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Degradation of dacarbazine in aqueous solution*

BHASKER V. SHETTY,‡§ RICHARD L. SCHOWEN,‡ MILAN SLAVIK‖ and CHRISTOPHER M. RILEY†‡

‡*Department of Pharmaceutical Chemistry and the Center for BioAnalytical Research, University of Kansas, Lawrence, KS 66046, USA*
‖*Department of Medicine and Department of Veterans Affairs, University of Kansas Medical Center, Wichita, KS 67218, USA*

Abstract: The effects of initial concentration (0.05–5.0 mg ml^{-1}, 2.5 × 10^{-4}–0.025 M) (pH 1–13), buffer concentration (0.01–0.075 M), light, antioxidants and co-solvents on the degradation of dacarbazine in aqueous solution were investigated at 37°C. Liquid chromatography was used to monitor the degradation of dacarbazine as well as the appearance of degradation products. The kinetics of hydrolysis of dacarbazine in the dark were pseudo first-order and independent of the initial concentration of the drug. The degradation of dacarbazine was accelerated by light and at low concentration proceeded by pseudo zero-order kinetics. The pH-rate profiles showed that both the photolytic and the hydrolytic reactions were dependent on the ionization state of the molecule. The main degradation product of both hydrolysis and photolysis was detected by liquid chromatography and confirmed by mass spectrometry to be 2-azahypoxanthine.

Keywords: *Dacarbazine; DTIC; antineoplastic; kinetics, pH effects; co-solvent effects; hydrolysis; photolysis; solubility.*

Introduction

Dacarbazine (**1**, DTIC, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, Fig. 1) is an anticancer drug currently used for the treatment of malignant melanoma, Hodgkin's disease [1], soft-tissue sarcoma [2] and childhood solid tumours [3]. Currently the drug is administered by intravenous bolus injection or by short term intravenous infusion as either a single dose or five single daily doses repeated every 3 or 4 weeks. The drug has a very short half-life in plasma of about 35 min [4], which means that proliferative tissues may be exposed to the drug for only short periods of time. Therefore low-dose continuous infusion therapy would seem to offer some advantages over high-dose bolus injections or short-term infusions because a constant level of **1** would be maintained over long periods of time. Additional potential benefits of infusion therapy would be the reduction in the normal toxicities arising from high-dose therapy and the opportunity for the drug to be delivered to outpatients by the use of portable infusion pumps.

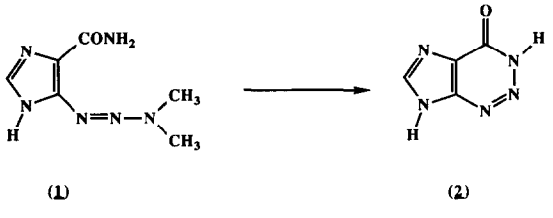

Figure 1
The structure of dacarbazine (**1**) and its main degradation product 2-azahypoxanthine (**2**).

Unfortunately, long-term continuous infusion therapy of **1** is not possible with the present commercial intravenous formulations, which have a shelf-life of only 8 h at room temperature after reconstitution. However, a clear understanding of the kinetics of degradation of **1** which is a prerequisite to the development of a stable formulation for long-term infusion therapy, is lacking.

Materials and Methods

Chemical and reagents

Dacarbazine was obtained from Sigma Chemical Company (St Louis, MO, USA) and was used as received. Dacarbazine (100 mg 10 ml^{-1} vial) injection manufactured by Quad

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†Author to whom correspondence should be addressed.
§Present address: Agouron Pharmaceuticals, 505 Coast Boulevard S, La Jolla, CA 92037, USA.

Pharmaceuticals was obtained from the University of Kansas Medical Center Pharmacy Department and was reconstituted with 5% Dextrose Injection USP. The commercial formulation also contained a citrate buffer (pH 3.5 after reconstitution) and 37.5 mg of mannitol. HPLC grade acetonitrile was obtained from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were reagent grade and were used as received from various commercial sources.

Buffer solutions

The following buffers were used to control the pH of the solutions used in the kinetic experiments: pH 1–2, HCl (0.01–0.1 M); pH 3–4, formate (0.025–0.075 M); pH 5, acetate (0.025–0.075 M); pH 6–8, phosphate (0.01–0.05 M); pH 9–11, carbonate (0.01–0.05 M); and pH 12–13, KOH (0.01–0.1 M). The ionic strength of the buffer solutions was adjusted to 0.15 with KCl. The pH was measured at the temperature of experiment with a combination pH electrode and a Model 150 Corning Ion Analyzer (Corning Scientific Instruments, Medfield, MA, USA).

Liquid chromatography

The modular liquid chromatograph comprised a Shimadzu SCL-6A system controller, a Shimadzu LC-6A solvent delivery pump, a Shimadzu SIL-6A auto-injector and a Perkin–Elmer LC235 diode-array detector. Data were collected and reduced with an Epson Equity 1+ microcomputer and Perkin–Elmer Omega 235 Software. Dacarbazine and its degradation product 2-azahypoxanthine (**2**, Fig. 1) were separated by isocratic elution (Fig. 2) on a 5-μm ODS Hypersil column (150 × 4.6 mm, i.d.) with a mobile phase of acetonitrile–phosphate buffer (0.1 M, pH 7.0) (10:90, v/v) containing 0.04 M triethylamine at a flow rate of 1.5 ml min^{-1}. Dacarbazine and **2** were detected at 330 nm (0.05 AUFS) and 250 nm (0.02 AUFS), respectively. The total run time was 3 min with **1** and **2** eluting with a retention time of 1.1 and 2.1 min, respectively (Fig. 2). Calibration curves for **1** and **2** were both linear from 0.25 to 3 μg ml^{-1} and, where necessary, samples were diluted within that range with the mobile phase. Within the linear range of the assay, the RSD of replicate injections (n = 6) was less than 2%.

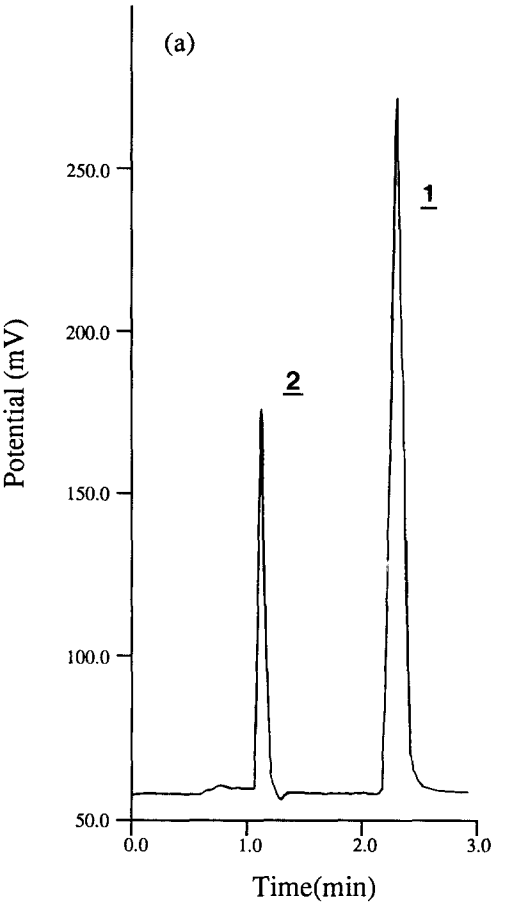

Figure 2
Separation of dacarbazine (**1**) and its main degradation product 2-azahypoxanthine (**2**) by reversed-phase liquid chromatography. (a) Standard calibration solutions of **1** and **2** both 35 μg ml^{-1}; (b) **1** in pH 10 buffer stored under fluorescent lighting (37.0 ± 0.5°C); (c) **1** in pH 10 buffer stored in the dark (37.0 ± 0.5°C). Chromatographic conditions: column, 5-μm ODS Hypersil (150 × 4.6 mm, i.d.); mobile phase, acetonitrile–phosphate buffer (0.1 M, pH 7.0) (10:90, v/v) containing 0.04 M triethylamine; flow rate, 1.5 ml min^{-1}; temperature, ambient.

Mass spectrometry

Electron ionization (EI) mass spectra of **2** in 0.001 M HCl were obtained on a Nermag (Paris, France) R10-10 quadrupole mass spectrometer with a Spectral 30 data system. Samples were evaporated from the direct insertion probe at 5°C s^{-1} to 500°C. Ionization was with 70 eV energy and 200 mA emission and the source block was kept at 250°C.

Kinetic studies

The degradation of **1** was studied in 13-ml stoppered glass test-tubes placed inside a Styrofoam box 5 cm from a normal, white, indoor fluorescent light (length 16.5 in., 15 W, Westinghouse Electric Company, Bloomfield, NJ, USA). It was found that the temperature

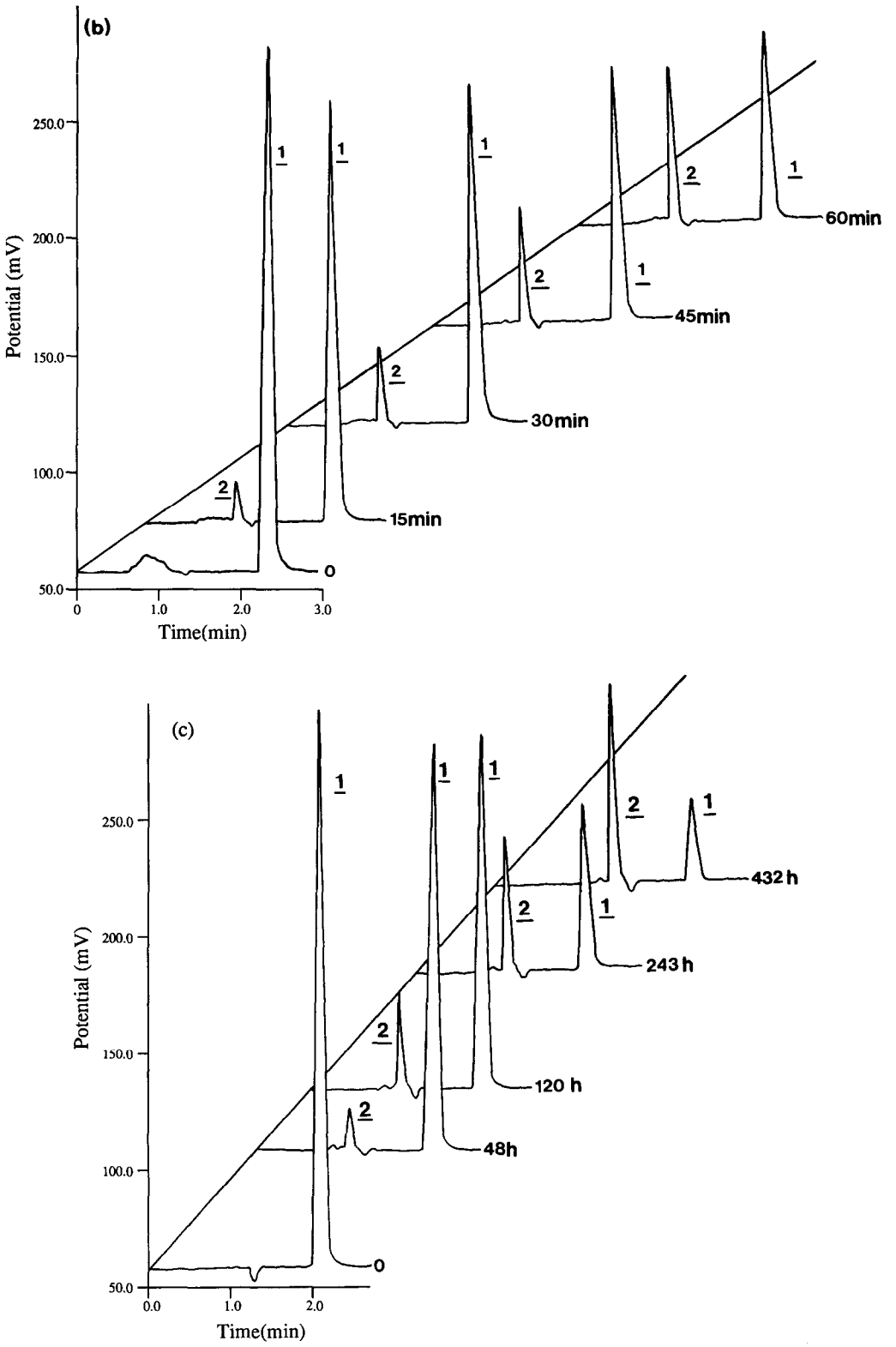

Figure 2(b) and (c)

inside the box could be maintained at 37.0 ± 0.5°C by circulating water at 30.0 ± 0.1°C through copper coils placed inside the box. Samples were placed at a fixed distance from the light source. Those samples protected from light were wrapped in aluminum foil.

The commercial formulation of **1** was reconstituted to concentrations of 0.05, 0.5 or 5 mg ml^{-1} to determine the effect of initial concentration on the rate of degradation. This study was actually conducted at 35.0° ± 0.5°C. Subsequent experiments were conducted at 37.0 ± 0.5°C using dacarbazine itself. The influence of pH (1–13), buffer type (HCl, formate, acetate, phosphate, carbonate and KOH), buffer concentration (0.01–0.10 M) and light on the degradation of **1** was studied at 37.0 ± 0.5°C and an ionic strength of 0.15. The initial concentration of **1** in these experiments was 0.05 mg ml^{-1} (2.5×10^{-4} M). A 50 μl volume of a 5 mg ml^{-1} stock solution of **1** in 0.01 M HCl was added to 4.95 ml of the appropriate, temperature-equilibrated buffer, and mixed by inversion. After removal of an initial sample, the reaction tube was placed in the light box and the reaction was followed for at least four half-lives by taking 30-μl samples at various intervals. The samples were immediately frozen at −20°C until analysis. Independent experiments confirmed that there was less than 2% degradation during the time of storage of the samples in the freezer. Immediately before analysis the samples were thawed to room temperature, diluted with 570 μl of the mobile phase and then injected into the chromatograph. The concentrations of **1** and **2** were determined by comparison with calibration curves using peak areas for quantification.

The effect of sodium bisulphite (1%, pH 4.0), sodium sulphite (0.2%), EDTA disodium salt (0.01%), ethanol (10%), ethanol–propylene glycol (10:40, v/v), and sodium carboxymethylcellulose (CMC) (0.01–1.0%, pH 4 and 7.0) on the degradation of **1** were investigated in the light. The experimental protocol to study the effect of additives and co-solvents on the degradation of **1** was essentially the same as that described above.

Spectrophotometric pK_a determinations

The pK_a values of **1** were determined spectrophotometrically at room temperature (22 ± 1°C) and 37.0 ± 0.1°C on a Hewlett–Packard 8451 diode-array spectrophotometer using the general procedures described by Albert and Sergeant [5]. Initial spectra (200–400 nm) of **1** obtained at pH 1, 7 and 13 were distinctly different (Fig. 3) and suggested at least two ionization steps. The apparent value of pK_1 was determined by adding 0.8 ml of a 0.05 mg ml^{-1} solution of **1** in water to 3.2 ml of acetate buffer (0.05 M) and measuring the absorbance at 238 nm as a function of pH (3.5–5.0). Similarly, the apparent value of pK_2 was determined by adding 0.8 ml of a 0.05 mg ml^{-1} solution of **1** in water to 3.2 ml of carbonate buffer (0.05 M) or aqueous NaOH and measuring the absorbance at 250 nm as a function of pH (10.0–12.5).

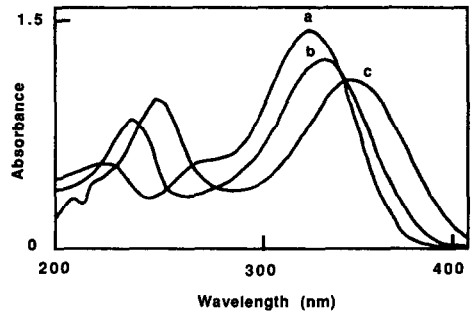

Figure 3
Spectra of **1** in 0.1 M HCl (a), phosphate buffer (pH 7.0) (b) and 0.1 M (KOH) (c) obtained by diode-array spectrophotometry.

Aqueous solubility

The aqueous solubility of **1** was determined at pH 4, 7 and 12 by adding excess solid to the appropriate buffer and allowing the suspension to equilibrate for at least 24 h in the dark. The suspension was filtered and the pH of the filtrate measured. An aliquot of the filtrate was diluted (1:50) with the mobile phase and the concentration of **1** determined by LC.

Data analysis

Pseudo first-order and pseudo zero-order rate constants were obtained by least-squares linear regression analysis of the concentration–time data. Data were fitted to the non-linear equations manually and the theoretical curves simulated using the spreadsheet program MS Works® version 2.0 and the graphics program DeltaGraph Professional® version 2.0 on a Macintosh IICX personal computer (Apple).

Results and Discussion

Effect of initial concentration

Figure 4(a) shows that the hydrolysis of **1** in

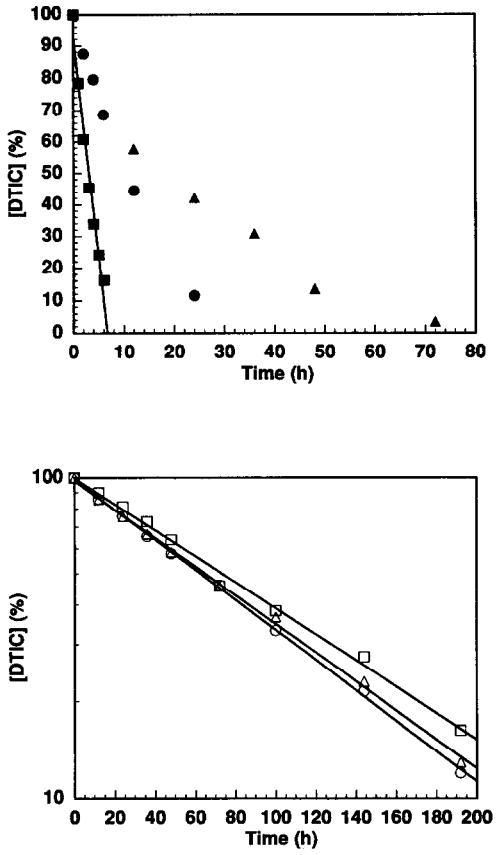

Figure 4
Effect of initial concentration and light on the degradation of **1** in citrate buffer (pH 3.5) and 35.0 ± 0.5°C. The open symbols refer to experiments conducted under fluorescent light and the closed symbols refer to experiments conducted in the dark. Concentrations: 5 mg ml^{-1}, triangles; 0.5 mg ml^{-1}, circles; 0.05 mg ml^{-1}, squares.

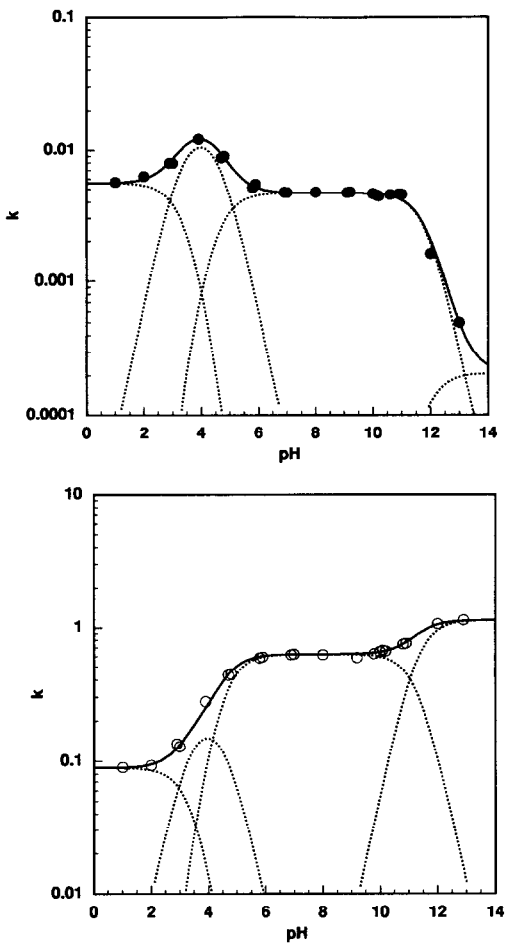

Figure 5
Log k–pH profiles for the hydrolysis and photolysis of **1** in aqueous solution (37.0 ± 0.5°C, μ = 0.15 M). The open symbols refer to experiments conducted under fluorescent light and the closed symbols to experiments conducted in the dark. The points are experimental and the lines are theoretical. The solid lines represent the overall relationship and the dotted lines represent the individual contributions to the overall rate.

the dark was pseudo first-order and independent of the initial concentration, $[\mathbf{1}]_0$, of the drug. In contrast, when **1** was exposed to fluorescent light, which was designed to simulate normal ambient conditions, the degradation kinetics were dependent on the initial concentration of the drug (Fig. 4b). At initial concentrations of less than 0.05 mg ml^{-1} (2.5×10^{-4} M) the loss of **1** followed pseudo zero-order kinetics. The reduction in the rate of photolysis at $[\mathbf{1}]_0 > 0.05$ mg ml^{-1} was attributed to absorption of the incident light by the degradation products. All further kinetic experiments were conducted with an initial concentration of 0.05 mg ml^{-1} of **1** to simplify the data analysis. The rate of degradation of **1** was considered to be the sum of the individual contributions from hydrolysis and photolysis:

$$v = k^{d}_{obs}[1] + k^{l}_{obs} \qquad (1)$$

where k^{d}_{obs} and k^{l}_{obs} are the pseudo first-order rate constant for hydrolysis and the pseudo zero-order rate constants for photolysis, respectively. When protected from light the contribution of photolysis to the overall rate of degradation was negligible and in the presence of light the contribution of hydrolysis to the overall reaction was negligible. Therefore, the two reactions could be studied independently. For convenience, the pseudo zero-order rate constant for the photolysis reaction was converted to a pseudo first-order rate constant using:

$$k'^{l}_{obs} = \frac{k^{l}_{obs}}{[1]_0} \qquad (2)$$

Effect of pH

The complete log k–pH profiles for the degradation of **1** at 37.0 ± 0.5°C in the dark and the light are given in Fig. 5. The points are experimental and the theoretical lines were obtained by manually fitting the data to equations (3) and (4), which describe the hydrolysis (d) and photolysis (l) reactions, respectively

$$k^{d}_{obs} = \frac{k^{d}_{1}[H^+]^3 + k^{d}_{2}K^{d}_{1}[H^+]^2 + k^{d}_{3}K^{d}_{1}K^{d}_{2}[H^+] + k^{d}_{4}K^{d}_{1}K^{d}_{2}K^{d}_{3}}{D} \quad (3)$$

$$k^{l}_{obs} = \frac{k^{l}_{1}[H^+]^3 + k^{l}_{2}K^{l}_{1}[H^+]^2 + k^{l}_{3}K^{l}_{1}K^{l}_{2}[H^+] + k^{l}_{4}K^{l}_{1}K^{l}_{2}K^{l}_{3}}{L}, \quad (4)$$

where

$$D = [H^+]^3 + K^{d}_{1}[H^+]^2 + K^{d}_{1}K^{d}_{2}[H^+] + K^{d}_{1}K^{d}_{2}K^{d}_{3} \quad (5)$$

and

$$L = [H^+]^3 + K^{l}_{1}[H^+]^2 + K^{l}_{1}K^{l}_{2}[H^+] + K^{l}_{1}K^{l}_{2}K^{l}_{3}. \quad (6)$$

The rate constants k^{d}_{1}, k^{d}_{2}, k^{d}_{3}, k^{d}_{4}, k^{l}_{1}, k^{l}_{2}, k^{l}_{3} and k^{l}_{4} as well as the apparent dissociation constants K^{d}_{1}, K^{d}_{2}, K^{d}_{3}, K^{l}_{1}, K^{l}_{2}, and K^{l}_{3} may, of course, be a complex combination of rate constants. Equations (3)–(6) imply that the effects of pH on the hydrolysis [equations (3) and (5)] and photolysis [equations (4) and (6)] can be explained in terms of the different reactivities of the four possible Bjerrum species (Fig. 6). The correlation between the hydrolytic rate constants (k^{d}_{n}) and the photolytic rate constants (k^{d}_{n}) (equation 7) was poor ($r^2 = 0.513$), suggesting that there was no apparent relationship between the hydrolytic reactivity of the four Bjerrum species and their susceptibility to photolytic degradation.

$$\log k^{l}_{n} = -0.433 \log k^{d}_{n} - 1.55; \; r^2 = 0.513. \quad (7)$$

The same values of pK_1 (3.35) and pK_2 (4.60) were obtained from the photolysis and hydrolysis experiments. Only one pK_a value in the range 3–5 could be determined spectrophotometrically. However, the value of 4.49 obtained spectrophotometrically was in good agreement with that of pK_2 (4.50) obtained kinetically. Values of 11.8 and 11.6 for the third pK_a were obtained from the hydrolytic and photolytic experiments, respectively. Those values were in good agreement with the value of 11.3 obtained spectrophotometrically.

The aqueous solubilities of **1** pH 4.6, 7.0 and 11.4 were 3.01 ± 0.30, 1.53 ± 0.15 and 3.18 ± 0.47 mg ml^{-1}, respectively. These solubilities are consistent with an ionization scheme (Fig. 6) involving protonation of the trienzyl side chain ($pK_1 = 3.35$), protonation of the imidazole ring ($pK_2 = 4.60$) and deprotonation of the imidazole ring ($pK_3 = 11.3$–11.8).

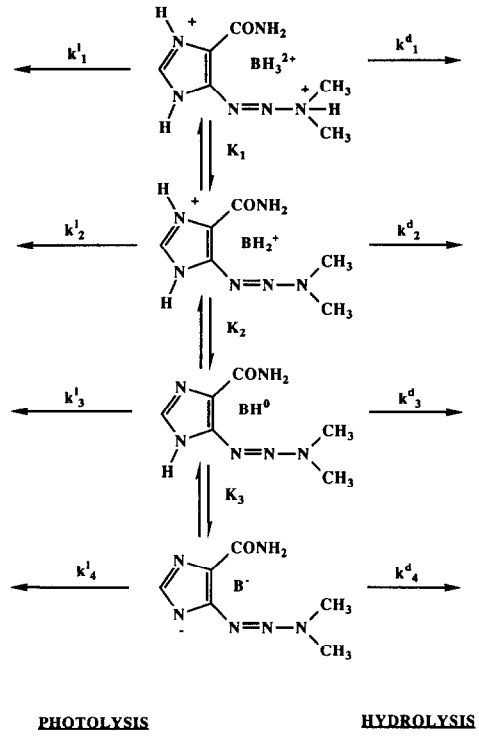

Figure 6
Proposed scheme for the photolysis (l) and hydrolysis of **1** in aqueous solution (d).

Product analysis

The product of the hydrolysis and the photolysis of **1** under all conditions (Table 1) was found to be 2-azahypoxanthine (**2**) (Fig. 1). The retention time of **2** and its UV spectra, obtained by on-line diode-array detection, in the degraded samples were the same as those for an authentic sample. The recovery of **2** from completely degraded samples of **1** was greater than 95% in all cases. The structure of **2** was confirmed as the main degradation product of **1** by comparing its mass spectrum with that of an authentic standard. The EI spectrum of the authentic standard of **2** showed a molecular ion at *m/z* 137 and characteristic fragment ions at *m/z* 110 and 94. The EI spectra of samples obtained after degradation of **1** in 0.001 M HCl in both the light and the dark were essentially the same as that of the standard. An ion of *m/z* 218 was observed in both the standard and the degraded samples; this ion could not be accounted for.

These results appear to contradict the previous results of Horton and Stevens [6] who reported that **2** was generally the major product of photolysis and hydrolysis of **1** over the pH range of 1–12. However, between pH 2 and 6 they claimed that the major product of photolysis was 4-carbamoylimidazolium-5-olate. In the present studies the only degradation product detected at all pH values from 1 to 13 was **2**. The reason for the apparent difference in the results of the two studies is not clear at the present time but may have arisen because of the different experimental conditions used in the two studies. In particular, it should be noted that Horton and Stevens [6] used diffused or direct sunlight, which has a significantly different spectral distribution from the indoor, white fluorescent tubes used

Table 1
Effect of light on the rate constants for the degradation of dacarbazine in various buffers at 37.0 ± 0.5°C (μ = 0.15 M)

	Buffer (μ = 0.15 M)*		k^{l}_{obs} (s^{-1})† × 10^{1}		k^{d}_{obs} (s^{-1})‡ × 10^{3}	
Initial pH	Type	Conc. (M)	Mean§	SD‖	Mean§	SD‖
1.00	HCl	0.100	0.904	0.01	5.69	0.34
2.00	HCl	0.010	0.932	0.02	6.29	0.68
2.97	Formate	0.025	1.29	0.03	7.97	0.24
2.92	Formate	0.050	1.36	0.07	7.96	0.04
2.91	Formate	0.075	1.43¶	0.06¶	8.05	0.12
3.85	Formate	0.050	2.78	0.06	12.1	0.64
4.75	Acetate	0.025	4.21	0.11	9.36	0.15
4.76	Acetate	0.050	4.44	0.00	8.99	0.11
4.70	Phosphate	0.075	4.33	0.03	8.72	0.34
5.85	Phosphate	0.025	5.86	0.08	5.16	0.06
5.89	Phosphate	0.050	6.21	0.11	5.58	0.09
5.91	Phosphate	0.075	5.67	0.34	5.46	0.32
6.98	Phosphate	0.010	6.03	0.29	4.68	0.15
6.97	Phosphate	0.025	6.43	0.15	4.84¶	0.00¶
6.93	Phosphate	0.050	6.16	0.38	4.75	0.11
7.91	Phosphate	0.050	6.16	0.16	—	—
8.00	Carbonate	0.050	—	—	4.76	0.05
9.13	Carbonate	0.075	—	—	4.69	0.09
9.22	Carbonate	0.025	—	—	4.54	0.22
9.22	Carbonate	0.050	5.89¶	0.26¶	4.76	0.05
9.78	Carbonate	0.050	6.28	0.20	—	—
9.96	Carbonate	0.010	6.53¶	0.23¶	4.63¶	0.05¶
10.08	Carbonate	0.025	6.71¶	0.12¶	4.53¶	0.03¶
10.14	Carbonate	0.050	6.66	0.19	4.43¶	0.07¶
10.55	Carbonate	0.010	—	—	4.55¶	0.16¶
10.80	Carbonate	0.010	7.47	0.01	—	—
10.85	Carbonate	0.025	7.13	0.44	4.61¶	0.14¶
10.88	Carbonate	0.050	8.05¶	0.22¶	4.58¶	0.00¶
12.00	NaOH	0.010	10.7	0.26	1.61¶	0.02¶
13.00	NaOH	0.100	11.4¶	0.08¶	0.50¶	0.01¶

* Adjusted with NaCl.
† Pseudo first-order rate constant for the degradation of dacarbazine in the light.
‡ Pseudo first-order rate constant for the degradation of dacarbazine in the dark.
§ Mean of three determinations except where indicated.
‖ Standard deviation (for $n = 3$ except where indicated).
¶ $n = 2$.

Table 2
Dissociation constants and rate constants for the photolytic and hydrolytic degradation of dacarbazine in aqueous solution at 37.0 ± 0.2°C (μ = 0.15)

Parameter	Value
Hydrolysis	
k_1^d (h^{-1})	5.60×10^{-3}
k_2^d (h^{-1})	1.54×10^{-2}
k_3^d (h^{-1})	4.70×10^{-3}
k_4^d (h^{-1})	2.80×10^{-4}
K_1^d (pK_1^d)	4.51×10^{-4} (3.35)
K_2^d (pK_1^d)	2.50×10^{-5} (4.60)
K_3^d (pK_3^d)	1.44×10^{-12} (11.8)
Photolysis	
k_1^l (h^{-1})	8.95×10^{-2}
k_2^l (h^{-1})	0.220
k_3^l (h^{-1})	0.625
k_4^l (h^{-1})	1.14
K_1^l (pK_1^l)	4.51×10^{-4} (3.35)
K_2^l (pK_1^l)	2.50×10^{-5} (4.60)
K_3^l (pK_3^l)	2.50×10^{-12} (11.6)
Spectrometric determination of pK_a values	
K_1 (pK_1)	*
K_2 (pK_2)	3.36×10^{-5} (4.49) (37°C)
	3.11×10^{-5} (4.51) (RT)†
K_2 (pK_3)	1.17×10^{-12} (11.9) (37°C)
	5.46×10^{-12} (11.3) (RT)†

*Could not be determined.
†Room temperature (22 ± 1°C).

in the present work (more UV content down to 290 nm and more irradiance below 350 nm).

Effect of additives and co-solvents

The effects of antioxidants (sulphite, bisulphite), complexing agents (EDTA, citrate and carboxymethylcellulose) and co-solvents (ethanol and propylene glycol) on the photolysis of **1** were studied (Table 3). Those particular experiments were studied at 37°C in the light box. Comparison of the $t_{10\%}$ values (Table 3) showed that addition of complexing agents, propylene glycol, carboxymethylcellulose or sodium bisulphite had no effect on the degradation of **1**. Addition of sodium sulphite and ethanol increased the shelf life by 25% when added separately. However, there was no synergism when both sodium sulphite and ethanol were included in the formulation.

Conclusions

The degradation of **1** in aqueous solution was significantly faster in the presence of light. The influence of pH on the rates of photolysis and hydrolysis of **1** could be explained in terms of the different reactivities of the four possible Bjerrum species of the drug present in solution. There was no apparent relationship between the rate of hydrolysis and the rate of photolysis of the various Bjerrum species, even though the final product of degradation, **2**, was the same for both reactions. When protected from light, injectable formulations of **1** (e.g. Dacarbazine for Injection, USP) have a pH of 3–4 after reconstitution. Relatively acidic solutions are used to achieve aqueous solubilities in excess of 10 mg ml^{-1}. However, this pH range of 3–4 actually corresponds to the pH of minimum stability where the shelf life $t_{10\%}$ is only 8 h at room temperature. If the product were to be reformulated at pH 7, the room

Table 3
Shelf-life ($t_{10\%}$) of dacarbazine at 37.0 ± 0.2°C in various solvent systems

Solvent system	$t_{10\%}$ (h)*	
	Light	Dark
Formate buffer (0.1 M, pH 3.9)	0.37	8.68
Citrate buffer (0.1 M, pH 4.0), sodium bisulphite (1%)	0.42	nd†
Citrate buffer (0.1 M, pH 4.0), carboxymethylcellulose (0.1%)	0.42	nd†
Citrate buffer (0.1 M, pH 4.0), carboxymethylcellulose (1.0%)	0.30	nd†
Phosphate buffer (0.1 M, pH 7.0)	0.18	24.8
Citrate buffer (0.1 M, pH 7.0)	0.17	nd†
Citrate buffer (0.1 M, pH 7.0), EDTA (0.01%)	0.18	nd†
Citrate buffer (0.1 M, pH 7.0), sodium sulphite (0.2%)	0.24	nd†
Citrate buffer (0.1 M, pH 7.0), ethanol (10%)	0.22	nd†
Citrate buffer (0.1 M, pH 7.0), sodium sulphite (0.2%), ethanol (10%)	0.26	nd†
Citrate buffer (0.1 M, pH 7.0), propylene glycol (40%), ethanol (10%)	0.21	nd†
Citrate buffer (0.1 M, pH 7.0), carboxymethylcellulose (0.1%)	0.21	nd†
Citrate buffer (0.1 M, pH 7.0), carboxymethylcellulose (1.0%)	0.17	nd†

*Mean of two determinations.
†Not done.

temperature shelf-life of the reconstituted injection would be more than 24 h, which is adequate for continuous infusions provided that they were protected from light. The major limitation to this approach would be the reduced aqueous solubility of **1** which is 1.53 mg ml^{-1} at pH 7.0. The effects of co-solvents and anti-oxidants on the stability of **1** in the light or the dark were slight and of no practical value in substantially increasing the shelf-life of the reconstituted product.

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