Degradation Kinetics and Mechanism of RH1, a New Anti-Tumor Agent: A Technical Note

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Neera Jain,[1] Stephen G. Machatha,[2] S. Esmail Tabibi,[3] and Samuel H. Yalkowsky[4]

[1]Wyeth Research, Pearl River, NY 10965
[2]Cydex Inc, Lenexa, KS 66214
[3]Pharmaceutical Resources Branch, National Cancer Institute, NIH, Bethesda, MD 20892
[4]Department of Pharmacy Practice and Science, College of Pharmacy, The University of Arizona, Tucson, AZ 85721

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INTRODUCTION

Recently, there has been great interest in bioreductive drugs for the treatment of cancer. Bioreductive drugs are designed to take advantage of the unique features of solid tumors, especially oxygen tension and the overexpression of certain reductase enzymes.[1] Of the several reductase enzymes, DTD (NAD(P)H: quinone oxidoreductase) is excessively found in solid tumors. Using NADPH and NADH as an electron donor, DTD is capable of reducing quinone-type compounds to DNA damaging species.[2]

RH1 is a water-soluble diaziridinyl benzoquinone derivative that has been shown to have preferential activity against human tumor cell lines that express high levels of the 2-electron reductase DTD.[3]

The chemical name of RH1 is 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone, and its structure is shown in Figure 1. It occurs as a reddish-brown odorless crystalline powder and has a molecular weight of 234.3 d. It is currently a candidate for clinical trials in both the United Kingdom and the United States.

The stability of diaziridinyl-benzoquinones has been reported previously by several workers,[4-11] including Cheung et al[4] who studied the degradation products of RH1. The degradation of RH1 is of concern because it is intended to be delivered in aqueous media. In this technical note, a degradation pathway for RH1 in acidic medium has been proposed.

MATERIALS AND METHODS

Materials

RH1 was used as received from the National Cancer Institute (Bethesda, MD). High-performance liquid chromatography (HPLC)-grade methanol and glacial acetic acid were purchased from Burdick and Jackson (Muskegon, MI) and Spectrum (Gardena, CA), respectively. Ammonium acetate was purchased from Chempure (Houston, TX). Citric acid, glycine, sodium hydroxide, and hydrochloric acid were purchased from Aldrich (Milwaukee, WI). Disodium hydrogen phosphate and sodium chloride were obtained from Sigma (St Louis, MO). All chemicals were used as received without any further purification.

Corresponding Author: Stephen G. Machatha, Cydex Inc, Lenexa, KS 66214. Tel: (913) 402-3522; Fax: (913) 685-8856; E-mail: smachatha@cydexinc.com

Methods

Drug Characterization

The melting point was determined using a DuPont 910 differential scanning calorimeter (DSC; TA Instruments Inc, New Castle, DE) with a heating rate of 10°C/min; standard aluminum pans were used.

High Performance Liquid Chromatography Analysis

Solutions of RH1 were analyzed[6] by HPLC instrument (Beckman Coulter Inc, Fullerton, CA). The data were analyzed using System Gold Chromatographic Acquisition software (Beckman Coulter Inc, Fullerton, CA).

The HPLC system consisted of:

- Column: C8 Adsorbosil 5 μm, 150 × 4.6 mm
- Mobile phase: 30/70 mixture of methanol/20 mM ammonium acetate, pH 4
- Injection volume: 20 μL
- Flow rate: 1 mL/min
- Detection: 254 nm

Stability Kinetics of RH1 in Aqueous Solutions

Buffers in the pH range of 3.0 to 8.0 were prepared using 0.01 M citric acid and 0.02 M disodium hydrogen phosphate. Basic buffers were prepared with 0.01 M glycine and 0.01N NaOH. The ionic strength was maintained by adding 0.2 M sodium chloride. Solutions of 100 μg/mL RH1 in each buffer were prepared and stored at room temperature (25°C).

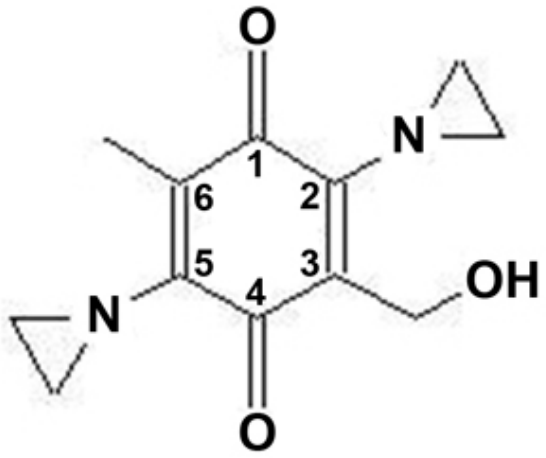


Figure 1. Chemical structure of RH1.

The molecular masses of the degradation products were determined by using HPLC and TSQ7000 triple quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA) with atmospheric pressure chemical ionization (APCI).

Effect of Antioxidants and Light

The effect of antioxidants was studied using solutions of 100 μg/mL RH1, buffered at pH 6.0 and 8.7 with and without 0.05% ascorbic acid. Duplicate samples were placed in the presence and absence of light.

Results and Discussion

The melting point of RH1 determined using DSC is 171°C to 172°C. RH1 is expected to be a weaker base than an aromatic amine such as aniline ($pK_a \approx 5$). Because of the electron withdrawing nature of the neighboring quinone groups, aziridine groups are easily protonated, hence the degradation at low pH.

The degradation reactions for a drug in aqueous solution can be affected by several factors including drug concentration, pH, temperature, light, and the presence of additives. The different elements that affect the stability of RH1 are considered in the following sections.

Effect of pH on Degradation

The pH of a solution is known to affect degradation of many drugs. This result can be attributed to the fact that degradation reactions can be catalyzed by hydrogen ions or hydroxide ions. The degradation of RH1 followed first-order kinetics, and the degradation rate constants in various buffers were calculated. These were then used to estimate the half-life and the amount of time required for loss of 10% of parent drug (t_{90}) at each pH value; data are shown in Table 1.

Using the rate constants and pH values in Table 1, the degradation of RH1 was shown to be catalyzed in both acid and base and the maximum stability is observed in neutral conditions. These data yielded slopes of −0.97 in acid pH and +0.90 in basic pH, which are in good agreement with the expected theoretical values of −1 and +1.

Poochikian and Kelley[11] noted that ionic strength does affect the degradation rate of aziridine-based compounds. They also showed that chlorides bind and degrade aziridines. None of these degradation products were observed with HPLC and liquid chromatography mass spectrometry (LC-MS).

Effect of Antioxidants

Antioxidants prevent degradation by being preferentially oxidized over other species. They essentially act as free radical scavengers. Ascorbic acid is commonly employed as an antioxidant in pharmaceuticals. The presence of ascorbic acid buffered at pH 6.0 and 8.75 showed no effect on the degradation rate of RH1. No effect of light on the degradation of RH1 in acid, neutral, and base conditions was observed, suggesting that light oxidation is not a major mechanism in the degradation of RH1. On the basis of the effect of light and antioxidants, oxidation is not likely to play a significant role in the decomposition of RH1 in aqueous solutions.

HPLC Analysis

A sample of RH1 was kept at pH 5 for 24 hours, and using the developed HPLC method, the parent and the 4 major degradation products were separated. The chromatogram is shown in Figure 2. Note that the peak associated with degradation product II has a shoulder, which has not been identified. The degradation products are numbered in order of their rate of appearance.

Table 1. Degradation Rate Constants, t_{90}, and Half-lives at Various pH Values*

pH	k ($week^{-1}$)	t_{90} (days)	$t_{1/2}$ (days)
4.16	73.48	0.01	0.07
4.95	9.85	0.07	0.49
5.95	1.12	0.66	4.36
6.96	0.13	5.47	36.13
7.33	0.11	6.59	43.52
7.78	0.11	5.99	39.52
8.96	1.05	0.70	4.64
9.82	5.08	0.14	0.96

*k indicates degradation rate constant; t_{90} refers to the loss of 10% of parent drug; and $t_{1/2}$ refers to the half-life.

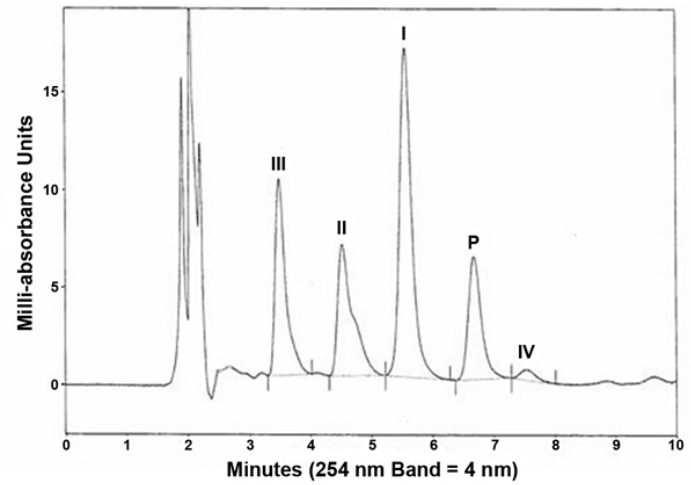


Figure 2. Chromatogram of degraded sample of RH1 at pH 5.0.

Degradation Products and Mechanism

The appearance of degradation products and the disappearance of the parent compound were analyzed on the basis of the peak areas. A plot of the percentage area versus time is shown in Figure 3. Note that the number of degradation products is the same as the number of peaks in the chromatogram in Figure 2. The degradation of RH1 at pH 5 follows first-order kinetics with a constant rate of 9.85 for week 1.

Using the information from Figure 3, the following schematic decomposition was proposed.

Hydrolytic cleavage of the aziridine rings is a likely reaction as the 3-membered ring is extremely strained. This leads to the formation of isomers IA and/or IB of molecular mass 252. Degradation product 1B is likely to form because of the hydrogen bond formation between the aziridine at the C2 position with the neighboring methylhydroxy group.

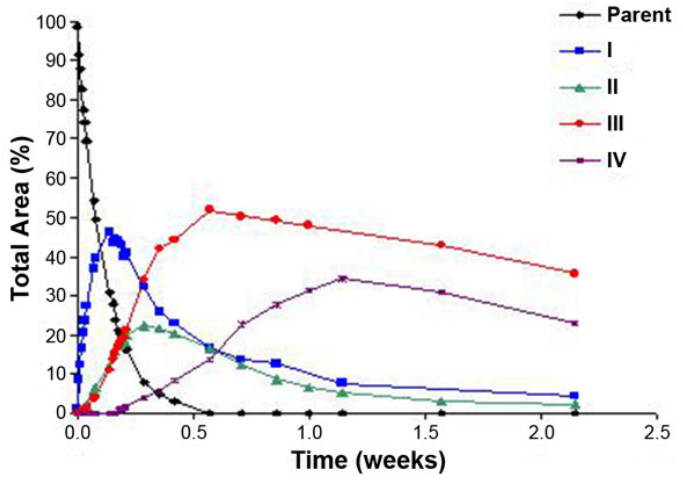


Figure 3. Kinetic profile of RH1 and its degradation products when solution is buffered at pH 5.0.

The next degradation product (IIIA and/or IIIB) with a molecular mass of 209 is the result of the nucleophilic substitution of the aziridine ring with a hydroxy group at the carbon attached to the nitrogen. The lag time for their formation suggests that IIIA and IIIB are not formed from the parent drug, but rather from the degradation of IA and IB. Degradation product IIIB is more likely to be formed for the same reason as 1B. The second aziridine group is hydrolyzed to produce degradation products IIA and/or IIB with a molecular mass of 227. Again, product IIB is more likely to form than IIA. The molecular masses were confirmed by the use of LC-MS. The molecular mass of degradation product IV was found to be 295, but its structure is not understood and is not included in Figure 4.

Note that degradation products V and VI of Figure 4 have not been detected by the chromatographic system or LC-MS. Compound V can be formed as a result of hydrolysis of both the aziridine rings. Compound VI would be formed by the nucleophilic substitution of hydroxyl group at the 2 carbons attached to the aziridine rings. Although substitution at one of the rings was observed, a di-hydroxy derivative was not detected. This finding supports the hypothesis that the nucleophile preferentially attacks at the C5 position

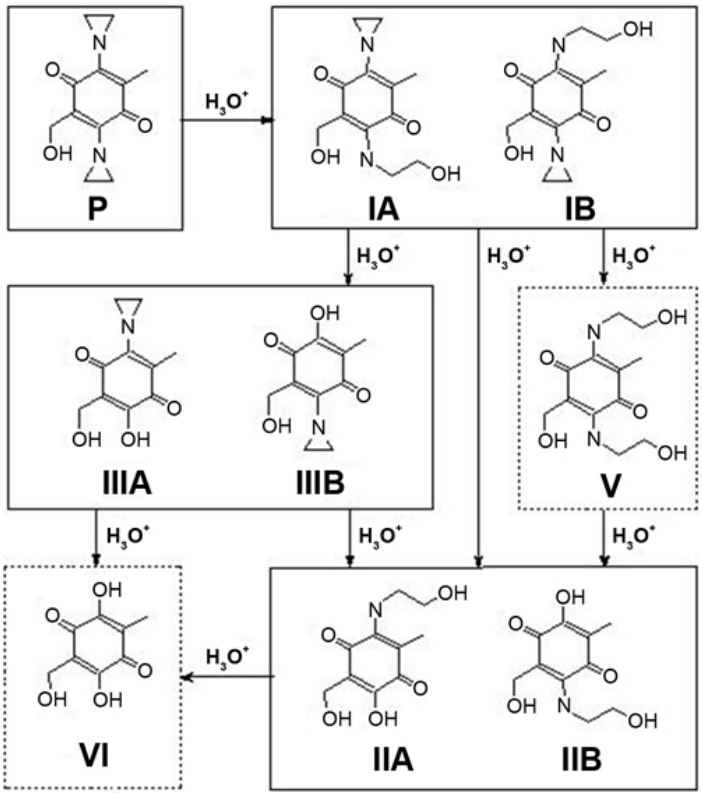
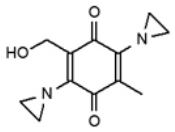
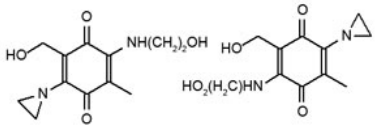
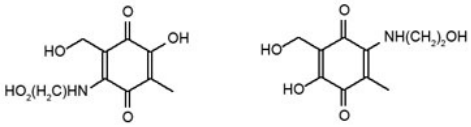
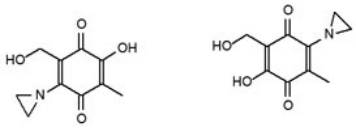
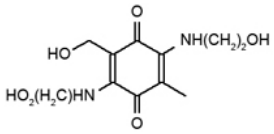
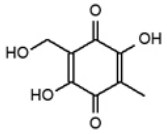


Figure 4. Proposed degradation pathway of RH1 in acid conditions, where P is the parent drug and numbers I to VI correspond to the subsequent degradation products. Products V and VI were not seen.

Table 2. Degradation Products

Name	Retention Time (minutes)	Molecular Mass	Proposed Structure
Parent	6.7	234	
IA and B	5.6	252	
IIA and B	4.5	227	
IIIA and B	3.5	209	
IV	8.0	295	Not known
V	Not found	270	
VI	Not found	184	

of IIA and IIIA. Cheung et al[4] do not show the degradation products IIIA and IIIB, which we observed. They also do not acknowledge that degradation product V could be formed.

A summary of the degradation product structures, their retention times as seen on the chromatogram, and the molecular masses from LC-MS are shown in Table 2.

Summary and Conclusions

The degradation of RH1 in aqueous solution is found to be both acid and base catalyzed. The maximum stability is obtained in neutral pH but still degrades by 10% (t_{90}) after just 1 week. The stability profile at pH 5 was done, and 4 major degradation products were observed in acid solutions. LC-MS was performed and the molecular weights determined, from which a degradation mechanism was proposed. Degradation products I, II, and III form 2 isomers each depending on which aziridine group is hydrolyzed. No significant effect of light or the presence of antioxidants was observed, indicating that photodegradation and oxidation are not likely degradation reactions.

Acknowledgment

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