Facilitated Intramolecular Conjugate Addition of Amides of 3-(3′,6′-Dioxo-2′,4′-dimethyl-1′,4′-cyclohexadienyl)-3,3-dimethylpropionic Acid. 2. Kinetics of Degradation

Michalis G. Nicolaou,† Janet L. Wolfe,†,‡ Richard L. Schowen, and Ronald T. Borchardt*

Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, Kansas 66047-2504

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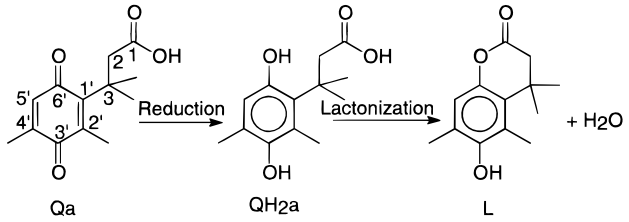
The chemical stability studies of amides of 3-(3′,6′-dioxo-2′,4′-dimethyl-1′,4′-cyclohexadienyl)-3,3-dimethylpropionic acid (Qa) [Qop(a−j)] were conducted in order to determine the utility of this redox-sensitive system as a potential prodrug promoiety or redox-sensitive protecting group in organic synthesis. This study showed that quinone propionic amides of aniline derivatives [Qop-(a−d)] underwent rapid degradation in mildly acidic conditions (pH 4.5−6.0) to yield degradation products resulting from the intramolecular 1,2- or 1,4- conjugate addition of the amide nitrogen to the quinone ring. This conjugate addition was found to be specific base-catalyzed and independent of the *para* substituent on the aromatic ring of the amine. The predominant route of degradation yielded a five-membered ring spirolactam. By altering the nature of the amine component of the amide, these degradation reactions were prevented. Amides of Qa other than those of the aniline type [Qop(e−j)] were found to be substantially more stable and were thus proposed as the more suitable candidates for this potential redox-sensitive prodrug system and redox-sensitive protecting group for amines and alcohols in organic synthesis.

Introduction

The unique chemical properties of 3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic acid (Qa, *Q*uinone *a*cid), which include ease of reduction either chemically or enzymatically to yield the hydroquinone QH_2a that rapidly lactonizes[1] (Scheme 1), have made this molecule useful as a redox-sensitive promoiety for amine-containing drugs.[2,3] In addition, Qa has been used as a redox-sensitive protecting group for amines[4,5] and alcohols[6] by synthetic chemists.

For Qa to be a useful redox-sensitive promoiety or a redox-sensitive protecting group, esters or amides of this quinone propionic acid need to be chemically stable and, upon reduction, to quantitatively release the amine or the alcohol. In an earlier study from our laboratory,[7] we observed that when the *p*-anisidine amide derivative, Qop(a), of this quinone propionic acid was dissolved in mildly acidic or basic aqueous solutions, it rapidly degraded to yield the products QAd(a), cy5en(a), cy5ke-(a), and cy6ke(a) shown in Scheme 2 (QAd, *Q*uinone *Ad*duct; cy5en, *five*-membered ring *cy*clic *en*ol; cy5ke, *five*-membered ring *cy*clic *ke*tone; cy6ke, *six*-membered ring *cy*clic *ke*tone). These products arise from the 1,2- or 1,4-conjugate addition of the amide nitrogen to the α,β-unsaturated carbonyl system present in the quinone propionic acid.

Scheme 1



The objectives of this study were to determine more extensively the aqueous stability of amides of Qa as a function of pH and buffer concentration and to ascertain how the structure of the amine affects the rates and the extent of these degradation reactions. Information forthcoming from this study should better define the limitations of Qa as a redox-sensitive promoiety for drug delivery and a redox-sensitive protecting group in organic synthesis.

Results

The kinetics of degradation of the quinone propionic amide Qop(a) were determined in aqueous buffered acetonitrile (90:10 v/v) in the pH range 4.5−6.0. Acetic acid and sodium acetate were used in the concentration range of 0.05−0.30 M. Scheme 2 illustrates the kinetic scheme that adequately describes the degradation based on the data generated in this study. The degradation of the starting material Qop(a) and the appearance of the degradation products, QAd(a), cy5en(a), cy5ke(a) and cy6ke(a), were followed by HPLC. In Table S1 (supporting information) are provided the retention times for Qop-(a−e) and their degradation products on the HPLC system employed for analysis. Characterization of the degradation products by ^{1}H NMR and UV spectroscopy was described earlier.[7] To further ascertain the identity of these degradation products, the HPLC system was

* To whom the correspondence should be addressed. Dr. Ronald T. Borchardt: Tel: (913)-864-3427. Fax: (913)-864-5736. e-mail: Borchardt@Smissman.hbc.ukans.edu.

† These authors contributed equally to this paper.

‡ Current Address: Department of Pharmaceutics, University of Tennessee, Memphis, TN 38163.

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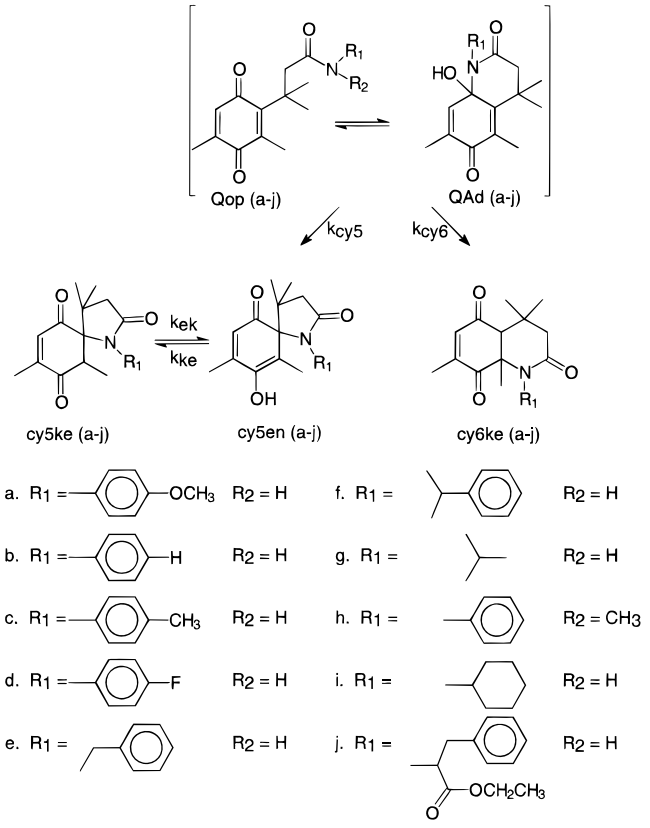
Scheme 2



equipped with a diode array spectrophotometric detector. Table S2 (supporting information) lists the observed λ_{max} values for the different degradation products. The structure of the product with retention time of 3.70 min is proposed to be cy6ke.

Figure 1 illustrates time profiles for the disappearance of the quinone propionic amide Qop(a) and its degradation products at the highest (6.0) and lowest (4.5) pH values employed in this study. In all cases, Qop(a) appeared to reach fast equilibration with the hydroxy dienone QAd(a). Thus, it was not possible to isolate the microscopic rate constants describing this equilibration, and both species were treated as a pool of a single reactant as illustrated by brackets in Scheme 2. The disappearance of Qop(a)−QAd(a) was accompanied by concomitant formation of the enol spirolactam cy5en(a) and the proposed lactam cy6ke(a), whereas the keto spirolactam cy5ke(a) appeared after a slight lag time (Figure 1, Scheme 2).

Table 1. Rate Constants for the Degradation of the Quinone Propionic Amide, Qop(a), at Different pH; in 0.1 M Acetate Buffer (μ = 0.3 M) at 37 °C

	$k \times 10^7$ s^{-1}			
pH	k_{cy5}	k_{cy6}	k_{ek}	k_{ke}
4.50	**285** ± 5	**51** ± 2	*a*	*a*
5.00	**1742** ± 16	**77** ± 8	**11622** ± 775	**1753** ± 178
5.50	**4101** ± 31	**196** ± 9	**21822** ± 977	**3761** ± 198
6.00	**14943** ± 417	**763** ± 54	**13537** ± 521	**2742** ± 135

[a] Due to the fast equilibration, k_{ek} and k_{ke} could not be adequately estimated. The equilibrium constant obtained from the ratio of the mole fractions of the keto and enol tautomers cy5ke(a) and cy5en(a) was 3.0 ± 0.8.

Mole fractions of the starting material and the degradation products were obtained from the relative HPLC peak areas. The sum of all peak areas of the starting material and the degradation products remained constant throughout the time course of the experiment, suggesting that all species absorbed similarly at a wavelength of 250 nm and that mass balance was maintained. The kinetic rate constants kcy5, kcy6, kek, and kke (Scheme 2) were estimated by nonlinear least-squares fits on the experimentally obtained mole fraction-time data. The kinetic equations describing the time dependence for the degradation of Qop(a) and appearance of its degradation products as well as the analytically derived integrated rate equations are listed in Appendix 1 (supporting information) (the analytically derived integrated rate equations define each species involved in the reaction with the letter S, followed by the specified species in parentheses). The values for the rate constants shown in Table 1, which were determined in different pH conditions while maintaining the buffer concentration at 0.1 M, indicate that both kcy5 and kcy6 increase as the reaction mixture becomes more basic. In addition, the magnitude of kcy5 was consistently larger at all pH values studied. The rate constants describing the enol−keto equilibration (Table 1), kek and kke, do not show

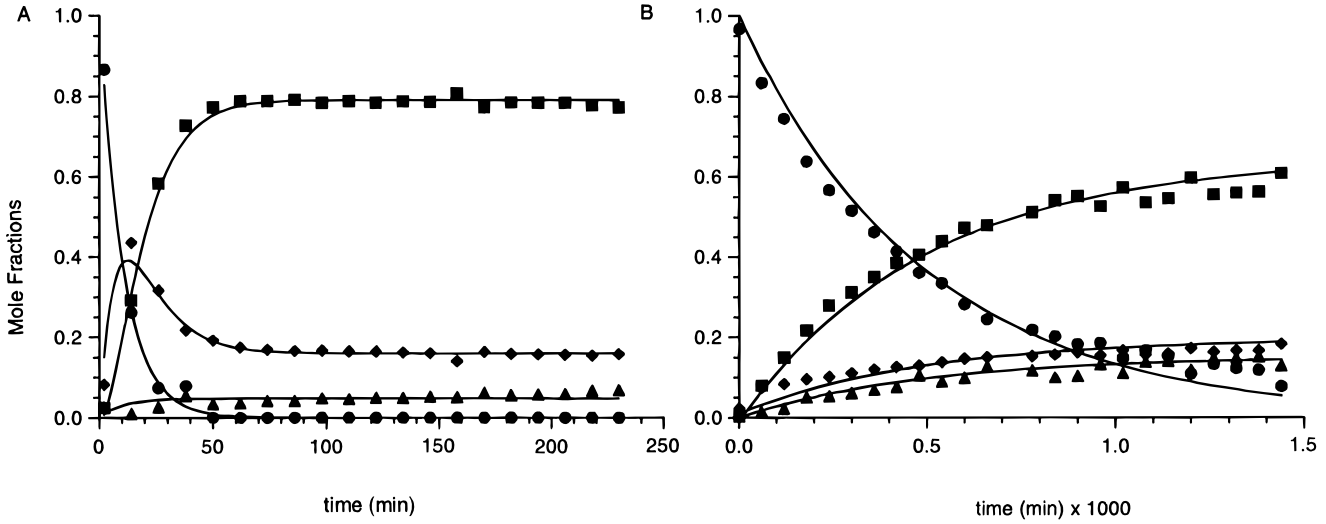


Figure 1. Representative time profiles for the degradation of Qop-a (●) and the formation of cy5en-a (◆), cy5ke-a (■), and cy6ke-a (▲) at 37 °C, 0.1 M acetate buffer and ionic strength (μ = 0.3 M): A. pH = 6.0, B. pH = 4.5. Data points represent experimentally obtained mole fractions, and lines are plots of equations from Appendix 1 with rate constants shown in Table 1.

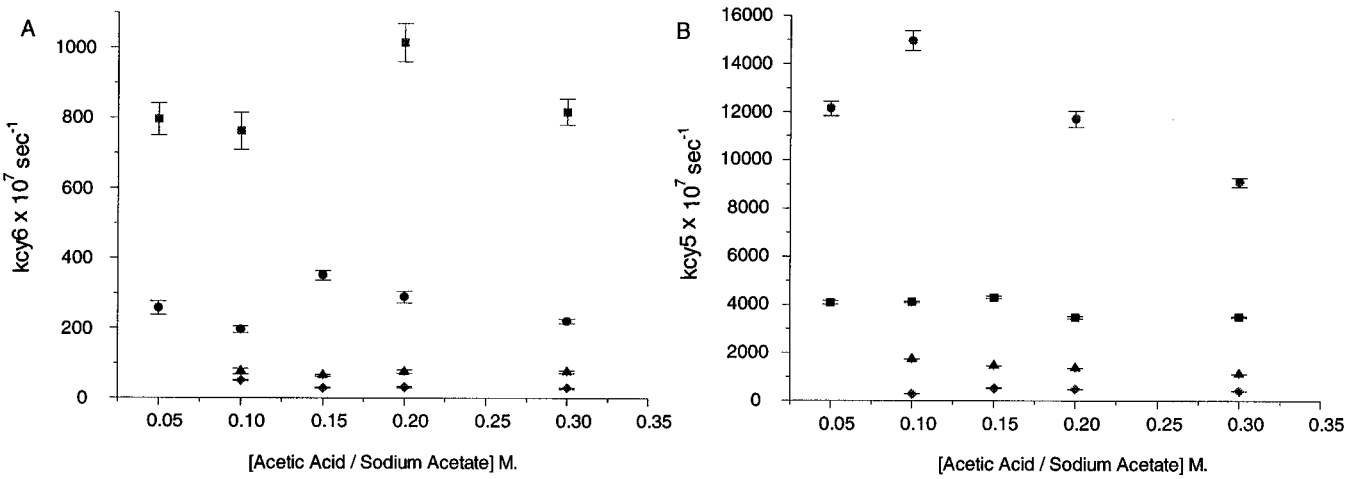


Figure 2. Plots for kcy6 (A) and kcy5 (B) as a function of buffer concentration. pH 6.0 (●), pH = 5.5 (■), pH = 5.0 (▲), and pH = 4.5 (◆) at 37 °C.

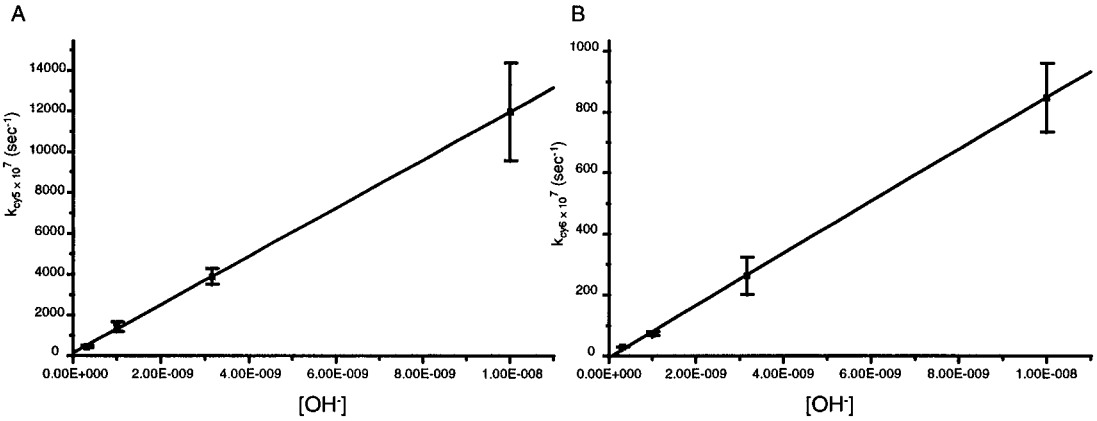


Figure 3. Plots of average values of kcy5 (A) and kcy6 (B) as a function of the hydroxide ion concentration.

any systematic increase or decrease with the pH. At pH 4.5, the values for kek and kke were accompanied by large standard deviations, and the equilibrium constant obtained from the ratio of the mole fractions of cy5ke(a) and cy5en(a) was reported instead.

The kinetics of degradation of Qop(a) were studied as a function of different acetic acid/sodium acetate buffer concentrations (0.05−0.30 M). Figure 2 illustrates that kcy5 and kcy6 were observed to be pH-dependent (pH range of 4.5−6.0) and buffer concentration-independent. Average values for kcy5 and kcy6 were calculated with reasonable standard deviations (<20% and 23%, respectively). Figure 3 illustrates linear dependence in both kcy5 and kcy6 with hydroxide ion concentration according to the following relationship:

$$\text{kcy5,6} = k_{\text{OH}} \times [\text{OH}^-]$$

Second order rate constants ($M^{-1} \times s^{-1}$) obtained by linear regression of the data shown in Figure 3 are $k_{\text{OHcy5}} = 1.2 \times 10^5$ and $k_{\text{OHcy6}} = 8.5 \times 10^3$, relating the average rate constants (kcy5 and kcy6) with the hydroxide ion concentration, respectively.

Enol−keto equilibration data at different pH and buffer conditions were also investigated. Rate constants kek and kke were found to be buffer-dependent, and they showed a systematic increase as buffer concentration increased. However, the slopes of the buffer plots became smaller as the pH increased, suggesting that acetic acid is a more effective catalyst than is acetate ion. Extrapolated data at zero buffer concentrations were found to be inconclusive to define pH dependencies for kek and kke.

The effect of the amide structure was also addressed. Different quinone propionic amides [Qop(a−j)] were studied in order to determine structural features that affect the rate of degradation of these types of compounds. The kinetics of disappearance of Qop(a−j) were studied at pH 5.0, 37 °C, constant ionic strength (μ = 0.3 M), and 0.1 M acetic acid/sodium acetate buffer. Analysis was conducted by HPLC. Table S2 (supporting information) shows retention times for all species of interest. The characterization of the degradation products was conducted by UV spectroscopy and λ_{max} values for the different species are shown in Table S1. First-order rate constants for the disappearance of Qop(a−j) were obtained by nonlinear least-squares fits on the HPLC peak area−time data. Only Qop(a−d) underwent rapid degradation. Disappearance rate constants were obtained as follows: Qop(a), *p*-oMe substituent, $1819 \pm 30 \times 10^{-7}$ s^{-1}; Qop(b), *p*-H, $2500 \pm 233 \times 10^{-7}$ s^{-1}; Qop(c), *p*-Me, $872 \pm 42 \times 10^{-7}$ s^{-1} and Qop(d), *p*-F, $2000 \pm 83 \times 10^{-7}$ s^{-1}. The change in reactivity is considered minimal. As illustrated in the Hammett plot in Figure 4, the substituent on the *para* position of the aromatic ring does not greatly affect the reactivity. By inserting a methylene group between the aromatic ring and the nitrogen, *e.g*, Qop(e), solution stability ($k = 24 \times 10^{-7}$ s^{-1}) was improved substantially. When steric bulk was introduced

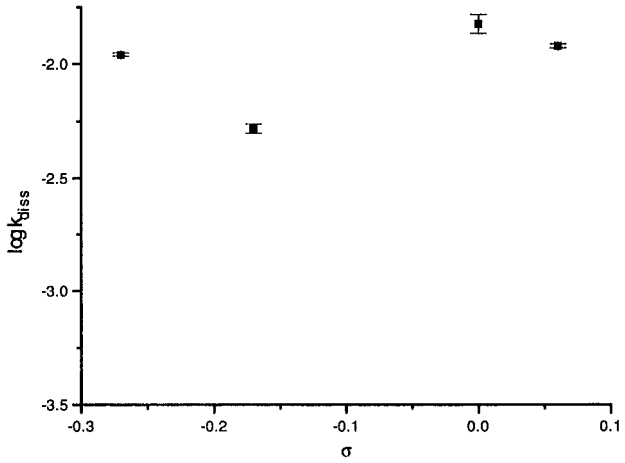


Figure 4. Plot of the log k_{dis} versus the Hammett value σ.

on the methylene group of compound Qop(e) to form Qop(f), as well as for compound Qop(g) and Qop(j), no detectable degradation in solution occurred within 24 h. When an amide was made from a secondary amine, *i.e.* Qop(h), or when cyclohexylamine was used in the place of aniline, *i.e.* Qop(i), no detectable degradation in solution occurred within 24 h. Finally, compounds Qop(i,j) were found to be equilibrated with their corresponding hydroxy dienones QAd(i,j), after 24 h in solution (Qop(i) and Qop(j) contained 11% and 15% of QAd(i) and QAd(j), respectively).

Discussion

Scheme 2 illustrates the degradation of the compounds of the Qop type. From this degradation scheme one may realize that there are six sites on the quinone ring that may undergo either 1,2 or 1,4 conjugate addition. However, only three sites undergo nucleophilic attack by the nitrogen, and the products formed contain five- and six-membered ring lactams. Attack at the other three sites of the quinone ring would result in products containing seven- and eight-membered ring lactams, which are thermodynamically less favorable.[8]

Initially, the starting material Qop(a) undergoes a fast intramolecular 1,2-conjugate addition by the amide nitrogen to yield the hydroxy-amide QAd(a). QAd(a) then undergoes ring opening to form what seems to be the thermodynamically more stable starting material Qop(a). This is consistent with an earlier observation of Dürckheimer and Cohen where a 9-hydroxy-α-tocopherone, a cyclic hydroxy dienone, spontaneously underwent ring opening and established a pH-dependent equilibrium with the corresponding open quinone structure.[9] Qop(a) can also undergo 1,4-conjugate addition by the amide nitrogen at positions 1′ and 2′ on the quinone ring. The major route of degradation in this case, however, is attack at position 1′ to yield formation of the five-membered ring spirolactam, which is present as both the enol and keto tautomer, cy5en(a) and cy5ke(a), respectively. Attack at the 2′ position is less favored, since <10% cy6ke(a) is formed at equilibrium.

The combination of the linear dependence of the rate constants kcy5 and kcy6 on the hydroxide ion concentration with zero intercept (Figure 3) and the buffer concentration independence (Figure 2) of these rate constants suggests that the reactions in these two routes of degradation are specific-base-catalyzed. Thus, the proton abstraction by the hydroxide ion occurs prior to the rate-determining step. Hydroxide ion-facilitated deprotonation of the amide nitrogen promotes the development of negative charge (on the nitrogen), forming a highly reactive nucleophilic species, which by subsequent attack at positions 1′ and 2′ of the quinone ring yields products cy5en and cy6ke. The formation of the five-membered ring product is the dominating degradation route of the reaction. Such degradation occurs not only in the case of amides, as illustrated in this study, but also in the case of the carboxylic acid, Qa. Borchardt and Cohen reported formation of a spirolactone, the product that results from 1,4-conjugate addition of the carboxylic acid oxygen to the 1′ position of the quinone ring, in equilibrium with Qa in acidic conditions.[1] Other possible degradation routes of Qa, such as attack at position 2′, to form a product analogous to cy6ke or at position 6′ to form QAd(a), were not reported. This degradation profile was explained by the existence of a "trimethyl lock", which resulted in such conformation of the carboxylic acid that favored the formation of the transition state, leading to the five- instead of the six-membered ring spirolactone.

In a recent study,[10] our laboratory determined the crystal structures of quinone propionic acid and several of its amides. The distance between the amide nitrogen, in the case of the quinone propionic amides and the carboxylic acid oxygen, in the case of the quinone propionic acid, and the positions 1′ and 6′ of the quinone ring is approximately 1 Å less than the distance from position 2′. Thus, in addition to unfavorable rotation that has to take place in order to form the six-membered ring lactone, simple propinquity of the reacting centers may explain the dominance of the five-membered ring spirolactams. In addition, the higher reactivity of the negatively charged nitrogen compared to the corresponding carboxylic acid oxygen could be the driving force for the formation of the less favorable degradation product cy6ke. The reason for the slower degradation of Qop(a) is that there is not enough base at low pH to promote amide deprotonation.

The predominant route of degradation, the formation of the five-membered ring spirolactam, results in the formation of the two possible tautomers: the enol form, which forms first, and the keto form, which appears after a brief lag time. Keto tautomers are generally found to be the thermodynamically most stable tautomers,[12−14] although rare exceptions have been observed.[15] It is interesting to note that approximately 10−15% of the total degradation product was isolated as the enol tautomer cy5en(a). This tautomer presents interesting stabilization features: first, the enol is cyclic and the enolic double bond is conjugated with another double bond in the ring and, second, the methyl substitution β to the enolic hydroxyl group provides the enol with

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additional stability.[16] The keto−enol equilibrium constant due to proton conservation was expected to be independent of pH.[12] Equilibrium constant data (Table 1 K_{equil} = kek/kke) show some variation at different pH values. This phenomenon may be due to possible ionization of the enolic hydroxyl group and, in such a case, the assumption of proton conservation does not hold.

Similar to that of Qop(a), degradation in aqueous solution occurred with structures Qop(b−e). The kinetics of disappearance of Qop(b−d) produced rate constants that showed minimal dependence when plotted against the Hammett σ value (Figure 4). This suggests that substitution on the *para* position of the aromatic ring of the amine group does not significantly alter the rate of degradation of these compounds. The benzyl amide Qop(e), having one methylene between the amide nitrogen and the aromatic ring, was expectedly found to degrade significantly slower than the aromatic amine substituents Qop(a−d). The minimal Hammett correlation (Figure 4) suggests that there is no difference in the amount of negative charge between the ground state of the starting material and the transition state in the formation of the different degradation products. This suggests that at the transition state a strong carbon−nitrogen bond is formed, resembling the structure of the products.

Compounds Qop(f−i) were found to be stable and did not significantly degrade in aqueous solution at pH 5.0. This suggests that this redox-sensitive system is stable when the amine is secondary [Qop(h)], also supporting the claim of the nitrogen deprotonation. Quinone propionic amides of aliphatic amines, such as Qop(g,i,j), did not undergo significant degradation, and only a minor amount of QAd(i,j) was observed. Finally, when the amide nitrogen is sterically hindered, such as the case of Qop(f), no degradation is observed.

The "trimethyl lock" was found earlier to facilitate acceleration of the rate of lactonization of hydroxypropionic acids such as Qa and amides such as Qop(a).[1,2] In this case, the great strain relief to form products such as cy5en(a), cy5ke(a), and cy6ke(a) is also of consideration. In a recent publication, the crystal structure of the aforementioned spirolactone revealed that the methyl groups of the "trimethyl lock" are no longer in close proximity as in the open structures; rather, the geminal dimethyl group at position 3 points in the opposite direction from the methyl group at position 2′.[11] Therefore, the major driving force for this reaction is possibly the relief of the strain energy involved because the close proximity between the methyl groups that form the "trimethyl lock" is lost.

Conclusion

In conclusion, the studies described here suggest that the instability problems reported previously with amide derivative Qop(a) of the quinone propionic acid Qa generated from anisidine[7] are unique to primary aromatic amines [Qop(a−d)]. In contrast, amides of Qa generated from secondary aromatic [Qop(h)] and aliphatic amines [Qop(e,f,g,i,j)] are generally stable. This result suggests that if amines are carefully chosen, derivatization with Qa could lead to useful redox-sensitive prodrugs or redox-sensitive protecting groups in organic synthesis.

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Experimental Section

All melting points were determined on a Meltemp apparatus and are uncorrected. Mass spectral analyses were conducted by The University of Kansas Mass Spectrometry Laboratory, Lawrence, KS, and elemental analyses were determined by Desert Analytics Organic Microanalysis, Tucson, AZ, or The University of Kansas Elemental Analyses Laboratory. Column chromatography was performed on silica gel (70−270 mesh) purchased from Aldrich Chemical Co., Milwaukee, WI. 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, Germany. ^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ on a Bruker AM 500 or a Varian XL-300 spectrophotometer. ^{1}H-NMR spectra were recorded at 500.13 or 300 MHz and chemical shifts were recorded in δ ppm relative to TMS (0.0 ppm) or H_2O (4.80 ppm). ^{13}C-NMR spectra were recorded at 125.77 MHz and chemical shifts were recorded in δ ppm relative to $CDCl_3$ (77.0 ppm).

General Method I for Preparation of Amides. 3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-diene)-3,3-dimethylpropionic acid Qa (0.29 g, 1.23 mmol), whose synthesis was previously described,[2] was dissolved in 25 mL of dry CH_2Cl_2, to which was added the amine (2 equiv). The mixture was cooled to 0 °C, and dicylohexylcarbodiimide (DCC), (0.30 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 removed by filtration and the CH_2Cl_2 removed under reduced pressure. EtOAc was added to the residue, the mixture was again filtered to remove DCU, and the solvent was again evaporated. This procedure was repeated until no additional DCU could be precipitated. The desired product was eluted from a silica gel column with 30% EtOAc in hexane. The fractions were concentrated and recrystallized in EtOAc/hexane.

General Method II for Preparation of Amides. In a Pierce reaction vial, the amine (2 equiv) was added to a solution of the *N*-hydroxysuccinimide ester of Qa (0.1 g, 0.3 mmol), whose synthesis was previously described,[3] in 2 mL benzene. The vial was sealed and placed in an oil bath at 80 °C for 15 min and then cooled to room temperature. A crystalline white precipitate was formed. The reaction mixture was then diluted with diethyl ether, washed with water (20 mL), 5% aqueous HCl (20 mL), water (20 mL), and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded a yellow solid that was recrystallized in EtOAc/hexane.

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid Amide *p*-Anisidine [Qop(a)]. The synthesis for Qop(a) has been previously reported by this laboratory using the general method I described above. Structural data for this compound are described elsewhere.[2,7]

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid Aniline Amide [Qop(b)]. Compound Qop(b) was prepared and purified according to general method I as described above. Analytically pure Qop(b) was obtained in 44% yield. Structural data for this compound are described elsewhere.[2]

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-diene)-3,3-dimethylpropionic Acid *p*-Methylaniline Amide [Qop(c)]. Compound Qop(c) was prepared and purified according to general method I as described above. Analytically pure Qop(c) was obtained in 20% yield: mp 157−159 °C; UV (CH_3CN) λ_{max} 250 nm (ϵ = 21876), 334 nm (ϵ = 954); MS (EI), *m/e* 325 (M); ^{1}H NMR δ 1.50 (6 H, s, $C(CH_3)_2$), 1.98 (3 H, d, J = 1.35 Hz, 4′-(CH_3)), 2.19 (3 H, s, 2′-(CH_3)), 2.28 (3 H, s, *p*-CH_3), 3.00 (2 H, s, 2-CH_2), 6.48 (1 H, d, J = 1.38 Hz, 5′-H), 7.08, 7.25 (4 H, m, ArH); ^{13}C NMR δ 14.80 (4′-C(CH_3)), 15.95 (2′-C(CH_3)), 21.22 (p-CH_3), 29.82 (3C(CH_3)$_2$), 39.27 (3-*C*(CH_3)$_2$), 50.76 (2-CH_2), 120.51 (Ar), 129.82 (Ar), 134.33 (Ar), 135.43 (Ar), 135.49 (5′-(CH)), 139.98 (2′-C), 144.05 (4′-C), 152.54 (1′-C), 170.42 (amide carbonyl), 188.64 (3′-carbonyl), 190.93 (6′-carbonyl). Anal. Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.67; H, 7.14; N, 4.12.

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid *p*-Fluoroaniline Amide [Qop-(d)]. Compound Qop(d) was prepared and purified according to general method I as described above. Analytically pure Qop-(d) was obtained in 22% yield: mp 126−128°C; UV (CH_3CN) λ_{max} 248 nm (ϵ = 15953), 330 nm (ϵ = 846); MS (EI), *m/e* 329 (M); ^{1}H NMR δ 1.48 (6 H, s, C(CH_3)$_2$), 1.95 (3 H, d, J = 1.43 Hz, 4′-(CH_3)), 2.15 (3 H, s, 2′-(CH_3)), 3.01 (2 H, s, 2-CH_2), 6.46 (1 H, d, J = 1.46 Hz, 5′-H), 6.93, 7.32 (4 H, m, ArH); ^{13}C NMR δ 14.39 (4′-C(CH_3)), 15.56 (2′-C(CH_3)), 29.37 (3-C(CH_3)$_2$), 38.79 (3-C(CH_3)$_2$), 50.22 (2-CH_2), 115.43 (Ar), 115.61 (Ar), 121.76 (Ar), 121.83 (Ar), 130.55 (Ar), 133.53 (Ar), 135.00 (5′-(CH)), 139.52 (2′-C), 143.73 (4′-C), 151.72 (1′-C), 170.10 (amide carbonyl), 188.17 (3′-carbonyl), 190.22 (6′-carbonyl). Anal. Calcd for $C_{19}H_{20}NO_3F$: C, 69.28; H, 6.12; N, 4.25; F, 5.77. Found: C, 69.25; H, 6.33; N, 4.64; F, 6.08.

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid Benzylamine Amide [Qop(e)]. Compound Qop(e) was prepared according to general method I as described above. Structural data for this compound are described elsewhere.[2]

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid (S)-(−)-α-Methylbenzylamine Amide [Qop(f)]. Compound Qop(f) was prepared and purified according to general method I as described above. Analytically pure Qop(f) was obtained in 24% yield as an orange oil: UV (CH_3CN) λ_{max} 254 nm (ϵ = 8088), 336 nm (ϵ = 532); MS (EI), *m/e* 339 (M); ^{1}H NMR δ 1.40 (3 H, s, α-CH_3), 1.41 (3 H, s, -C(CH_3)$_2$), 1.48 (3 H, s, C(CH_3)$_2$), 1.96 (3 H, d, J = 1.37 Hz, 4′-(CH_3)), 2.12 (3 H, s, 2′-(CH_3)), 2.84 (2 H, q, 2-CH_2), 5.01 (1 H, m, α- proton), 6.42 (1 H, d, J = 1.43 Hz, 5′-H), 7.22−7.30 (5 H, m, ArH); ^{13}C NMR δ 14.80 (4′-C(CH_3)), 15.95 (2′-C(CH_3)), 21.22 (*p*-CH_3), 29.82 (3-C(CH_3)$_2$), 39.27 (3-C(CH_3)$_2$), 50.76 (2-CH_2), 120.51 (Ar), 129.82 (Ar), 134.33 (Ar), 135.43 (Ar), 135.49 (5′-(CH)), 139.98 (2′-C), 144.05 (4′-C), 152.54 (1′-C), 170.42 (amide carbonyl), 188.64 (3′-carbonyl), 190.93 (6′-carbonyl). Anal. Calcd for $C_{21}H_{25}NO_3$: C, 74.31; H, 7.42; N, 4.13. Found: C, 74.59; H, 7.71; N, 3.98.

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid L-Phenylalanine Ethyl Ester Amide [Qop(j)]. Compound Qop(j) was prepared and purified according to general method I as described above. Analytically pure Qop(j) was obtained in 27% yield as an yellow oil: UV (CH_3CN) λ_{max} 256 nm (ϵ = 8663), 338 nm (ϵ = 425); MS (EI), *m/e* 411 (M); ^{1}H NMR δ 1.21 (3 H, t, J = 7.11 Hz, $CO_2CH_2CH_3$), 1.37 (3 H, s, 3-CH_3), 1.41 (3 H, s, 3-CH_3), 1.97 (3 H, d, J = 1.21 Hz, 4′-(CH_3)), 2.12 (3 H, s, 2′-(CH_3)), 2.86 (2 H, q, 2-CH_2), 3.01 (2 H, m, CH_2Ph), 4.12 (2 H, q, J = 7.12 Hz, $CO_2CH_2CH_3$), 4.78 (1 H, q, a-proton), 6.13 (1 H, d, amide proton), 6.47 (1 H, d, J = 1.33 Hz, 5′ proton), 6.98−7.19 (5 H, m, ArH); ^{13}C NMR δ 13.84 ($CO_2CH_2CH_3$), 14.04 (4′-C(CH_3)), 15.34 (2′-C(CH_3)), 28.82 and 28.89 (3-C(CH_3)$_2$), 37.70 (3-C(CH_3)$_2$), 38.35 (NHCH), 48.81 (2-CH_2), 52.54 (CH_2Ph), 61.16 ($CO_2CH_2CH_3$), 126.76 (Ar), 128.23(Ar), 129.03 (Ar), 134.97 (5′-(CH)), 135.68(Ar), 138.68 (2′-C), 143.06 (4′-C), 151.89 (1′-C), 171.16 ($CO_2CH_2CH_3$), 171.28 (amide carbonyl), 187.96 (3′-carbonyl), 189.61 (6′-carbonyl). Anal. Calcd for $C_{23}H_{27}NO_5$: C, 70.05; H, 7.10; N, 3.40. Found: C, 69.85; H, 7.36; N, 3.74.

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid Isopropylamine Amide [Qop-(g)]. Compound Qop(g) was prepared and purified according to general method II as described above. The analytically pure Qop(g) was obtained in 95% yield: mp 121−122 °C; UV (CH_3-CN) λ_{max} 262 nm (ϵ = 6757), 336 nm (ϵ = 570); MS (EI), *m/e* 277(M); ^{1}H NMR δ 1.05 (6 H, d J = 6.54 Hz, CH(CH_3)$_2$), 1.44 (6 H, s, C(CH_3)$_2$), 1.96 (3 H, d, J = 1.41 Hz, 4′-(CH_3)), 2.17 (3 H, s, 2′-(CH_3)), 2.78 (2 H, s, 2-CH_2), 3.95 (1 H, m, CH(CH_3)$_2$), 5.17 (1 H, d, NH), 6.52 (1 H, d, J = 1.41Hz, 5′-H); ^{13}C NMR δ 14.68 (4′-C(CH_3)), 15.65 (2′-C(CH_3)), 23.15 (CH(CH_3)$_2$), 29.59 (3-C(CH_3)$_2$), 39.19 (3-C(CH_3)$_2$), 49.86 (2-CH_2), 135.66 (5′-CH), 139.19 (2′-C), 143.41 (4′-C), 152.85 (1′-C), 171.19 (amide carbonyl), 188.63 (3′ carbonyl), 190.43 (6′ carbonyl). Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.31; H, 8.30; N, 5.05. Found: C, 69.38; H, 8.68; N, 4.78.

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid Cyclohexylamine Amide [Qop-(i)]. Compound Qop(i) was prepared and purified according to general method II as described above. The analytically pure Qop(i) was obtained in 95% yield: mp 142−143 °C; UV (CH_3-CN) λ_{max} 256 nm (ϵ = 9198), 332 nm (ϵ = 650); MS (EI), *m/e* 317 (M); ^{1}H NMR δ 0.90−1.90 (10 H, m, cyclohexyl CH_2), 1.45 (6 H, s, 3-C(CH_3)$_2$), 1.98 (3 H, d, J = 1.41 Hz, 4′-C(CH_3)), 2.15 (3 H, s, 2′-C(CH_3)), 2.78 (2 H, s, 2-CH_2), 6.52 (1 H, d, J = 1.53 Hz, 5′-CH); ^{13}CNMR δ 14.14 (2′-C(CH_3)), 15.48 (4′-C(CH_3)), 24.69, 25.29, 33.05 (cyclohexyl CH_2), 47.69 (HNCHCH$_2$), 28.96 (3-C(CH_3)$_2$), 38.62 (3-C(CH$_3$)$_2$), 49.34 (2′-CH_2), 135.20 (5′-CH), 138.46 (2′-C(CH$_3$)), 143.12 (4′-C(CH$_3$)), 152.45 (1′-C), 170.60 (amide carbonyl), 188.11 (3′ carbonyl), 189.85 (6′ carbonyl). Anal. Calcd for $C_{16}H_{23}NO_3$: C, 71.90; H, 8.50; N, 4.42. Found: C, 72.19; H, 8.89; N, 4.69.

3-(3′,6′-Dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic Acid *N*-Methylaniline Amide [Qop-(h)]. 3-(3′,6′-Dioxo-2′,4′ dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic acid Qa (0.18 g, 0.76 mmol) was dissolved in 50 mL of CH_2Cl_2, to which was added *N*-methylmorpholine (0.23 g, 2.28 mmol). The mixture was cooled to −55 °C in a dry ice−2-propanol bath. Isobutyl chloroformate (0.092 g, 0.91 mmol) was then added, followed by addition of *N*-methylaniline (0.24 g, 2.28 mmol) after 10 min. The reaction mixture was stirred for 30−40 min and filtered. The residue obtained by evaporation of the solvent under reduced pressure was dissolved in 30 mL $CHCl_3$. The solution was then washed with water (50 mL), 5% HCl (50 mL), 5% $NaHCO_3$ (50 mL), and saturated NaCl (50 mL) solutions. The product Qop(h) was eluted from a silica gel column with 40% methyl tertiary butyl ether in hexane (25% yield). All characterization data for this compound are described elsewhere.[2]

HPLC Assay Conditions. The kinetics of degradation of the quinone propionic amides [Qop(a−j)] were monitored by HPLC according to a previously described analytical method.[7] The concentrations of the Qop(a−j) and their degradation products [QAd(a−j), cy5en(a−j), cy5ke(a−j), cy6ke(a−j)] shown in Scheme 2 were quantified by measuring peak areas.

Kinetics of Degradation. Degradation kinetics were carried out using acetate buffers (pH 4.5−6.0) diluted with 10% acetonitrile. The concentration of acetate was varied from 0.05 M to 0.03 M, while maintaining a constant ionic strength of 0.3 M with sodium chloride. Reactions were initiated by adding 150 μL of 1.0 mM solutions of the quinone propionic amide [Qop(a−j)] to 1.35 mL of acetate buffer. Samples were incubated at 37 °C. Aliquots of reaction mixtures at pH 5.0, 5.5, and 6.0 were removed at T = 2 min and every 13 min thereafter, up to 236 min. For pH 4.5 reaction mixtures, aliquots were removed at T = 2 min and every 30 min thereafter, up to 572 min.

Supporting Information Available: Tables of HPLC retention time data and of UV absorbance data; equations describing the kinetics of the reaction in Scheme 2 (3 pages). This material is contained in 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.

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