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An HPLC and spectrophotometric study of the hydrolysis of ICRF-187 (dexrazoxane, (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane) and its one-ring opened intermediates

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Abstract

The hydrolysis of the cardioprotective agent dexrazoxane ICRF-187 ((+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane) and its one-ring open intermediates has been examined over a wide pH range using both HPLC and spectrophotometric methods. The isolation of these one-ring open intermediates by HPLC allowed a study of their hydrolysis reactions to be carried out spectrophotometrically without interference from other competing hydrolysis reactions. In 40 mM NaOH (25°C) the half-life for the hydrolysis of ICRF-187 is 0.3 h, at pH 7.4 (37°C) 9.3 h and in 1.0 M HCl (25°C) 560 h. However, at pH 7.4 (37°C) the half-time for the production of the fully ring-opened hydrolysis product, which is presumably the active metal ion binding form of the drug, is 28 h. A full kinetic analysis was carried out within a reaction scheme that included both parallel and consecutive reaction steps and allowed the determination of the individual ring-opening reaction rate constants.

Key words: ICRF-187; Dexrazoxane; Antioxidant; Radical; Doxorubicin; Hydrolysis; Razoxane; Imide

1. Introduction

ICRF-187 has shown great promise in phase III clinical trials (Speyer et al., 1988, 1992; Koning et al., 1991) where it is being used to protect against the dose-limiting cardiotoxicity caused by doxorubicin. There is now a considerable body of evidence to indicate that anthracycline-induced toxicity is due to iron-based oxidative stress (Myers et al., 1982; Demant and Jensen, 1983; Gianni et al., 1983; Gutteridge, 1984; Halliwell and Gutteridge, 1989) on the relatively unprotected heart muscle. ICRF-187 is the (+)-(*S*)-enantiomer of the racemic ICRF-159 (razoxane) (Fig. 1), which upon full hydrolysis gives the (*S*)-enantiomer of ICRF-198 (ADR-925). ICRF-198

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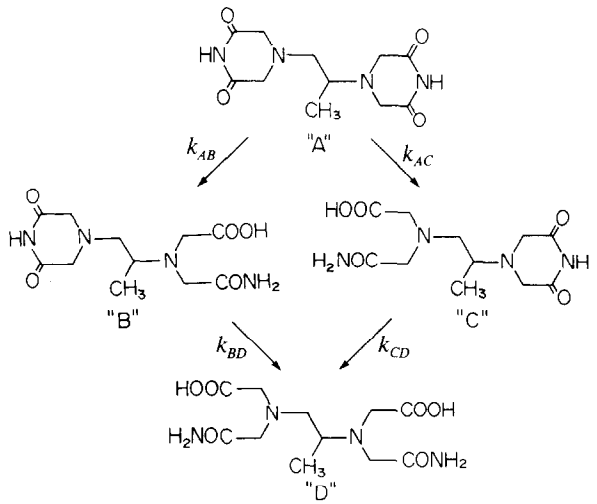

Fig. 1. Reaction scheme for the hydrolysis of ICRF-187. If the pH of the reaction mixture is held constant the rate constants for the conversion of **A** into **B** and **C** (k_{AB} and k_{AC}) and **B** into **D** (k_{BD}) and **C** into **D** (k_{CD}) are pseudo-first order.

is structurally similar to EDTA and is also a powerful metal ion chelating agent (Huang et al., 1982). While the charged ring-opened hydrolysis product ICRF-198 is likely too polar to cross cell membranes, the less polar ICRF-159 is able to (Dawson, 1975). Thus, ICRF-187 may be acting by diffusing into the cell, hydrolyzing to its ring-opened metal-ion binding form and chelating free iron or iron bound to the iron-anthracycline complex (Myers et al., 1982; Gianni et al., 1983; Gutteridge, 1984; Hasinoff, 1989, 1990b), preventing iron-based oxygen radical production. Since the active form of ICRF-187 is likely its ring-opened hydrolysis products, it is important to characterize this hydrolysis reaction. In a previous spectrophotometric study the hydrolysis of ICRF-187 showed both base- and water-catalyzed terms (Hasinoff, 1990a,b). However, since the spectra of the rings-closed parent and intermediates are so similar, it was not possible to characterize individual ring opening reactions. We have also shown that ICRF-187 is enzymatically hydrolyzed by the enzyme dihydropyrimidine amidohydrolase, which is present in the liver and kidney, but not in the heart (Hasinoff et al., 1991; Hasinoff, 1993). The products of the hydrolysis of ICRF-187 have been separated by HPLC and characterized by fast atom bombardment mass spectrometry (Burke et al., 1991). The hydrolysis of ICRF-187 under non-physiological conditions has been followed, but the two single-ring open intermediates were not identified or quantitated (Sisco and Stella, 1992a,b). This study quantitates the hydrolysis of ICRF-187 and its ring-opened intermediates under physiological conditions and over a wide pH range both spectrophotometrically and by using improved HPLC methodology.

2. Materials and methods

2.1. Materials

The ICRF-187 (ADR-529) and ADR-925 were a gift from Adria Laboratories (Columbus, OH). Na_2EDTA was obtained from BDH Chemicals (Toronto, Canada), HPLC grade methanol from Mallinckrodt (Mississauga, Canada), Tris from Sigma (St. Louis, MO) and KH_2PO_4 from Fisher Scientific (Fair Lawn, NJ). All other chemicals were of the highest grade available. Water for HPLC and reagent preparation was deionized and then glass distilled. Non-linear least-squares curve fitting and plotting were performed using SigmaPlot 5.0 (Jandel Scientific, San Rafael, CA).

2.2. Methods

The HPLC analyses were carried out as previously described (Hasinoff, 1993) on a Waters μBondapak C18 10 μm 3.9 × 300 mm reversed phase column. The compounds **B**, **C**, and **D** (Fig. 2) were determined at 205 nm and **A** at 215 nm because of its higher extinction coefficient (Hasinoff, 1990a, 1993). Identification of peaks for **B** and **C** were based on their elution order, as determined from the results of the HPLC-FAB mass spectrometry study of Burke et al. (1991). A clean separation of all four species was achieved with the following elution profile. Starting from 100% 0.5 mM Na_2EDTA (pH 4.5), methanol was increased linearly from 0 to 10 min to 8%, and at 10 min increased linearly to 80% at 20 min. In the absence of EDTA in the eluent, the strongly metal ion complexing product **D** (ADR-925) scavenged iron from the flow system interfering with its determination and the determination of the other hydrolysis products. Baseline separation of the reactant and three products was achieved and they eluted in the order **D** (3.4 min), **B** (5.2 min), **C** (6.6 min) and **A** (16.6 min). Peak area counts, which were shown to be linear with concentration over the concentration range used (1–32 nmol for **A**, 0.2–22 nmol for **B** and **C**, and 0.8–108 nmol for **D**), were used to quantitate all species. The HPLC calibration factors for ICRF-187 (**A**) and ADR-925 (**D**) were obtained from pure samples of these compounds. Calibration factors for **B** and **C** were obtained from a ^{1}H-NMR determination of the concentrations of each component of a partially hydrolyzed ICRF-187 sample which was simultaneously determined by HPLC, as previously described (Hasinoff, 1993).

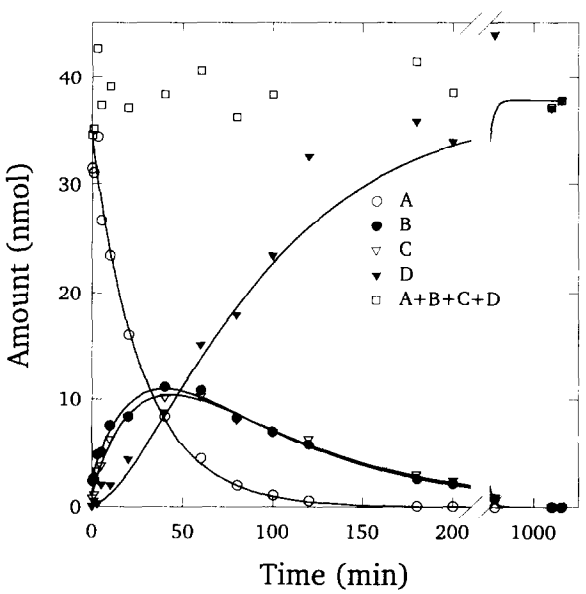

Fig. 2. Time course for the hydrolysis of ICRF-187 in 40 mM NaOH at 25°C as quantitated by HPLC. The amount in nmol refers to the amount contained in 10 μl of the reaction mixture applied to the column: (○) **A**, (●) **B**, (▽) **C**, (▼) **D**, and (□) the sum of $A + B + C + D$. The solid lines are least-squares calculated for the reaction scheme shown in Fig. 1 and were obtained from a fit to Eq. 5–8 with the best fit parameters in Table 1. The least-squares calculated values of A_0, B_0 and C_0 were 34.2 ± 0.5, 2.6 ± 0.2 and 1.1 ± 0.2 nmol, respectively. D_0 was set equal to 0 as no D could be detected at the start of the reaction.

The spectrophotometric analyses were conducted on a computer-controlled Cary 1 UV-visible double beam spectrophotometer in 1 cm stoppered silica cells in a thermostatted cell holder. Complete UV spectra were run at timed intervals and absorbances at a given wavelength were read from a family of spectra to obtain the absorbance-time curves. Pure samples of **B** and **C** were obtained by collecting and combining 0.8 mL peak fractions of a partially NaOH-hydrolyzed ICRF-187 sample injected onto the column. As monitored spectrophotometrically, approximately the center 70% of each peak was collected. The partially hydrolyzed ICRF-187 was prepared by adding 40 μl of 1 M NaOH to 500 μl of 5 mg/ml ICRF-187, letting the reaction proceed for 40 min at 25°C, quenching it with 40 μl of 1 M HCl, and storing the resulting mixture at 4°C to prevent further hydrolysis. The **B** and **C** so obtained were rechromatographed to check their purity. The purity of **B** was greater than 99.8% and contained no detectable amounts of **C** or **D**, and **C** was 98.9% pure with the remaining 1.1% being **B** due to a small amount of tailing that occurred at the high sample loadings used. Quantitation of **B** and **C** allowed determination of their extinction coefficients at 227 nm of 19 and 22 mM^{-1} cm^{-1}, respectively, in 47 mM NaOH. The buffer denoted Tris/NaCl was 100 mM Tris-

HCl/80 mM NaCl, Tris was 150 mM Tris and phosphate buffer was 50 mM KH_2PO_4-KOH.

3. Results and discussion

3.1. Hydrolysis of ICRF-187 and its one-ring open intermediates B and C under basic conditions

The time course of the hydrolysis of ICRF-187 in 40 mM NaOH at 25°C is shown in Fig. 2. **A** was observed to decrease with time, whereas intermediates **B** and **C** increased at approximately equal rates, reached a maximum and then decreased. The final hydrolysis product **D** was observed to increase sigmoidally throughout the course of the reaction. Assuming pseudo-first order rate constants in the reaction scheme shown in Fig. 1, the solutions to the linear first-order differential rate equations, Eq. 1–4, are given by Eq. 5–8 for the general case where initial concentrations of A_0, B_0, C_0 and D_0 are present at $t = 0$

$$-\mathrm{d}A/\mathrm{d}t = k_{AB}A + k_{AC}A \quad (1)$$

$$\mathrm{d}B/\mathrm{d}t = k_{AB}A - k_{BD}B \quad (2)$$

$$\mathrm{d}C/\mathrm{d}t = k_{AC}A - k_{CD}C \quad (3)$$

$$\mathrm{d}D/\mathrm{d}t = k_{BD}B + k_{CD}C \quad (4)$$

$$A = A_0 e^{-(k_{AB}+k_{AC})t} \quad (5)$$

$$B = \frac{k_{AB}A_0}{(k_{BD} - k_{AB} - k_{AC})} e^{-(k_{AB}+k_{AC})t} + \left\{ B_0 - \frac{k_{AB}A_0}{(k_{BD} - k_{AB} - k_{AC})} \right\} e^{-k_{BD}t} \quad (6)$$

$$C = \frac{k_{AC}A_0}{(k_{CD} - k_{AB} - k_{AC})} e^{-(k_{AB}+k_{AC})t} + \left\{ C_0 - \frac{k_{AC}A_0}{(k_{CD} - k_{AB} - k_{AC})} \right\} e^{-k_{CD}t} \quad (7)$$

$$D = A_0 + B_0 + C_0 + D_0 - A - B - C \quad (8)$$

A good fit to the exponential decay (Eq. 5) was obtained for A in a preliminary analysis. This indicates that the hydrolysis reactions are truly pseudo-first order. In the final analysis the whole data set for A–D as a function of time was concatenated and fitted, using non-linear least-squares analysis, to Eq. 5–8 simultaneously. The best-fit calculated values of each reactant or product are shown in Fig. 2 and the best-fit parameters are given in Table 1. A statistical weight that was equal to the reciprocal of the square of the HPLC conversion factor was used in the analysis to compensate for the differing extinction coefficients.

Isolation of the hydrolysis intermediates **B** and **C** (Fig. 1) by HPLC allowed a direct independent spectrophotometric determination of the pseudo-first order rate constants k_{BD} and k_{CD}. As shown

Table 1
Rate constants for the ring opening reactions of ICRF-187 and its intermediates [a]

Reaction conditions and method	k_{AB} (h^{-1})	k_{AC} (h^{-1})	k_{BD} (h^{-1})	k_{CD} (h^{-1})	$t_{1/2}$ [b] (h)
40 mM NaOH, 25°C HPLC	1.07 ± 0.06	1.08 ± 0.05	0.80 ± 0.04	0.75 ± 0.04	0.32
40 mM NaOH, 25°C spec.	–	–	1.21 ± 0.01	0.91 ± 0.01	–
pH 7.39 Tris/NaCl, 37°C HPLC	0.052 ± 0.001	0.022 ± 0.001	0.040 ± 0.001	0.082 ± 0.004	9.3
pH 7.40 phosphate, 37°C HPLC	0.059 ± 0.002	0.020 ± 0.002	0.037 ± 0.001	0.073 ± 0.001	8.8
pH 7.40 phosphate, 37°C spec.	–	–	0.080 ± 0.001	0.183 ± 0.001	–
pH 7.00 Tris, 25°C HPLC	0.0095 ± 0.0009	0.0028 ± 0.0009	–	–	71
0.001 M HCl, 25°C HPLC	0.0041	0.00135	–	–	127
0.01 M HCl, 25°C HPLC	0.00316	0.00100	–	–	167
0.1 M HCl, 25°C HPLC	0.00196	0.00099	–	–	235
1.0 M HCl, 25°C HPLC	0.00095	0.00028	–	–	563

[a] The errors quoted are fitting errors only from nonlinear least-squares analysis.
[b] The $t_{1/2}$ are half-times for the disappearance of ICRF-187 (**A**), from the reaction mixture and are calculated from $0.693/(k_{AB} + k_{AC})$.

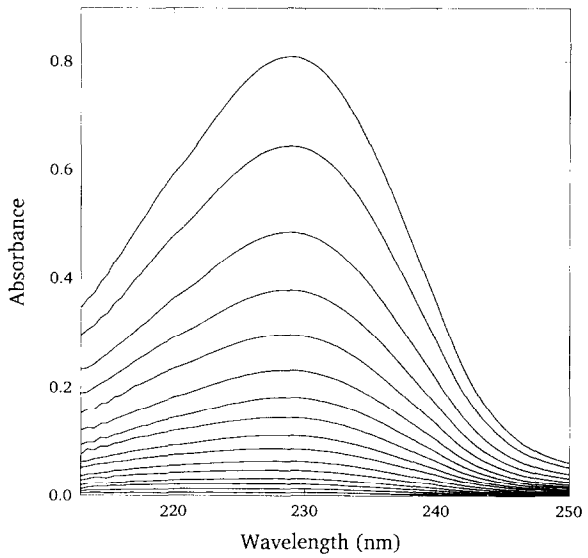

Fig. 3. Spectrum of 43 μM ***B*** in 40 mM NaOH at 25°C, recorded at intervals of 12 min. The absorbance decreased continuously with time. The spectra were corrected for a sloping baseline by subtracting from each spectrum the spectrum observed at 192 min.

in Fig. 3, in 40 mM NaOH the spectrum of **B**, which exhibited a peak at 229 nm (228 nm for **C** and **A**, data not shown), decreased with time. The spectrum is very similar to that found previously for ICRF-187 (Hasinoff, 1990a). The spectrum of **C** (data not shown) displayed very similar behaviour with time. The change in absorbance with time at 227 nm is shown in Fig. 4 for both **B** and

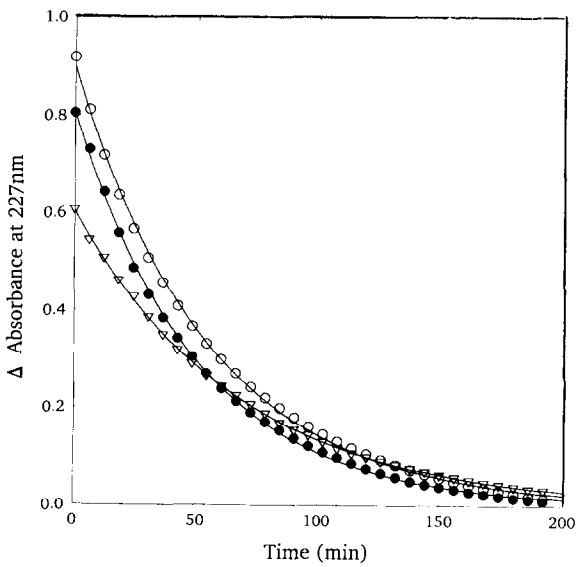

Fig. 4. Change in absorbance with time for **A** (○), **B** (●), and **C** (▽) in 40 mM NaOH at 25°C. The solid lines are least-squares calculated for a fit to the three-parameter exponential decay (Eq. 9). The initial concentrations of A, B, and C were 25, 43, and 28 μM, respectively.

C, and for comparison **A** as well. Since both **B** and **C** undergo hydrolysis without forming intermediates, their hydrolysis is a simple first-order reaction and thus the absorbance was fitted to Eq. 9.

$$A = A_0 e^{-k_{obs}t} + A_\infty \quad (9)$$

where A, A_0 and A_∞ are the absorbance at time t, 0, and infinity, respectively, and k_{obs} denotes an observed pseudo-first order rate constant. The rate constants k_{BD} and k_{CD} are thus obtained directly by non-linear least-squares fitting to Eq. 9 and may be compared (Table 1) to those obtained by analysis of the data of Fig. 2. The agreement of k_{BD} and k_{CD} using these two methods is reasonable considering the differences in the methods used and the errors inherent in fitting complex multiparameter functions (Eq. 5–8). Also shown in Fig. 4 is a fit to Eq. 9 for the decrease in absorbance for ICRF-187 (**A**). Fitting these data to a single exponential decay equation is approximate at best as an inspection of Eq. 5–8 indicates that the absorbance decrease would best be described by an equation with three exponential terms and extinction coefficients for each of **A–D**. The fact that under these reaction conditions the decrease in absorbance of **A** can be approximately fitted to a single exponential equation indicates that k_{AB}, k_{AC}, k_{BD} and k_{CD} are similar in magnitude as was assumed previously (Hasinoff, 1990a). Thus, the 'apparent' first-order rate constant for the decrease in absorbance (Fig. 4) when ICRF-187 is hydrolyzed is 1.1 h^{-1} and is very similar in size to the values (0.75–1.07 h^{-1}) of the first-order rate constants measured by HPLC (Table 1).

3.2. Hydrolysis of ICRF-187, B and C at pH 7.4 and 37°C

The time course for the hydrolysis of ICRF-187 at pH 7.39 in Tris/NaCl buffer at 37°C (Fig. 5) was much slower than in 40 mM NaOH, even though the general shape of the progress curves is similar. It is noteworthy that while in 40 mM NaOH the curves for both **B** and **C** (Fig. 2) follow

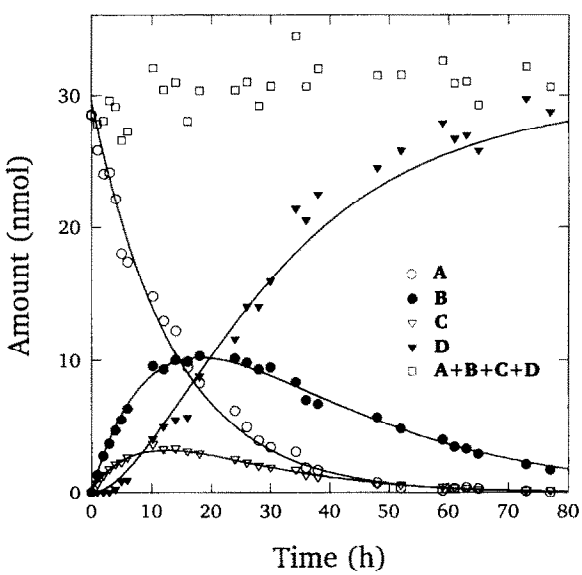

Fig. 5. Time course for the hydrolysis of ICRF-187 in Tris/NaCl buffer (pH 7.39) at 37°C as quantitated by HPLC. The amount is the number of nmol contained in 10 μl of the reaction mixtures applied to the column. (○) **A**, (●) **B**, (▽) **C**, (▼) **D**, and (□) the sum of $A + B + C + D$. The solid lines are least-squares calculated for the reaction scheme shown in Fig. 1 with the best fit parameters of Table 1. The least-squares calculated values of A_0, B_0 and C_0 were 29.8 ± 0.3, 0.0 ± 0.1 and 0.24 ± 0.1 nmol, respectively. D_0 was set equal to 0 as no D could be detected at the start of the reaction.

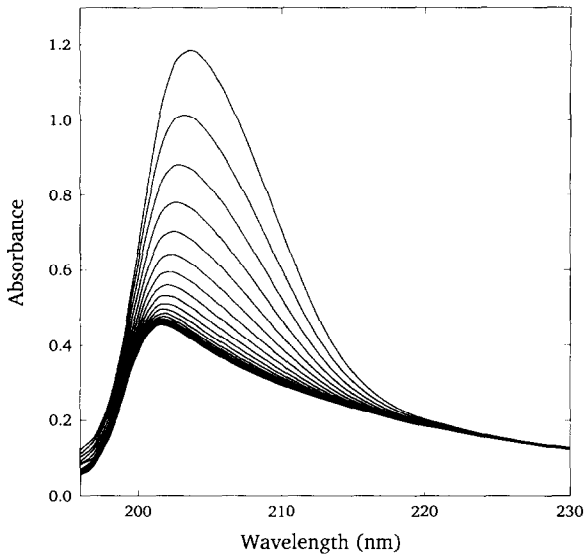

Fig. 6. Spectrum of **C** in phosphate buffer at 37°C (pH 7.40) recorded at intervals of 1.5 h. The absorbance decreased continuously with time over the whole spectral region.

similar time courses, at pH 7.39 significantly greater amounts of intermediate **B** accumulate in the reaction mixture. The fit of all the data to Eq. 5–8 yielded the best fit lines of Fig. 5 and the best fit parameters given in Table 1. The time course of ICRF-187 hydrolysis was also deter-

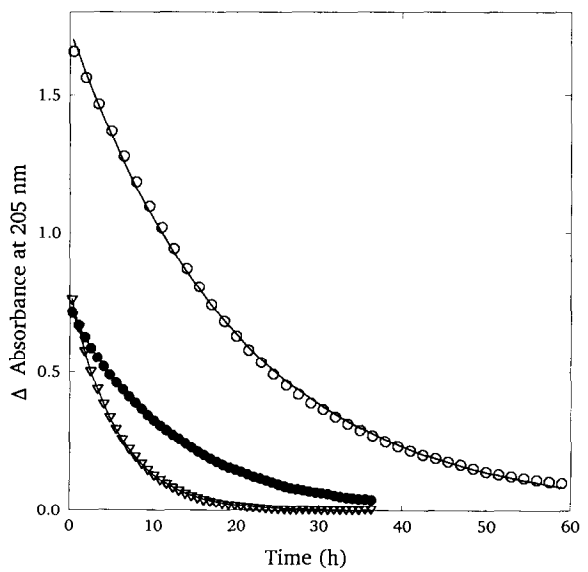

Fig. 7. Change in absorbance with time for **A** (ICRF-187) (○), **B** (●), or **C** (▽) in phosphate buffer at 37°C. The solid lines are least-squares calculated for the three-parameter decay (Eq. 9). The value of A_∞ has been subtracted from each data point. The initial concentration of A was 80 μM.

mined in phosphate buffer by HPLC as well (data not shown) yielding the best fit parameters given in Table 1.

The isolation of **B** and **C** by HPLC also allowed a direct spectrophotometric determination of the rate constants k_{BD} and k_{CD}. The spectral changes that occur with time accompanying the hydrolysis of **C** are shown in Fig. 6. Under these conditions the peak maxima for each of **A**, **B** and **C** were found at 204, 203 and 203 nm, respectively. The changes in absorbance with time at 205 nm for **B** and **C**, and for comparison **A** as well, are given in Fig. 7. The absorbance-time data were fitted by non-linear least-squares analysis to Eq. 9 to obtain the rate constants k_{BD} and k_{CD} of Table 1. The apparent rate constant obtained from fitting the decrease in absorbance of ICRF-187 (Fig. 7) of 0.050 h^{-1} is approximately in the middle of the range of values (0.020–0.073 h^{-1}) of k_{AB}, k_{AC}, k_{BD} and k_{CD} (Table 1), determined by HPLC under the same conditions. The agreement of the spectrophotometric and HPLC-determined values is reasonable considering the strong pH dependence of the rate constants at this pH (Hasinoff, 1990a), the strong temperature dependence of these reactions (Hasinoff, 1990a), and as noted previously, the errors involved in fitting complex multiparameter equations.

3.3. Hydrolysis of ICRF-187 under acidic conditions at 25°C

Since the previous spectrophotometric study (Hasinoff, 1990a) had indicated that the hydrolysis of ICRF-187 was considerably slower under relatively acidic conditions, the stability of ICRF-187 was studied at several concentrations of HCl by HPLC. Hydrolysis in HCl was studied at various times up to 218 h at 25°C and resulted in hydrolysis of **A** ranging from 69 to 27% for the lowest to highest acid concentrations studied (Table 1). The extreme slowness of the reactions under these conditions resulted in no detectable amounts of **D** being produced over the time these reactions were followed and thus Eq. 5–8 could not be used to analyze the time course of the hydrolysis of ICRF-187. Instead A was fitted to Eq. 5 by non-linear least-squares analysis to obtain the single parameter $k_{AB} + k_{AC}$. From Eq. 2 and 3 under initial velocity conditions, the terms $k_{BD}B$ and $k_{CD}C$ are not significant. Thus, values of dB/dt and dC/dt were obtained from slopes of plots of B and C against time. The ratio of dB/dt to dC/dt is given by:

$$(dB/dt)/(dC/dt) = k_{AB}/k_{AC} \quad (10)$$

Thus, with the ratio of the slopes from Eq. 10 and the parameter $k_{AB} + k_{AC}$, the individual values of k_{AB} and k_{AC} given in Table 1 were obtained. These results indicate that over the range of acidities studied, ICRF-187 is the most stable in 1.0 M HCl with a half-life for its hydrolysis of 563 h at 25°C.

In a previous spectrophotometric study (Hasinoff, 1990a) it was shown that the sigmoidal pH dependence of the hydrolysis of ICRF-187 could be explained by a scheme in which the neutral form of the ring undergoes attack by hydroxide, but with the anionic form being unreactive. Additionally, at pH 7.4 water also acts as a nucleophile. The pK_a for deprotonation of the imide hydrogen was determined to be 9.6 at 37°C (Hasinoff, 1990a). These conclusions were subsequently substantially confirmed (Sisco and Stella, 1992a,b), but a more detailed analysis of the pH-rate profiles including additional protonated and deprotonated species in the reaction scheme was given. This study, by allowing the quantitation of the intermediate hydrolysis products **B** and **C**, has allowed a determination of the individual rate constants of Fig. 1.

Quantitation of intermediates **B** and **C** also allowed a determination of their differential rate of production. It is seen from Table 1 that under basic conditions the ratio k_{AB}/k_{AC} is close to unity and thus both rings undergo hydrolysis at almost the same rate. Under basic conditions the rate of hydrolysis of the one-ring opened intermediates occurs at about 70% of that for **A**. However, the ratio of rates for ring opening of **C** compared to **B** is (from the ratio k_{CD}/k_{BD}) close to unity. However, at a pH closer to neutral the ratio of k_{AB}/k_{AC} and k_{CD}/k_{BD} is 2.4 and 2.1, respectively. This change in the ratio from unity at high pH is probably due to reaction of differ-

ent protonated species and also to water acting as a nucleophile at lower pH. It can also be seen from the rate constants of Table 1 that the reason for the preferential accumulation of **B** in the reaction mixture at neutral pH is not only because it is produced more rapidly than is **C**, but also because it decays more slowly than **C**. Under basic conditions, by contrast, both **B** and **C** are produced at the same rate and also decay at the same rate. Thus, under these conditions neither intermediate preferentially accumulates in the reaction mixture. Under acidic conditions (Table 1) the ratio k_{AB}/k_{AC} is slightly larger than at pH 7.4 with the ratio having values of 3.0, 3.2, 2.0 and 3.4 in 0.001, 0.01, 0.10 and 1.0 M HCl, respectively, indicating that protonation of ICRF-187 with a pK_a of 2.5 (Hasinoff, 1990a; Sisco and Stella, 1992b) for the protonated piperazine nitrogen affects the rate of hydrolysis.

The data of Table 1 indicate a strong pH dependence for hydrolysis of ICRF-187 with the half-time varying by a factor of 1800 over the pH range studied. The strong pH dependence is due to the differing reactivities of the various ionizable species present over the pH range (Hasinoff, 1990a; Sisco and Stella, 1992a,b) studied and is also due to water acting as a nucleophile at neutral pH and below, where the hydroxide concentration is very small. The ionization of the imide hydrogens under basic conditions produces a dianionic ICRF-187 species that is almost unreactive to OH^- attack (Hasinoff, 1990a; Sisco and Stella, 1992a,b). Under very acidic conditions the piperazine nitrogen protonates and this produces a positively charged ICRF-187 species which is less reactive to nucleophilic attack by water.

It can also be seen that under basic conditions the reaction of OH^- with neutral ICRF-187 is slightly faster than with the anionic **B** and **C** species. At neutral pH, however, this order is reversed and the one-ring open products undergo hydrolysis 1.6- and 1.8-times faster, as measured from the ratios k_{CD}/k_{AB} and k_{BD}/k_{AC}, respectively. Thus, under these conditions the presence of a negative charge on **B** or **C** accelerates the rate by a small amount. This result is also consistent with neutral water acting as a nucleophile at neutral pH.

In conclusion, this study has examined the stability of ICRF-187 and its hydrolysis intermediates over a wide pH range. At pH 7.4 and 37°C the half-time for the hydrolysis is 9.3 h but the half-time for the production of the fully ring-opened hydrolysis product (**D**), which is presumably the active metal ion binding form of the drug, is 28 h as can be seen from Fig. 5. Hydrolysis of ICRF-187 under acidic conditions occurs very slowly and thus storage of samples containing ICRF-187 obtained from pharmacokinetic studies, for example, under acidic conditions is indicated from this study.

Acknowledgments

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References

Burke, T.G., Lee, T.D., Van-Balgooy, J. and Doroshow, J.H., Characterization of the aqueous decomposition products of (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)-propane (ICRF-187) by liquid chromatographic and mass spectral analysis. *J. Pharm. Sci.*, 80 (1991) 338–340.

Dawson, K.M., Studies on the stability and cellular distribution of dioxopiperazines in cultured BHK-21S cells. *Biochem. Pharmacol.*, 24 (1975) 2249–2253.

Demant, E.J.F. and Jensen, P.K., Destruction of phospholipids and respiratory-chain activity in pig-heart submitrochondrial particles induced by an adriamycin-iron complex. *Eur. J. Biochem.*, 132 (1983) 551–556.

Gianni, L., Corden, B.J. and Myers, C.E., The biochemical basis of anthracycline toxicity and anti-tumor activity. *Rev. Biochem. Toxicol.*, 5 (1983) 1–82.

Gutteridge, J.M.C., Lipid peroxidation and possible hydroxyl radical formation stimulated by the self-reduction of a doxorubicin-iron(III) complex. *Biochem. Pharmacol.*, 33 (1984) 1725–1728.

Halliwell, B. and Gutteridge, J.M.C., *Free Radicals in Biology and Medicine*, 2 Edn., Clarendon, Oxford, 1989, pp. 489–492.

Hasinoff, B.B., The enzymatic ring-opening reactions of the chiral cardioprotective agent (+) (*S*)-ICRF-187 and its

(−) (*R*)-enantiomer ICRF-186 by dihydropyrimidine amidohydrolase. *Drug Metab. Dispos.*, 21 (1993) 883–888.

Hasinoff, B.B., The hydrolysis-activation of the doxorubicin cardioprotective agent ICRF-187 ((+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane). *Drug Metab. Dispos.*, 18 (1990a) 344–349.

Hasinoff, B.B., The interaction of the cardioprotective agent ICRF-187 ((+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane), its hydrolysis product ICRF-198, and other chelating agents with the Fe(III) and Cu(II) complexes of adriamycin. *Agents Actions*, 26 (1989) 378–385.

Hasinoff, B.B., The iron(III) and copper(II) complexes of adriamycin promote the hydrolysis of the cardioprotective agent ICRF-187 ((+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane). *Agents Actions*, 29 (1990b) 374–381.

Hasinoff, B.B., Reinders, F.X. and Clark, V., The enzymatic hydrolysis-activation of the adriamycin cardioprotective agent (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane. *Drug Metab. Dispos.*, 19 (1991) 74–80.

Huang, Z.-X., May, P.M., Quinlan, K.M., Williams, D.R. and Creighton, A.M., Metal binding by pharmaceuticals: 2. Interactions of Ca(II), Cu(II), Fe(II), Mg(II), Mn(II) and Zn(II) with the intracellular hydrolysis products of the antitumor agent ICRF-159 and its inactive homologue ICRF-192. *Agents Actions*, 12 (1982) 536–542.

Koning, J., Palmer, P., Franks, C.R., Mulder, D.E., Speyer, J.L., Green, M.D. and Hellmann, K., Cardioxane-ICRF-187, towards anticancer drug specificity through selective toxicity reduction. *Cancer Treat. Rev.*, 18 (1991) 1–19.

Myers, C.E., Gianni, L., Simone, C.B., Klecker, R. and Greene, R., Oxidative destruction of erythrocyte ghost membranes catalyzed by the doxorubicin-iron complex. *Biochemistry*, 21 (1982) 1707–1713.

Sisco, J.M. and Stella, V.J., An unexpected hydrolysis pH-rate profile, at pH values less than 7, of the labile imide, ICRF-187: (+)-1,2-bis(3,5-dioxopiperazin-1-yl)propane. *Pharm. Res.*, 9 (1992b) 1209–1214.

Sisco, J.M. and Stella, V.J., Is ICRF-187 [(+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane] unusually reactive for an imide? *Pharm. Res.*, 9 (1992a) 1076–1082.

Speyer, J.L., Green, M.D., Kramer, E., Rey, M., Sanger, J., Ward, C., Dubin, N., Ferrans, V., Stecy, P., Zeleniuch-Jacquotte, A., Wernz, J., Feit, F., Slater, W., Blum, R. and Muggia, F., Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. *N. Engl. J. Med.*, 319 (1988) 745–752.

Speyer, J.L., Green, M.D., Zeleniuch-Jacquotte, A., Wernz, J.C., Rey, M., Sanger, J., Kramer, E., Ferrans, V., Hochster, H., Meyers, M., Blum, R.H., Feit, F., Attubato, M., Burrows, W. and Muggia, F.M., ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. *J. Clin. Oncol.*, 10 (1992) 117–127.