= 2.85 mm^{-1}, $F(000)$ = 656, T = 22 °C.

A clear colorless 0.18- × 0.32- × 0.60-mm crystal, in the shape of an irregular prism, was used for data collection on an automated Siemens R3m/V diffractometer equipped with an incident beam monochromator. Lattice parameters were determined from 25 centered reflections within $27.0 \leq 2\theta \leq 38.5°$. The data collection range of hkl was: $-14 \leq h \leq 0$, $0 \leq k \leq 14$, $-15 \leq l \leq 16$, with $[(\sin\theta)/\lambda]_{max} = 0.59$. Three standards, monitored after every 97 reflections, exhibited random variations with deviations up to ±2.5% during the data collection. A set of 3736 reflections was collected in the $\theta/2\theta$ scan mode, with scan width $[2\theta(K_{\alpha 1}) - 1.2]$ to $[2\theta(K_{\alpha 2}) + 1.2]°$ and ω scan rate (a function of count rate) from 7.5 to 30.0 deg/min. For this compound, there were 3262 unique reflections, and 2020 were observed with $F_o > 3\sigma(F_o)$. Data were corrected for Lorentz and polarization effects. An empirical absorption correction was also applied (maximum and minimum transmission factors were 0.964 and 0.637, respectively). The structure was solved and refined with the aid of the SHELXTL system of programs.[12] The full-matrix least-squares refinement varied 163 parameters, including atom coordinates and anisotropic thermal parameters for all non-H atoms. H atoms were included using a riding model [coordinate shifts of C applied to attached H atoms, C–H distance set to 0.96 Å, H angles idealized, constant U_{iso}]. Final residuals were R = 0.050 and R_w = 0.041 with final difference Fourier excursions of 0.44 and −0.47 e Å^{-3} in the vicinity of the Br atom.

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Acknowledgment. We thank J. Klose for collecting the NMR data, J. Roman for mass spectral measurements, and J. A. Hrabie for helpful discussions. Work was supported in part by the Office of Naval Research.

Registry No. **2a**, 91725-44-9; **2b**, 112753-63-6; **2c**, 112753-64-7; **2d**, 143706-20-1; **2e**, 143706-21-2; **2f**, 143706-22-3; **2f'**, 143706-19-8; **2g**, 143706-23-4; **2h**, 143706-24-5; **2i**, 143706-25-6; **2j**, 143706-26-7; **2k**, 143706-27-8; **2l**, 143706-28-9; **2m**, 143706-29-0; $Et_2N(N_2O_2)Na$, 92382-74-6.

Supplementary Material Available: Complete crystal structure parameters for **2l**, including atomic positional and thermal parameters (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Facilitated Intramolecular Conjugate Addition of *N*-(*p*-Methoxyphenyl)-3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionamide. 1. Product Characterization

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N-(*p*-Methoxyphenyl)-3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionamide (1), a pro-prodrug model, was chosen to study the reaction of quinone propionic amides in mildly acidic aqueous solution. The quinone propionic amide 1 equilibrates rapidly with its hydroxy dienone 6 and undergoes a much slower 1,4 conjugate addition to form its enol spirolactam 4. The enol spirolactam 4 then tautomerizes to give the keto spirolactam 5. Spectroscopic evidence suggests that the keto spirolactam 5 is present as a diastereomeric mixture, wherein the preferred diastereomer has the 2′-methyl and the lactam nitrogen anti to each other.

Introduction

Bioreversible derivatives of drugs, commonly referred to as prodrugs, have been shown to improve the physicochemical (e.g., solubility, lipophilicity) and biological (e.g., membrane permeability, metabolic stability) properties of many compounds.[1-4] Whereas there is considerable published research on ester prodrugs, less is available on prodrugs of amines.[1-4] One approach to development of amine prodrugs is acylation; however, amides are typically too stable in vivo to be useful prodrug forms for amines.

Recently, our laboratory described the synthesis of a highly chemically reactive hydroxy amide, which lactonized with a half-life of approximately 1 min at near physiological pH and temperature.[5] In order to transform this hydroxy amide into useful prodrug model systems for amines, methods were developed for converting this hydroxy amide into chemically stable yet enzymatically labile pro-prodrugs.[6,7] Using the hydroxy amide template,[5] ester-sensitive[7] and redox-sensitive[6] pro-prodrug systems for amines were developed by our laboratory. The redox-sensitive pro-prodrug system consists of amides of 3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic acid (e.g., **1**, Figure 1). The chemical reduction of the quinone moiety of **1** was shown to generate the intermediate hydroquinone **2**, which rapidly lactonizes, yielding the lactone **3** and the amine (e.g., *p*-anisidine).[6] A similar redox-sensitive system has been described by Carpino et al.[8]

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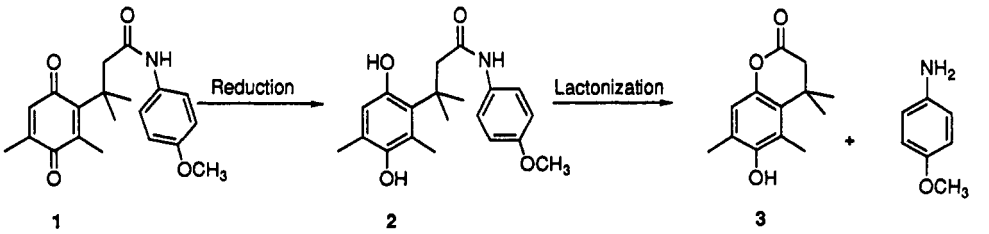


Figure 1. Proposed conversion mechanism for redox-sensitive model pro-prodrugs of amines.

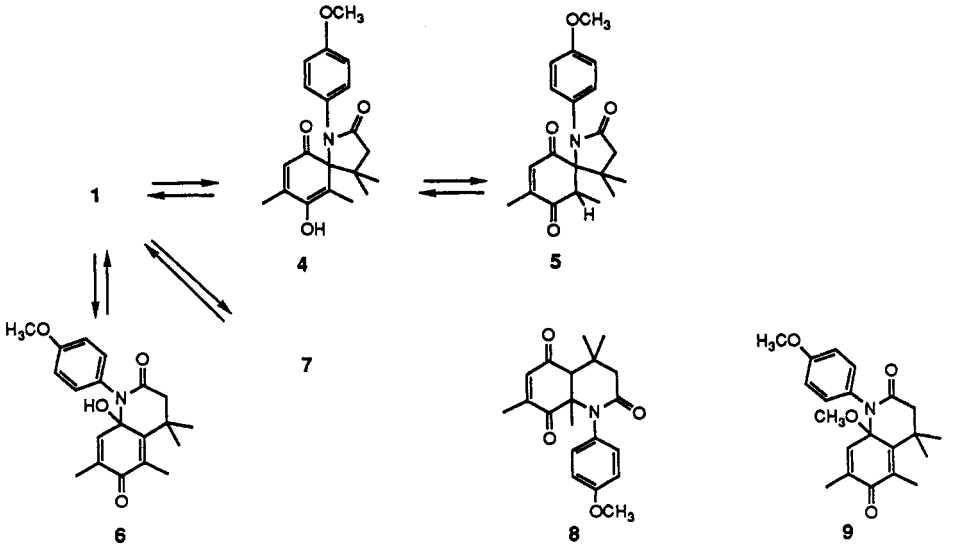


Figure 2. Degradation scheme of 1 in aqueous solution in the absence of reducing agent.

However, preliminary stability studies in aqueous solution in the absence of a reducing agent showed that the quinone propionic amide 1 rapidly undergoes degradation to yield products other than the lactone 3 and the amine.[9] The instability of this pro-prodrug system in aqueous solution could prevent the utilization of this system for delivery of amine-containing drugs. In the present study, chemical and spectral evidence are provided for the degradation pathway depicted in Figure 2 which involves the quinone propionic amide 1 equilibrating rapidly with its hydroxy dienone 6 and undergoing 1,4 conjugate addition to form the enol spirolactam 4. This spirolactam tautomerizes to give the keto spirolactam 5.

Results and Discussion

The quinone propionic acid, prepared according to a literature procedure,[6] was coupled with *p*-anisidine in the presence of dicyclohexylcarbodiimide and hydroxybenzotriazole to give 1. The quinone propionic amide 1 was characterized by standard spectroscopic methods. Thus, the UV spectrum of 1 had an absorption maximum at 252 nm (ϵ = 20 400 M^{-1} cm^{-1}) and the characteristic quinone absorption maximum at 338 nm (ϵ = 770 M^{-1} cm^{-1}). The ^1H NMR spectrum of 1 exhibited three methyl signals, a methylene signal, a vinyl proton signal, and aromatic proton signals. The ^{13}C NMR spectrum of 1 showed 17 resonance signals, those of interest assigned to the three carbonyls, four olefinic carbons, and four methyl lines. The geminal methyl groups in 1 have the same magnetic environment and, therefore, appear as a singlet. The short-range carbon–proton correlations were obtained using inverse-detected heteronuclear multiple-quantum

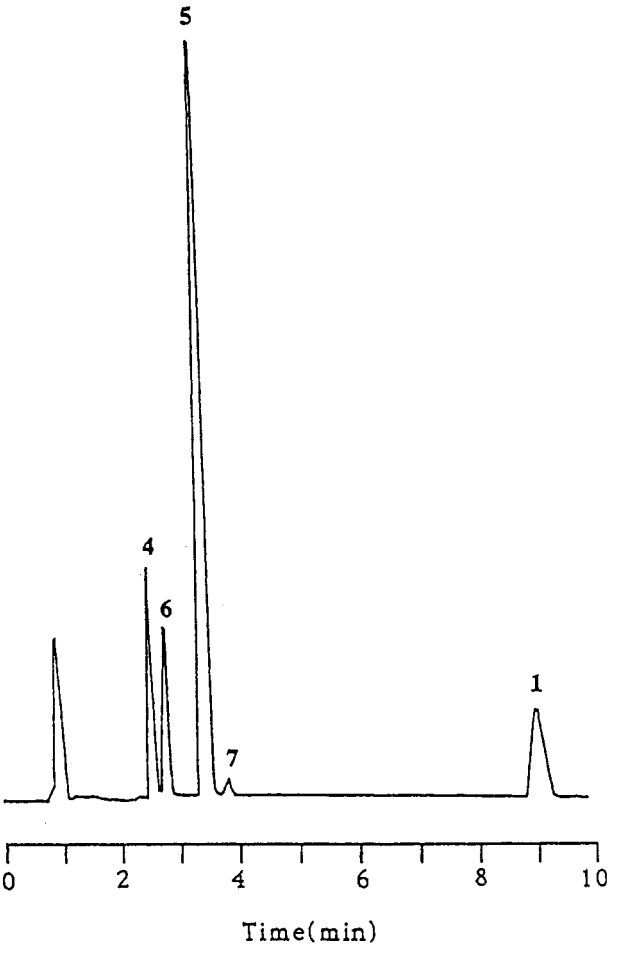


Figure 3. Representative chromatogram of an aliquot of the reaction mixture of 1 at 37 °C in 10% acetonitrile, 90% buffer (pH 5.0, 0.1 M acetate, μ = 0.3 M) after 50 min.

(9) Preliminary results of this work were presented at the 4th Annual Meeting of the American Association of Pharmaceutical Scientists, Las Vegas, NV, November, 1990; Wolfe, J. L.; Borchardt, R. T. *Pharm. Res.* 1990, 7, S-126.

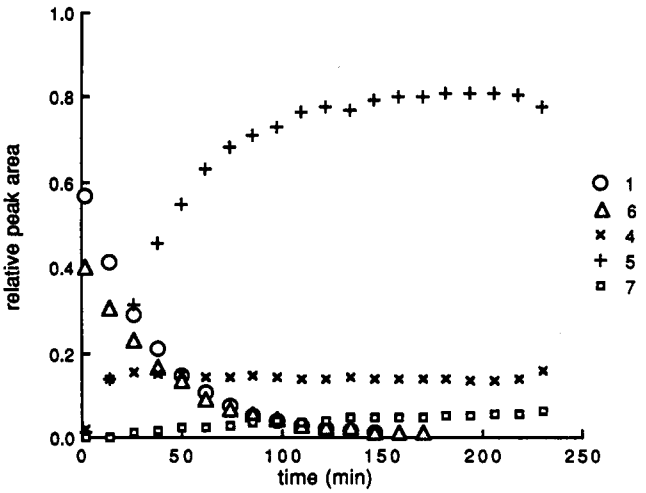


Figure 4. Representative time course illustrating the degradation of the quinone propionic amide 1 and the formation of the hydroxy dienone 6, the enol spirolactam 4, and the keto spirolactam 5.

coherence (HMQC) experiments,[10] and the long-range correlations through two and three bonds were obtained using inverse-detected heteronuclear multiple bond correlation (HMBC) experiments[11] (Table S1).

HPLC analysis resulted in the separation of the quinone propionic amide 1 from its degradation products as shown in Figure 3. Because the reaction proceeded so rapidly at pH 7.0, it was not feasible to study the degradation of 1 under more physiological conditions. However, it was found that the quinone propionic amide 1 produced the same products at pH 5.0 as at neutral pHs, resulting in the choice of slightly acidic conditions to study the reaction. Figure 4 shows a typical time course for the degradation of 1 at pH 5.0 (0.1 M acetate buffer) and at 37 °C. As shown in Figure 4, a very rapid equilibrium was established between the hydroxy dienone 6 and the quinone propionic acid amide 1. The other products 4, 5, and 7 formed more slowly, but in this time frame an equilibrium between the reactant and products was clearly established.

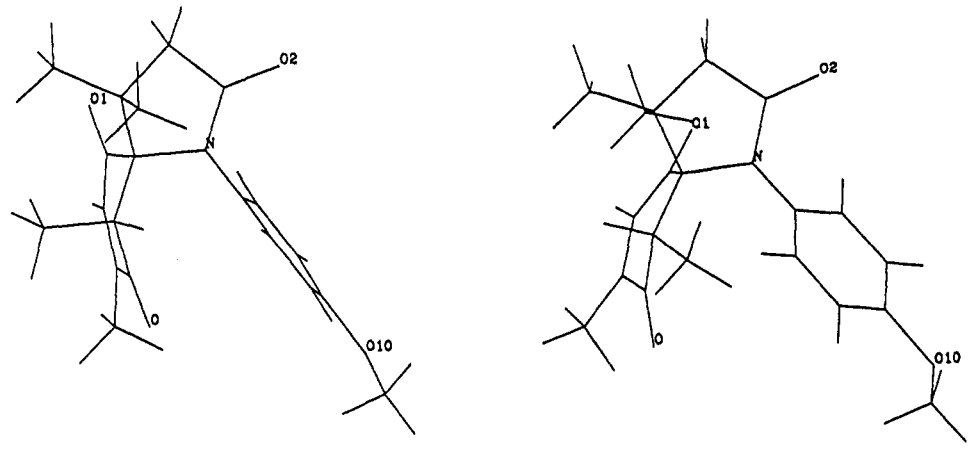
Although attempts to isolate the enol spirolactam 4 by preparative HPLC were unsuccessful, it was possible to form a reaction mixture in which the enol spirolactam 4 was the major product, thus allowing characterization of 4 by NMR experiments. For example, reaction of 1 in D_2O/CD_3OD (50/50) at 37 °C for 6 h afforded a mixture containing approximately 75% of the enol spirolactam 4, as measured by HPLC (data not shown). The mixture was cooled to 10 °C for the duration of the NMR experiments, maintaining the high concentration of 4. The ^1H and ^{13}C NMR spectra are shown in Figures S1a and S1b, respectively. Short- and long-range proton–carbon connectivities were established with the results of inverse-detected HMQC and HMBC experiments, respectively (Table S2). The ^{13}C NMR spectrum showed 18 lines, including signals for two quaternary carbons, four olefinic carbons, and five methyl groups. The ^{13}C spectrum of the enol spirolactam 4 contained resonance signals for only two carbonyl carbons but provided evidence for an olefinic carbon bonded to a hydroxyl. It is important to note that there were five methyl lines in this spectrum, as opposed to only four in that of the starting material, indicating a change in the magnetic environment of the geminal methyl groups. Also, C1′ is quaternary rather than olefinic, which is expected if this were the electrophilic site in a 1,4 conjugate addition. Finally, and most significantly, there were two carbonyl carbon signals in the ^{13}C NMR spectrum of this compound and a gain of a hydroxyl-bearing olefinic carbon signal, leading to the ultimate assignment of the structure as the enol spirolactam 4.

A pure sample of the keto spirolactam 5 was obtained by dissolving 1 in 50% aqueous acetonitrile at 37 °C. Aliquots of the reaction mixture were injected onto a preparative HPLC column and the peak eluting at 10.1 min (the keto spirolactam) was collected, extracted into ether, and concentrated in vacuo. The ^1H and ^{13}C NMR spectra are shown in Figures S2a and S2b, respectively. Proton–carbon one bond connectivities of the keto spirolactam 5 were established using inverse-detected HMQC experiments. Long-range couplings were obtained using inverse-detected HMBC experiments (Table S3). The ^{13}C NMR spectrum revealed that the compound had three carbonyls, five distinct methyl groups, and only two olefinic carbons. The ^1H NMR spectrum revealed two geminal methyl singlets, the 4′ methyl doublet split by the vinyl proton which appeared as a doublet, the methylene protons which appeared as two doublets, and the aromatic protons. The ^1H NMR spectrum also showed a three-proton doublet and a one-proton quartet, corresponding to the 2′-methyl group and the 2′-proton. With the assignments obtained from the one-dimensional spectra and the proton–carbon connectivities from the two-dimensional spectra, the keto spirolactam structure 5 was unambiguously confirmed.[12] The fused bicyclic structure 8 was excluded because it does not have a 2′-proton that would cause the 2′-methyl proton signals to be split.

In the NMR spectrum of the keto spirolactam 5, small signals were observed that had similar characteristics to each major peak, indicating the presence of diastereomers. On the basis of the intensity of the signals, one diastereomer was preferentially formed in a 14:1 ratio. The stereochemical assignments for the major isomers are those with the C-2′ methyl anti to the nitrogen atom. Minor isomers were assigned the syn configuration. These assignments were based on the unusual upfield shift of the three-proton doublet of one of the isomers. In most cases, signals attributable to minor isomers were no more than 0.1 ppm removed from the major isomer. The exception was the three-proton doublet (δ 1.54). The doublet attributed to the minor isomer was shifted upfield to δ 0.57. It is hypothesized that the minor diastereomer has the 2′-methyl perpendicular and in close proximity to the phenyl ring and that ring current effects cause this large upfield shift. Molecular modeling of the syn isomer revealed a close spatial proximity between the phenyl ring and the C-2′ methyl, consistent with this hypothesis (Figure 5). Using molecular mechanics, the energy levels of the models were optimized and revealed that the syn isomer had a greater energy level than the anti isomer, thus providing thermodynamic support for the proposed anti isomer.

The observation of a parallel degradative reaction of 1 was also observed. The quinone propionic amide 1 had degraded to less than 60%, as measured by the relative peak area, and product 6 had formed to more than 40%, as measured by the relative peak area, within 2 min. Evidence for a rapid equilibration between quinone 1 and

(10) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.
(11) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(12) Borchardt, R. T.; Cohen, L. A. *J. Am. Chem. Soc.* **1972**, *94*, 9175. A similar phenomenon was observed when a quinone propionic acid was exposed to aqueous conditions. Ring–chain tautomerism resulted from conjugated addition to one of the quinone double bonds, forming the corresponding spirolactone. This reversible reaction was highly dependent on pH. At pH 4, 87% of the spirolactone formed, and at pH 8, only 1% of the spirolactone formed.



Anti diastereomer *Syn* diastereomer

Figure 5. Molecular models of the syn and anti forms of **5**.

adduct **6** is provided by their similar rates of degradation (Figure 4). Presumably, as **1** undergoes 1,4 conjugate addition to form the enol spirolactam **4**, the hydroxy dienone **6** reconverts to **1** to maintain the dynamic equilibrium.

Attempts to isolate dienone **6** by preparative HPLC were unsuccessful because of its instability. Collection of the fraction containing **6** and subsequent extraction into diethyl ether resulted in its conversion to quinone propionic amide **1**. Modifications of acetonitrile–water ratios so that **6** would be the predominant product formed were unsuccessful. In all cases, other products formed to such an extent that it was not possible to characterize the hydroxy dienone. However, using diode array UV spectroscopy, it was possible to observe the rapid formation of a new absorption maximum at 228 nm and a concomitant decrease in the absorbance maximum at 252 nm (Figure 6).

The absorbance maximum of the putative hydroxy dienone **6** (λ_{max} (predicted) = 230 nm) is consistent with the absorbance maxima of 9-hydroxy-α-tocopherone and structurally similar systems, with λ_{max} = 239–242 nm.[12-14] The absorbance maximum of the hydroxy dienone **6** was expected to have a bathochromic shift of approximately 12 nm because it lacks the methyl group at the 5′ position.[15] Attempts to extract **6** into ether were unsuccessful possibly because of reversion to **1**; synthesis of methoxy dienone **9** also was unsuccessful. However, because α-tocopheryl quinone and 9-hydroxy-α-tocopherone[13] are structurally related to **1** and **6**, respectively, and the two sets of compounds have similar spectral properties, it is reasonable to suspect that **1** is in equilibrium with its hydroxy dienone **6**.

Because so little of **7** was formed, it was not possible to isolate sufficient quantities to elucidate the structure. Attempts to manipulate the reaction conditions so that **7** would be the major degradation product were also unsuccessful. Possibly, **7** could be the alternative 1,4 conjugate product (i.e., bicyclic **8**); however, there is no evidence to support this proposal.

In conclusion, under mildly acidic aqueous conditions, the quinone propionic amide **1** undergoes a very rapid equilibration with its hydroxy dienone **6** and also undergoes a slower 1,4 conjugate addition to form enol spirolactam **4**. The enol spirolactam tautomerizes to give a diastereomeric mixture of keto spirolactam **5**, wherein the predominant diastereomers exist with the 2′ methyl and the lactam nitrogen anti to each other.

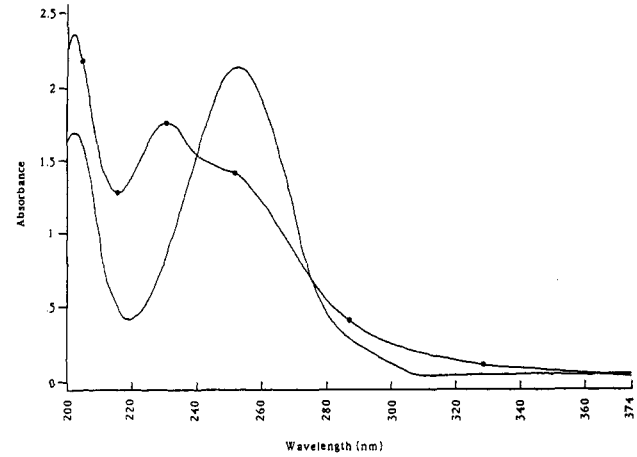


Figure 6. UV spectra of quinone propionic amide **1** in acetonitrile (—) and in 90% acetate buffer (pH 5.0, 0.1 M, μ = 0.3 M)–10% acetonitrile after 30 s (–●–) at room temperature. Subsequent experiments using an HPLC equipped with diode array detection provided a UV spectrum of the hydroxy dienone **6** exclusively (data not shown).

Experimental Section

All melting points are uncorrected. Mass spectral analyses were conducted by the University of Kansas Mass Spectral Laboratory, Lawrence, KS, and elemental analyses were determined by Desert Analytics Organic Microanalysis, Tucson, AZ. Column chromatography was performed with silica gel purchased from Aldrich Chemical Company (70–270 mesh). Thin-layer chromatography was performed with TLC plates consisting of aluminum sheets precoated with silica gel 60 F_{254}, which were purchased from EM Science, West Germany. ^1H, ^{13}C, HETCOR, HMBC, and HMQC NMR spectra were obtained in $CDCl_3$.

HPLC Assay Conditions. The degradation of the quinone propionic amide was monitored through the use of an HPLC system consisting of a system controller, pump, UV detector, integrator, and an autoinjector equipped with a circulating water bath. The detection wavelength was 250 nm. The concentrations of the quinone propionic amide and its degradation products were quantified by measuring peak areas. The isocratic assay was conducted on an ODS Hypersil (C-18) column with a mobile phase consisting of 45% CH_3CN (HPLC grade, Fisher Scientific) in pH 3, 0.01 M phosphate buffer, with a flow rate of 1 mL/min, resulting in retention times of less than 11 min for all compounds.

The keto spirolactam **5** was isolated using the same instrumentation as described above, with the exception of the column used. A Hamilton PRP preparative column was used to effect

(13) Durckheimer, W.; Cohen, L. A. *J. Am. Chem. Soc.* **1964**, *86*, 4388.
(14) Borchardt, R. T.; Cohen, L. A. *J. Am. Chem. Soc.* **1973**, *95*, 8303.
(15) Woodward, R. B. *J. Am. Chem. Soc.* **1941**, *63*, 1123. Woodward, R. B. *J. Am. Chem. Soc.* **1942**, *64*, 72.

the separation of the keto spirolactam 5 from the reaction milieu. The mobile phase composition and detection wavelength were the same as previously described. The flow rate was 3 mL/min, resulting in retention times of less than 16 min for all compounds.

The characterization of degradation products by UV spectroscopy was accomplished in part by HPLC with diode array detection. The system included a pump, a diode array detector, and a personal computer to record and integrate the chromatograms.

***N*-(*p*-Methoxyphenyl)-3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionamide (1).** The synthesis of 3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic acid has previously been reported by this laboratory.[6] 3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic acid (0.29 g, 1.23 mmol) was dissolved in 25 mL of CH_2Cl_2, to which was added 4-anisidine (0.30 g, 2.46 mmol). The mixture was cooled to 0 °C, and dicyclohexylcarbodiimide (0.54 g, 2.60 mmol) and 1-hydroxybenzotriazole (0.18 g, 1.36 mmol) were added. The mixture was shielded from light and allowed to warm to room temperature. After 12 h, the insoluble white crystalline side product, dicyclohexylurea (DCU), was filtered and the CH_2Cl_2 removed under reduced pressure. EtOAc (20 mL) was added to the residue and the mixture was again filtered to remove DCU and the solvent again evaporated. This procedure was repeated until no more DCU could be precipitated. The product 1 was eluted from a silica column (45 × 3.75 cm) with 30% EtOAc in hexane. The fractions were concentrated and recrystallized in EtOAc/hexane to afford the analytically pure yellow crystalline product (0.23 g, 56% yield): mp 149–150 °C; UV (CH_3CN) λ_{max} 252 nm (ϵ = 20 400), 338 nm (ϵ = 770); MS (EI), *m/e* 341 (M); ^1H NMR δ 1.49 (6H, s, $C(CH_3)_2$), 1.96 (3H, s, 4′-(CH_3)), 2.17 (3H, s, 2′-(CH_3)), 3.00 (2H, s, 2-CH_2), 3.75 (3H, s, OCH_3), 6.49 (1H, s, 5′-H), 6.80, 7.29 (4H, m, Ar-H); ^{13}C NMR δ 14.4 (4′-C(CH_3)), 15.6 (2′-C(CH_3)), 29.4 (3-C(CH_3)$_2$), 38.9 (3-*C*(CH_3)$_2$), 50.2 (2-CH_2), 55.5 (-OCH_3), 114.1 (Ar), 122.0 (Ar), 130.6 (Ar), 135.1 (5′-(CH)), 139.4 (Ar), 143.6 (4′-C), 151.9 (2′-C), 156.4 (1′-C), 169.9 (amide carbonyl), 188.3 (3′-carbonyl), 190.2 (6′-carbonyl). Anal. Calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10. Found: C, 70.42; H, 7.09; N, 4.21.

3′-Hydroxy-4,4,2′,4′-tetramethyl-1-(*p*-methoxyphenyl)-1-azaspiro[4.1′]dec-2′,4′-ene-2,6′-dione (4). 1 (6.2 mg, 19.91 μmol) was added to a solution of D_2O (0.5 mL) in CD_3OD (0.5 mL) at 37 °C for 6 h. The solution was cooled to 10 °C. HPLC analysis gave peaks as follows (retention times, area %): quinone 1 (9.5 min, 17.9%); enol spirolactam 4 (2.5 min, 76.3%); keto spirolactam 5 (3.2 min, 4.9%). ^1H NMR (D_2O/CD_3OD, 50/50): δ 1.01 (3H, s, 4-(CH_3)), 1.16 (3H, s, 4-(CH_3)), 1.50 (3H, s, 2′-(CH_3)), 2.13 (3H, s, 4′-(CH_3)), 2.22 (1H, d, J = 17.5 Hz, 3-(CH_2)), 2.62 (1H, d, J = 17.5 Hz, 3-(CH_2)), 3.72 (3H, s, OCH_3), 5.99 (1H, s, 5′-H), 6.8–6.9 (5H, m, Ar-H). ^{13}C NMR δ 15.4 (2′-C(*C*H_3)), 19.2 (4′-C(*C*H_3)), 25.7 (3-C(*C*H_3)), 27.8 (3-C(*C*H_3)), 44.4 (2-CH_2), 46.3 (3-*C*(CH_3)$_2$), 55.8 (OCH_3), 82.9 (1′-C), 114.7 (Ar), 118.8 (2′-C), 123.6 (5′-CH), 125.3 (Ar), 131.8 (ipso), 148.9 (3′-C), 157.4 (4′-C), 158.7 (para), 177.3 (amide carbonyl), 202.5 (6′-carbonyl).

4,4,2′,4′-Tetramethyl-1-(*p*-methoxyphenyl)-1-azaspiro[4.1′]dec-4′-ene-2,3′,6′-trione (5). A solution of 1 (20 mg, 64 mmol) in 2 mL of acetonitrile and 2 mL of sodium phosphate buffer (pH 7.0) was warmed to 37 °C for 24 h. The keto spirolactam 5 was purified on a PRP preparative HPLC column. After eluting at 10.1 min, the keto spirolactam was extracted into Et_2O. Removal of the solvent under reduced pressure afforded 5 mg (25%) of the keto spirolactam 5: ^1H NMR δ 1.05 (3H, s, 4-C(CH_3)), 1.15 (3H, s, 4-C(CH_3)), 1.54 (3H, d, J = 6.75 Hz, 2′-CH(C*H*$_3$)), 2.02 (3H, d, J = 1.46 Hz, 4′-C(CH_3)), 2.32 (1H, d, J = 16.8 Hz, 3-(CH_2)), 2.57 (1H, d, J = 16.8 Hz, 3-(CH_2)), 3.40 (1H, q, J = 6.8 Hz, 2′-C*H*(CH_3)), 3.80 (3H, s, methoxy), 6.62 (1H, d, J = 1.47 Hz, 5′-CH), 6.91–7.24 (4H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 11.1 (2′-C(*C*H_3)), 17.0 (4′-C(*C*H_3)), 23.5 (4-C(*C*H_3)$_2$), 30.8 (4-C(*C*H_3)$_2$), 40.9 (4-*C*(CH_3)$_2$), 46.7 (3-(CH_2)), 50.0 (2′-*C*(CH_3)), 82.6 (1′-C), 114.2 (Ar), 128.8 (ipso), 130.5 (Ar), 159.1 (para), 137.6 (5′-CH), 151.3 (4′-*C*(CH_3)), 175.0 (amide carbonyl), 194.3 (6′-carbonyl), 198.5 (3′-carbonyl).

Time Course of Degradation of 1 in Acetate Buffer, pH 5.0. Reactions were followed by HPLC after the addition of 0.150 mL of 1.0 mM 1 in CH_3CN to 1.35 mL of 0.1 M acetate buffer (pH 5.0). The reactions were conducted at 37 °C. The ionic strength was maintained at 0.3 M with NaCl. An aliquot of the reaction mixture was injected onto the HPLC after 2-min reaction time, and every 13 min thereafter, for a total reaction time of 236 min.

Molecular Modeling. The molecular structures of the keto spirolactam diastereomers 5 were constructed using the molecular modeling package INSIGHT, from Biosym, Inc. These structures were consequently optimized using the molecular mechanics program DISCOVER, from Biosym, Inc. The energy minimization was achieved using a combination of steepest descent and conjugated gradient minimizers to obtain reasonable energetic structures.

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Supplementary Material Available: Proton–carbon assignments for 1, 4, and 5 obtained from 2D NMR experiments and NMR spectra of compounds 4 and 5 (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.