubility studies, a 0.2 μm Nylon filter (Acrodisc, Gelman Sciences, Ann Arbor, MI). A 200 μl aliquot of filtered emulsion was then diluted with 5 ml absolute ethanol and 250 μl of this solution was applied to a pre-wetted Sep-pak C_{18} cartridge (Waters Associates, Milford, MA). The drug was eluted with 10 ml of 60% acetonitrile/pH 4 phosphate buffer and analyzed directly by HPLC. Recovery of drug from spiked emulsions over a concentration range of 0.09–0.75 mg/ml was 101% (±1.3%, SD).

Isolation/synthesis of degradation products

*pH 1 degradation product (benzoic acid, 2-chloro-5-[3-hydroxy-2-(2-hydroxyethylthio)-2-butenoyl]aminoisopropyl ester) (**III**)* The major degradation product formed in a solution of **I** (198 mg) in 200 ml 40% THF (pH 1) after 3 days reaction at 50°C was isolated by preparative chromatography (Partisil 10 ODS-3 column, 22 mm × 25 cm, Whatman, Inc., Clifton, NJ) using 50% acetonitrile/water as a mobile phase. LC/MS under discharge assisted thermospray ionization conditions gave *m/z* (ion, %): 372 ($MH^+ - 2$ for ^{35}Cl, 20), 374 ($MH^+ - 2$ for ^{37}Cl, 9), 373 ($M^{+\cdot}$ for ^{35}Cl, 5.3), 375 ($M^{+\cdot}$ for ^{37}Cl, 1.6); ^{1}H-NMR ($CDCl_3$): δ 1.3 (d, 6H, $(CH_3)_2C$), 2.3 (s, 3H, CH_3), 2.7–3.1 (m, 2H, CH_2O), 4.3–4.6 (m, 2H, CH_2S), 5.2 (m, 1H, COOCH), 7.2–7.8 (m, 3H, Ar-H), 8.6 (s, 1H, NH).

*5-Amino-2-chlorobenzoic acid, isopropyl ester, HCl salt (**IV**).* A 1.7 g sample of 5-amino-2-chlo-

robenzoic acid was placed in 200 ml isopropanol, 2 ml conc. sulfuric acid was added as a catalyst, and the suspension was refluxed in a Soxhlet extraction apparatus containing 4 Å molecular sieves until HPLC analysis indicated that product formation was complete (several days). The reaction mixture was neutralized with 1 N NaOH and filtered to yield 1.76 g crude product. A 0.5 g portion of the crude product was purified by extraction from ethyl acetate/heptane with 0.5 N HCl followed by adjustment of the combined aqueous layers to a pH of 6.1 and extraction into ethyl acetate. The organic extract was washed with deionized water and solvent was removed on a rotary evaporator. The oil residue was redissolved in isopropanol and adjusted to an apparent pH of 1 with 1 N HCl. Solvent was again removed and the solid was crystallized from CH_3CN. Sublimation in vacuo at 50 °C resulted in a white crystalline solid (270 mg). Equivalent weight (titration with 0.1 N NaOH), 251.6 (theory = 250.1); HPLC purity, > 99.6% (determined from peak areas at 254 nm); mp, 160.5–163°C (recrystallized melt); ^{1}H-NMR (DMSO-d_6): δ 1.3 (d, 6H, $(CH_3)_2C$), 5.1 (m, 1H, COO-CH), 6.6–7.2 (m, 3H, Ar-H).

Results and Discussion

Solubility and partitioning behavior

The intrinsic solubility of oxathiin carboxanilide in water was found to be extremely low (1.3 μg/ml) – well below the desired concentration for preclinical toxicology studies of > 5 mg/ml. This, coupled with the absence of ionizable functional groups, renders **I** unsuitable for formulation in classical aqueous systems. Given the molecular structure and the relatively low melting point (129–131 °C) of oxathiin carboxanilide, the low aqueous solubility may be due to the hydrophobic nature of the compound. This was supported by the partition coefficients of **I** which were determined at 25°C in octanol/water and soybean oil/water to be 8.0×10^3 ($\pm 0.7 \times 10^3$, SD) and 3.6×10^3 ($\pm 1.1 \times 10^3$, SD), respectively. These data suggested that solubilization could be achieved by any of several techniques which are sensitive to solute lipophilicity, including the use of mixed co-solvent systems, complexation, and emulsion formulations. All of these techniques were explored in this study.

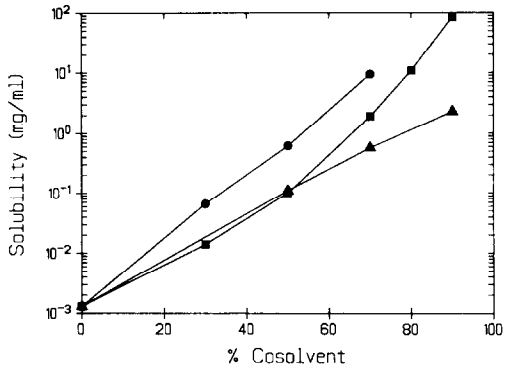


Fig. 1. Solubility of oxathiin carboxanilide vs % (v/v) co-solvent at 25°C: (●) DMA/water; (■) DMSO/water; (▲) PG/10% EtOH/water.

Various co-solvent/water systems were explored for solubilizing oxathiin carboxanilide, including dimethylacetamide (DMA)/water, dimethylsulfoxide (DMSO)/water, and ternary co-solvent systems containing 10% ethanol and varying in their percentages of propylene glycol and water. Fig. 1 displays the results of these studies as semi-logarithmic plots of the solubility of **I** vs the % (v/v) of co-solvent (i.e., % of water miscible non-aqueous solvent). Several reports have indicated that relatively lipophilic solutes exhibit approximately exponential increases in solubility with the fraction of co-solvent (Yalkowsky et al., 1976; Yalkowsky and Roseman, 1981; Gould et al., 1984). The data in Fig. 1 are qualitatively consistent with this expectation. The highest solubilities at a given % co-solvent were obtained for the DMA/water systems, consistent with the fact that DMA is the least polar of the co-solvents investigated (Rubino and Yalkowsky, 1987).

The size and lipophilicity of oxathiin carboxanilide make it a possible candidate for solubilization via inclusion complex formation with β-cyclodextrins (Szejtli, 1982; Brewster et al., 1989). While β-cyclodextrin itself has limited utility as a solubilizing agent due to its low water solubility and associated toxicity (Frank et al., 1976), the hydroxyalkyl derivative 2-hydroxypropyl-β-cyclodextrin (HPCD) is readily soluble in water (1

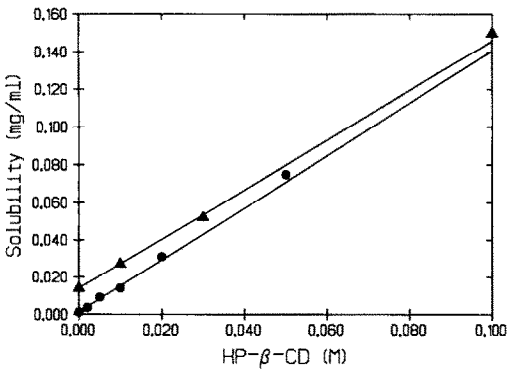


Fig. 2. Solubility of oxathiin carboxanilide in aqueous solutions containing various concentrations of 2-hydroxypropyl-β-cyclodextrin at 25°C: (●) 100% H_2O; (▲) 30% DMSO/H_2O.

g/ml) (Brewster et al., 1990), forms complexes with a variety of drugs with complexation constants similar to those found for β-cyclodextrin (Yoshida et al., 1988; Brewster et al., 1989), and has exhibited much improved safety after intravenous administration (Brewster et al., 1990). The solubility of **I** in either aqueous or 30% DMSO/water solutions containing various concentrations of HPCD is shown in Fig. 2. The linear increase in solubility with increasing HPCD concentration can be described by the following equation, which assumes that 1 : 1 complexes are formed:

$$S = S_0 + K_{1:1} \cdot S_0 \cdot [\text{HPCD}] \quad (1)$$

where S_0 is the intrinsic solubility of **I** in the solvent in the absence of complexing agent, $K_{1:1}$ is the equilibrium constant between the cyclodextrin and **I**, and [HPCD] is the molar concentration of 2-hydroxypropyl-β-cyclodextrin. The intrinsic solubility of **I** increased from 1.3 μg/ml in water to 14 μg/ml in 30% DMSO/water, but $K_{1:1}$ decreased from 1100 M^{-1} in water to 95 M^{-1}, consistent with the change in activity of **I** in the mixed co-solvent system. Thus, the combination of these two modes of solubilization resulted in only a modest advantage. The maximum solubility of **I** which could be expected assuming an HPCD solubility of 1 g/ml and a molecular weight of 1540 (corresponding to a degree of substitution of 7, 2-hydroxypropyl residues per molecule) is 0.9 mg/ml. Thus, complex formation could not be used to achieve concentrations of > 5 mg/ml.

Although it was possible to obtain solubilities exceeding 5 mg/ml in a variety of co-solvent systems, the percentage of organic solvent required was typically greater than 60 or 70%. Further increases in solubility of approx. 1.3–2-fold were attainable at 37°C. For example, the solubility of **I** in 70% DMSO/water increased from 1.9 mg/ml at 25°C to 3.8 mg/ml at 37°C. Thus, a 75% DMSO/water co-solvent system was required for attaining drug concentrations of 5 mg/ml.

Another approach which was evaluated for the solubilization of **I** was incorporation into a commercially available lipid emulsion (Liposyn II). The equilibrium solubility of **I** in 10 and 20% Liposyn II emulsions was determined to be 0.32 mg/ml and 0.76 mg/ml, respectively, at 25°C. These results were confirmed by adding an excess of drug dissolved in DMA to 10 and 20% Liposyn emulsions to a final theoretical concentration of 10.5 mg/ml and monitoring the apparent solubility over a 2 day period. Although the initial concentrations exceeded the equilibrium solubility, after 2 days the solution concentration of **I** was 0.39 and 0.80 mg/ml in the 10 and 20% emulsions, respectively. The solubilities observed in 10 and 20% Liposyn II emulsions are very close to those predicted from the soybean oil/water partition coefficient, if one assumes that the increase in solubility of the drug is due to partitioning into the internal oil phase. The predicted values were 0.36 mg/ml in a 10% emulsion and 0.73 mg/ml in a 20% lipid emulsion. These data indicate that there is little, if any, interfacial contribution to the solubilization of oxathiin carboxanilide in lipid emulsions.

Degradation of oxathiin carboxanilide

The degradation of **I** was monitored over a temperature range of 25–70°C and a pH range of 1–12. Semi-logarithmic plots of concentration remaining vs time were linear, indicating that the reaction was first-order with respect to drug. The apparent pseudo-first order rate constants determined from the slopes of such plots are shown in Table 1. The coefficients of variation de-

TABLE I

Apparent first-order rate constants for the degradation of oxathiin carboxanilide in aqueous solutions at various temperatures

Temp. (°C)	Buffer [a]	pH [b]	k_{obs} (h^{-1}) [c]
25	HCl	1.0	0.013
		2.02	3.99×10^{-3}
	Chloroacetate	2.97	9.18×10^{-4}
	Acetate	4.64	2.78×10^{-4}
		5.07	1.94×10^{-4}
	Phosphate	6.92	6.62×10^{-4}
	Borate	8.80	3.72×10^{-4}
	Carbonate	9.65	1.97×10^{-3}
	NaOH/NaCl	10.79	2.22×10^{-2}
	NaOH	12.0	0.302
37	HCl	1.0	0.036
		1.98	4.16×10^{-3}
	Chloroacetate	2.94	2.39×10^{-3}
		3.02	7.78×10^{-4}
	Acetate	4.61	3.74×10^{-4}
		5.03	2.87×10^{-4}
	Phosphate	6.91	8.10×10^{-4}
	Borate	8.72	7.92×10^{-4}
	Carbonate	9.49	5.54×10^{-3}
	NaOH/NaCl	10.35	6.32×10^{-2}
	NaOH	11.59	0.702
50	HCl	1.0	0.247
		2.00	1.48×10^{-2}
	Chloroacetate	2.86	1.91×10^{-3}
		2.98	2.00×10^{-3}
	Acetate	4.60	4.97×10^{-4}
		5.03	3.79×10^{-4}
	Phosphate	6.90	4.87×10^{-4}
	Borate	8.62	4.40×10^{-3}
	Carbonate	9.58	6.52×10^{-2}
	NaOH/NaCl	9.98	0.23
	NaOH	11.26	1.02
70	HCl	1.0	0.922
		2.02	0.117
	Chloroacetate	2.93	0.010
		3.00	0.013
	Acetate	4.62	1.36×10^{-3}
	Phosphate	6.97	1.32×10^{-3}
	Borate	8.49	0.036
	Carbonate	9.43	0.106
	NaOH/NaCl	9.55	0.676
	NaOH	10.82	3.67

[a] Buffers were 0.01 ionic strength (Perrin and Dempsey, 1974) with the exception of the pH 1 solutions which were 0.1 μ.
[b] Values reflect the pH at the temperature listed.
[c] Data represent 1–2 independent determinations.

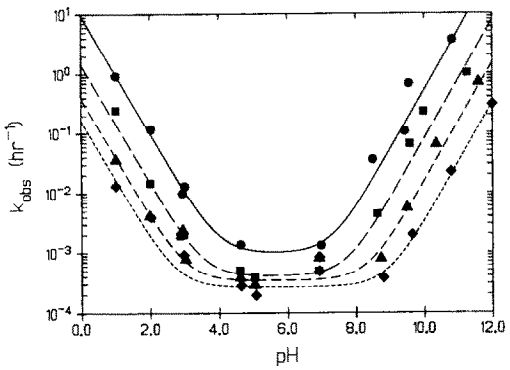


Fig. 3. pH-rate profiles for the degradation of oxathiin carboxanilide at 25 °C (◆); 37 °C (▲); 50°C (■); and 70 °C (●).

termined for these rate constants averaged 10–15% – somewhat larger than typically seen by the authors in related studies. A possible explanation for the higher than usual uncertainties in these parameters is that minor but not negligible adsorption of **I** to the walls of the glass ampules occurred. In a separate study of this phenomenon at a similar concentration (1 μg/ml), 7 ± 4% (SD, $n = 5$) loss of **I** due to adsorption was observed at room temperature. As no measures were taken to prevent such adsorption, it probably contributed to decreased precision in the data.

Fig. 3 displays the pH-rate profiles generated from the data in Table 1. Oxathiin carboxanilide exhibits maximum stability in aqueous solutions between pH 5 and 7 with an estimated shelf-life (time required for 10% decomposition) of ≈ 16 days at 25°C. The pH-rate profiles were analyzed according to Eqn 2:

$$k_{obs} = k_{H^+}\cdot[H^+] + k_{H_2O}\cdot[H_2O] + k_{OH^-}\cdot[OH^-] \quad (2)$$

where k_{H^+}, k_{H_2O}, and k_{OH^-} are bimolecular rate constants for specific acid, neutral, and specific base catalyzed reaction pathways, respectively. The bimolecular rate constants obtained are listed in Table 2.

Enthalpies and entropies of activation were calculated for each reaction pathway from Eyring plots, shown in Fig. 4. The parameter values obtained from regression analysis of these data are

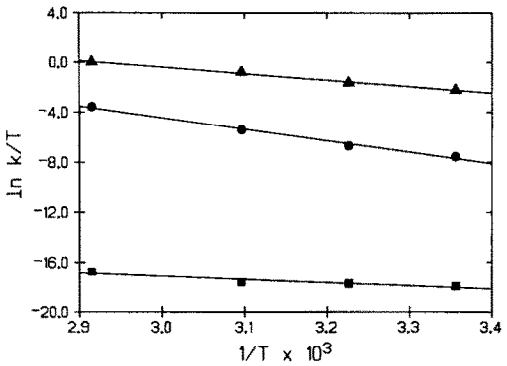


Fig. 4. Eyring plots of k_{H^+} (●), k_{H_2O} (■), and k_{OH^-} (▲) for the decomposition of oxathiin carboxanilide in aqueous solutions.

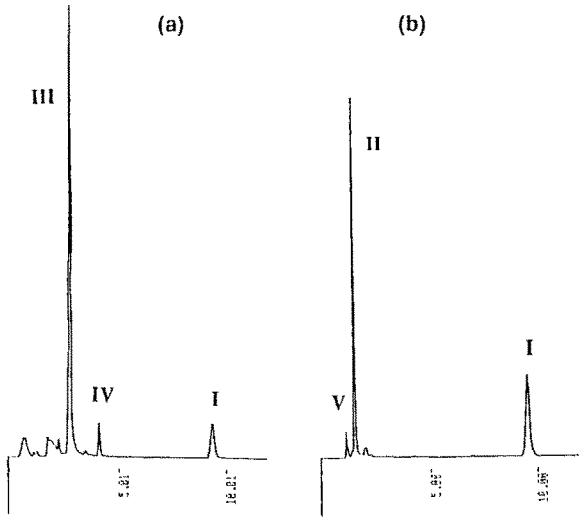


Fig. 5. (a) HPLC chromatogram of a 40% THF/water solution of **I** after 5 days at pH 1.1 and 50 °C; (b) HPLC chromatogram of a 40% THF/water solution of **I** after 1 day at pH 12.6 and 50 °C.

also listed in Table 2. It is evident from the small enthalpy of activation for the neutral pathway that the decomposition of **I** exhibits only a small dependence on temperature in the pH range of optimum stability.

Identification of oxathiin carboxanilide decomposition products

Shown in Fig. 5 are chromatograms of reaction mixtures of **I** in 40% THF/water after storage for (a) 5 days at pH 1.1 and 50°C; and (b) 1 day at pH 12.6 and 50°C. The structures of the major decomposition products and proposed pathways for the specific acid and specific base catalyzed hydrolysis of **I** are shown in Scheme 1. The major decomposition pathway under acidic conditions is believed to involve acid catalyzed water addition to the oxathiin enol ether double bond resulting in the formation of a cyclic hemiacetal which then decomposes to the ring-opened enol product (**III**). LC/MS and ^{1}H-NMR spectra were consistent with the proposed structure. A similar reaction has been reported for the acid catalyzed degradation of carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide), a widely used fungicide (Suokas et al., 1984). Compound **III** further degrades in acidic solution to form a product with the same retention time as the isopropyl ester of 5-amino-2-chlorobenzoic acid (**IV**). Initial rate studies indicated

TABLE 2

Bimolecular rate constants and thermodynamic parameters for the hydrolysis of oxathiin carboxanilide

Rate constant [a]	k (l mol^{-1} h^{-1}) (±SD)				Activation parameters [b]	
	25 °C	37°C	50 °C	70°C	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (eu)
k_{H^+}	0.16 (±0.05)	0.4 (±0.1)	1.5 (±0.3)	10.0 (±2.0)	18 (±1)	−18 (±4)
k_{H_2O}	5×10^{-6} ($\pm1\times10^{-6}$)	6×10^{-6} ($\pm1\times10^{-6}$)	7×10^{-6} ($\pm2\times10^{-6}$)	1.8×10^{-5} ($\pm0.7\times10^{-5}$)	5 (±1)	−82 (±4)
k_{OH^-}	32 (±9)	60 (±14)	145 (±33)	346 (±78)	10.3 (±0.5)	−33 (±2)

[a] Eqn 1.

[b] $\ln(k/T) = 31.95 + \Delta S^{\ddagger}/1.987 - \Delta H^{\ddagger}/(1.987T)$.

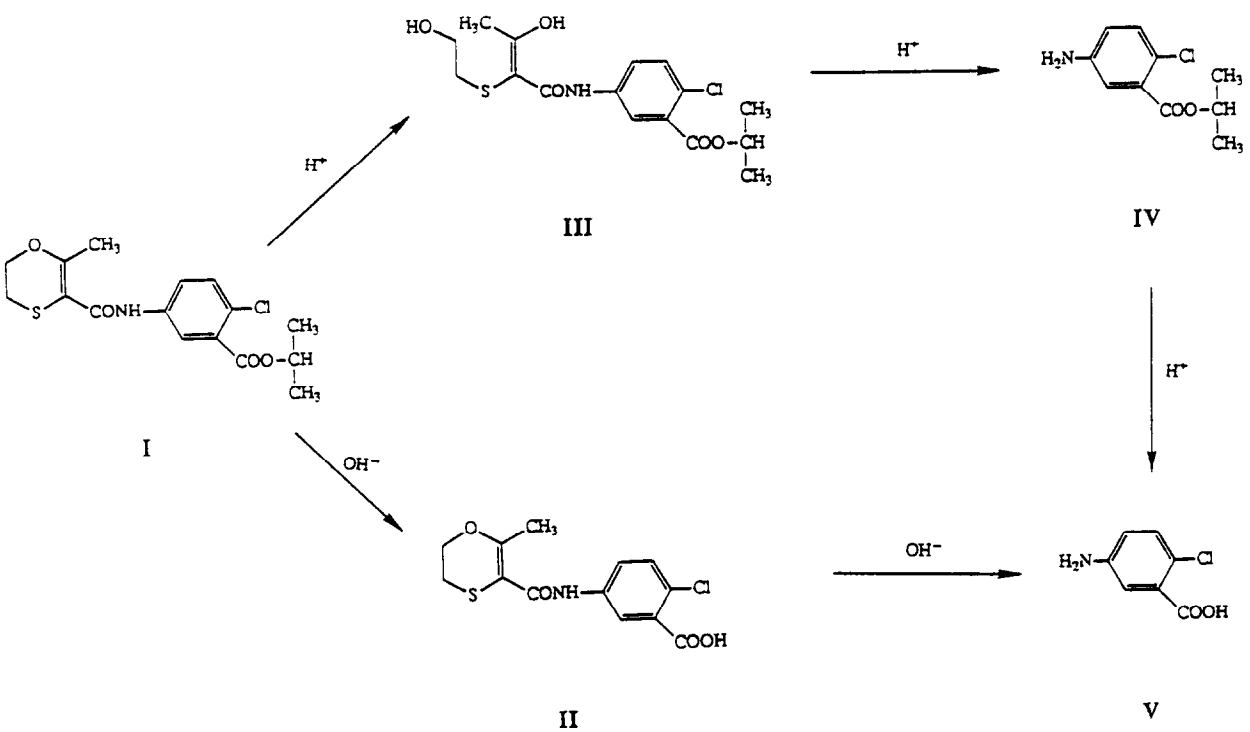
that there is a lag time in the formation of **IV**, so its formation occurs more rapidly from **III** than from **I**. Although mass balance studies were not performed at pH 1.1, the peak area (by HPLC) of **III** formed after 90% of **I** had decomposed represented 75% of the initial peak area of **I**. Since the UV spectra of **I** and **III** should be similar, this suggests that the formation of **III** is the predominant route for loss of **I**.

At pH 12 the principal degradation product exhibited the same retention time by HPLC and the same molecular weight, determined by LC/MS, as the ester hydrolysis product (**II**). An initial rate study of the formation of **II** at pH 12 indicated that ester hydrolysis accounted for > 90% of the overall decomposition reaction under these conditions.

Stability of emulsion formulations of oxathiin carboxanilide

A number of previous investigators have demonstrated the utility of parenteral emulsions for overcoming solubility and/or stability related problems of drugs which are to be administered intravenously (Fortner et al., 1975; El-Sayed and Repta, 1983; Singh and Ravin, 1986; Tarr et al., 1987). For the early stages of evaluation of new drug candidates, it is advantageous to prepare extemporaneous formulations using commercially available parenteral fat emulsions (El-Sayed and Repta, 1983). Such formulations may be prepared by slowly adding a concentrated solution of the drug in a suitable solvent directly to the emulsion with rapid mixing. Such a formulation was developed for the oxathiin carboxanilide for possible use in early clinical studies.

In order to prepare an emulsion formulation, 1 ml of a 75 mg/ml solution of **I** in DMSO or DMA was added slowly to 100 ml of Liposyn II 20% with continuous mixing to produce a final formulation containing 0.75 mg/ml of drug and 1% co-solvent. Addition of a smaller volume (0.375 ml) of a more concentrated solution of **I** in DMSO or DMA (200 mg/ml) resulted in precipitation of



Scheme 1. Pathways for the specific acid and specific base catalyzed hydrolysis of **I**.

the drug which required several hours shaking to re-dissolve. The stability of drug was monitored over a period of 6 months in DMA solution and for 48 h after preparation of emulsions. In DMA, there was no evidence of decomposition of **I** after 6 months at 25 and 37°C; < 3% decomposition at 50°C; and ≈ 26% decomposition at 70°C. The apparent shelf-life of **I** in extemporaneous emulsions prepared using Liposyn II 20% was 62 h, suggesting that the decomposition is accelerated in the emulsion formulation relative to aqueous solution.

The mean particle sizes in various emulsions were determined at the time of preparation and after storage at 25°C. The mean particle size of Liposyn II without added drug was 274 ± 2 nm, consistent with previous results using the same method (Caldwell and Li, 1989). Immediately after the addition of drug in DMSO, the mean particle size was 277 ± 2 nm. The mean size remained constant over several weeks storage at 25°C. The chemical and physical stability data suggested that extemporaneous emulsion formulations of **I** could be prepared and used up to 2 days without excessive degradation or physical deterioration. Such information may be valuable if oxathiin carboxanilide is selected as a candidate for further clinical testing after the preclinical studies have been completed.

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