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Solution Kinetics of CC-1065 A-Ring Opening: Substituent Effects and General Acid/Base Catalysis

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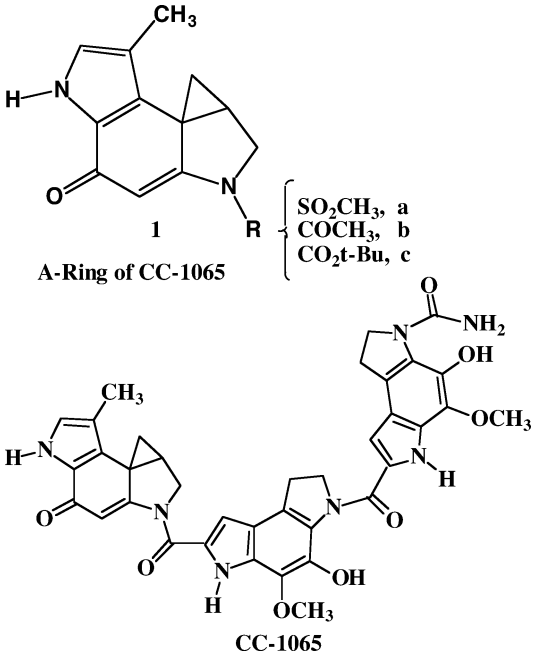
Abstract: pH−rate studies were carried out on 2-substituted derivatives of the CC 1065 A-ring over the range of pH 5 to 0. The goals were to document the presence of general-acid-catalyzed cyclopropyl ring opening near neutrality and to assess the role of the 2-substituent on the rate of nucleophile trapping by the cyclopropyl ring. Our studies show that the cyclopropyl group undergoes reversible general-acid/base-catalyzed trapping of chloride nucleophiles above pH 4. This finding implies that the general-acid-catalyzed alkylation of DNA minor groove by the A-ring is feasible. pH−rate studies also showed that the 2-substituent does not influence the pK_a of the protonated carbonyl of the A-ring (p$K_a \sim 1$) and has relatively little effect on the rate of nucleophile trapping. This finding is consistent with calculations that show the fused pyrrolo ring as the source of carbonyl oxygen electron density. The 2-nitrogen, on the other hand, only releases electrons to the carbonyl of the 2-substituent and does not greatly influence trapping of nucleophiles by the A-ring cyclopropyl group.

Introduction

The chemistry and biology of the natural product CC-1065 (Chart 1) has been the subject of intense study, largely because of its interesting DNA alkylation chemistry.[1−3] The alkylation mechanism first postulated[4,5] involved cyclopropyl ring activation by general acids in the DNA minor groove, resulting in adenine N (3) alkylation (structure A, Chart 2).[6] However, the absence of detectable general-acid-catalyzed cyclopropyl ring opening,[7] as well as the stability of CC-1065 toward nucleophiles at neutrality, necessitated an alkylation mechanism dependent on DNA binding.[8] Thus, it was postulated that the conformational change upon binding of CC-1065 to the minor groove removes the stabilizing influence of the vinylogous amide and thereby activates the cyclopropylcyclohexadienone system (structure B, Chart 2). This mechanism, referred to as shape-dependent catalysis, has been validated with 1,2,9,9a-tetrahydrocyclopropa[1,2-*c*]benz[1,2-*e*]indol-4-one (CBI) analogues, wherein the A-ring pyrrolo was replaced with a fused benzene ring.[8]

A postulate made at the outset of this study was that the fused pyrrolo group of the A-ring, not the vinylogous amide, supplies electrons to the cyclopropylcyclohexadienone system and thereby influences its alkylation reactivity. This postulate is based on Hartree−Fock calculations made on the A-ring analogues **1** shown in Chart 1. In contrast, replacing the A-ring pyrrolo with a fused benzene ring to afford CBIs will cause the vinylogous amide to supply electrons to the cyclopropylcyclohexadienone system instead. Proving this postulate has a bearing on the importance of shape-dependent catalysis in alkylation reactions mediated by CC-1065 and its analogues.

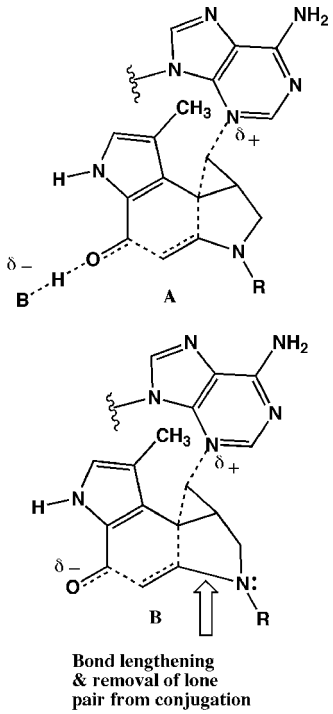
Chart 1. Structures of A-Ring (CPI) Analogues Used in This Study and the CC-1065 Structure



(1) Boger, D. L.; Johnson, D. S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1438−1474.
(2) Cacciari, B.; Romagnoli, R.; Baraldi, P. G.; DaRos, T.; Spalluto, G. *Expert Opin. Ther. Patents* **2000**, *10*, 1853−1871.
(3) Searcey, M. *Curr. Pharm. Des.* **2002**, 1375−1389.
(4) Lin, C. H.; Beale, J. M.; Hurley, L. H. *Biochemistry* **1991**, *30*, 3597−3602.
(5) Eis, P. S.; Smith, J. A.; Rydzewski, J. M.; Case, D. A.; Boger, D. L.; Chazin, W. J. *J. Mol. Biol.* **1997**, *272*, 237−252.
(6) Warpehoski, M. A.; Harper, D. E. *J. Am. Chem. Soc.* **1994**, *116*, 7573−80.
(7) Boger, D. L.; Garbaccio, R. M. *J. Org. Chem.* **1999**, *64*, 8350−8362.
(8) Boger, D. L.; Garbaccio, R. M. *Acct. Chem. Res.* **1999**, *32*, 1043−1052.

10.1021/ja057289a CCC: $33.50

Chart 2. Mechanisms of A-Ring Alkylation: (A) General-Acid Catalysis and (B) Shape-Dependent Catalysis



Another postulate made was that cyclopropyl ring activation by general acids does in fact occur at or near neutrality. The direct observation of general-acid-catalyzed alkylation of DNA by CC-1065 has remained elusive.[9] However, a pK_a value of ~1 for the protonated carbonyl of the A-ring suggests that general-acid catalysis can occur at neutrality. Our proposed mechanism involves the general-acid-catalyzed cyclopropyl ring opening by nucleophilic attack in rapid equilibrium with general-base-catalyzed re-formation of the cyclopropyl ring. This mechanism could only be detected by measuring equilibrium constants as a function of pH rather than by simply measuring rate constants of reactions at various buffer dilutions. Proving this postulate has a bearing on the feasibly of the general-acid-catalyzed DNA alkylation reactions.

To address the mechanistic postulates outlined above, a comprehensive pH−rate study was undertaken utilizing spectral global fitting and chloride nucleophile trapping[10] of CC-1065's A-ring bearing the 2-substituents shown in Chart 1. Spectral global fitting[11,12] was developed to study intermediates in complex photochemical reactions. This technique afforded accurate rate constant data for nucleophile-mediated cyclopropyl ring opening and provided evidence of intermediates in the course of these reactions. Chloride trapping has been used to study the nucleophile trapping of aziridinyl quinones[10] as well as in previous CC-1065 studies.[6] The advantages of using chloride as a nucleophile are that its protonation does not occur over the pH range studied and that second-order rate constants for chloride trapping can be readily separated from those of water and buffer species used to hold pH.

Experimental Section

General Methods. All TLCs were run with VWR silica gel 60 F_{254} plates employing various solvents. Atlantic Microlab Inc. (Norcross, GA) performed elemental analyses. IR spectra were taken as KBr pellets using a Nicolet MX-1 FT IR spectrophotometer; the strongest IR absorbances are reported. ^{1}H NMR spectra were obtained with a Varian Gemini 300 spectrometer. 2D NMR spectra were obtained with a Varian Gemini 500 spectrometer. All NMR chemical shifts (δ) were recorded relative to tetramethylsilane (TMS). Uncorrected melting points were determined with an electro Thermal Melt Temp apparatus. Measurements of pH were made with a Radiometer pHM 220 lab pH meter equipped with a Radiometer GK2401C combination electrode. The synthesis of the CPI analogues **1a**−**c** was carried out using the methodology reported by Warpehoski et al.[13]

Kinetic Studies of A-Ring (CPI) Cyclopropyl Opening. The stock solutions of *N*-sulfonamido-CPI (**1a**), *N*-acetamido-CPI (**1b**) and *N*-Boc-CPI (**1c**) were prepared by diluting 3 mg in a 3 mL volumetric flask with dry dimethyl sulfoxide (DMSO) to afford solutions with concentrations of 3.33−4.1 mM. All kinetic studies were carried out with buffers prepared from doubly distilled water. Final concentrations of the CPIs in reaction mixtures ranged from 55.5 to 68.3μM. DMSO concentrations in reaction mixtures were kept to <2% to minimize solvent effects. The following buffer systems were used to hold pH: 1 M acetate buffer (pH 3.61, 4.05, and 4.2), 1 M formate buffer (pH 2.69 and 3.10), 1 M chloroacetate buffer (pH 1.83), and H_2SO_4 buffers adjusted with concentrated sulfuric acid (pH 1.87, 1.34, 1.09, and 0.5). At each pH value, the concentrations of the buffers and CPIs were held constant while the concentration of the KCl was varied for each reaction. In addition, the concentrations of CPIs and KCl were kept constant, and the concentration of buffer was varied while a constant pH was maintained. Reactions were monitored between 280 and 400 nm with an Olis Cary 14 UV−visible spectrophotometer equipped with Olis Global Works software. The temperature of all the kinetic measurements was maintained at 30.0 ± 0.3 °C with circulating water from a thermostatic water bath. The reaction times varied depending on the pH of the buffers used and range from minutes (at low pH ≈1) to 24 h (high pH ≈4). When the reactions were complete, the pH of the samples was taken and recorded

Calculations. The absorbance vs time vs wavelength surfaces obtained as described above was fit with Global fitting software[11,12] available from Olis Inc. These fits provide first-order and two consecutive first-order rate constants along with intermediate spectra, if any. The log k_{obsd} vs pH data were fit with Graph Pad Prism 3 software, which was also used to generate the solid curve shown in the pH−rate profiles. Electrostatic potential maps of the A-ring (CPI) and the benzo analogue (CBI) were obtained with Spartan 04 software. Hartree−Fock calculations were carried out using the 3-21G(*) basis set.[14]

Results

The cyclopropyl opening reactions of the 2-substituted A-ring analogues **1** were studied over the pH range from ~0 to 5 in aerobic aqueous buffer at 30 °C with varying amounts of KCl added as the nucleophile. The use of KCl permitted the separation of second-order rate constants for cyclopropyl ring opening from those for secondary reactions such as trapping by water and buffer nucleophiles. Previous pH−rate studies of opening of the A-ring utilized chloride as the nucleophile, and the chloride trapping products have been characterized.[6] The goals of this study were to determine pK_a values for the

(9) Boger, D. L.; Boyce, C. W.; Johnson, D. S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 233−238.
(10) Skibo, E. B.; Xing, C. *Biochemistry* **1998**, *37*, 15199−15213.
(11) Beecham, J. M.; Brand, L. *Photochem. Photobiol.* **1986**, *44*, 323−329.
(12) Matheson, I. B. C. *Anal. Instrum.* **1987**, *16*, 345−373.
(13) Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Prairie, M. D.; Wicnienski, N.; Wierenga, W. *J. Med. Chem.* **1988**, *31*, 590−603.
(14) Durig, J. R.; Berry, R. J.; Durig, D. T.; Sullivan, J. F.; Little, T. S. *Teubner-Texte Phys.* **1988**, *20*, 54−68.

2-Sulfonylmethyl Derivative, 1a

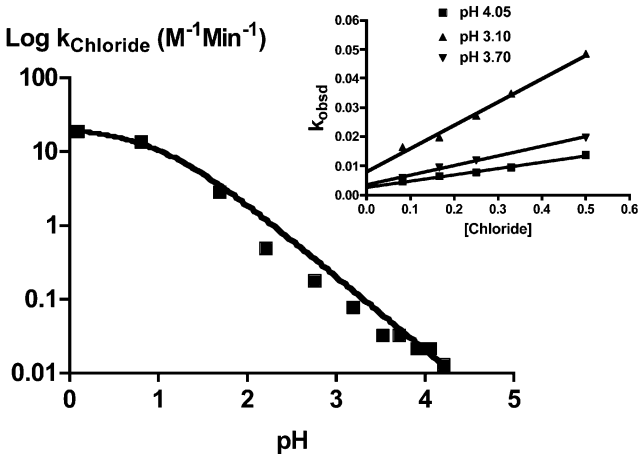


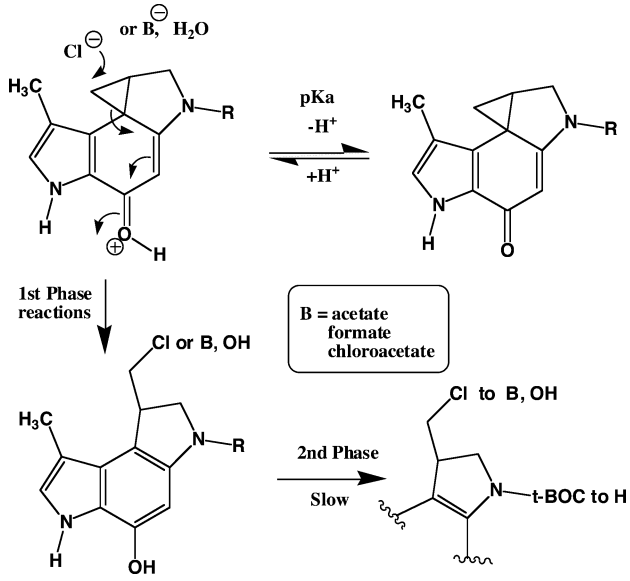
Figure 1. Plot of the log second-order rate constant for chloride-mediated cyclopropyl ring opening of **1a** vs pH. These second-order rate constants were determined from slopes of chloride dilution plots (see inset), obtained at constant pH values. The solid line was computer-generated with the equation shown in Table 1.

protonated C(4) carbonyl from pH−rate data, to determine the relationship between the electronic character of the 2-substituent and the second-order rate constants for cyclopropyl ring opening, and to understand the complex reactions occurring at pH values >4. The N(2)-substituents of **1** represent a range in electronic character, from sulfonylmethyl ($\sigma_{para} = 0.72$) to acetyl ($\sigma_{para} = 0.50$) and t-BOC ($\sigma_{para} = 0.45$) to determine substituent effects.

Global fitting[11,12] was carried out from 280 to 400 nm over a time period dependent on the pH of the reactions (from 5 min at pH 0−1 to 12 h at pH 4−5), and the resulting absorbance vs time vs wavelength surfaces were fit to a single or double exponential (biphasic) rate law. The bisphasic kinetics was due to slower secondary reactions following the rapid opening of the cyclopropyl ring by chloride, buffer, and water nucleophiles during the first kinetic phase. The secondary reactions were independent of chloride concentration and were attributed to nucleophilic substitution at the chloromethyl group of the chloride trapping product by buffer species (acetate, formate, and chloroacetate). The intermediate spectrum, obtained from global fitting of the biphasic reaction, closely resembles that of the final product, indicating that only a minor structural change had occurred during the second phase. At pH values <3, the reaction obeys a single-exponential rate law because the chloromethyl group does not react with the major buffer base species (water) at this pH.

First phase rates, plotted against chloride concentrations, were observed to be linear at pH values <∼4 but nonlinear and saturating with respect to chloride above this pH (see insets of Figures1 and 5, respectively). The linear plots were interpreted as the specific acid second-order opening of the A-ring by chloride (slope = M^{-1} min^{-1}) and cyclopropyl ring opening mediated by buffer species and water (*y*-intercept = min^{-1}) (Scheme 1). In addition, *y*-intercept values included rates for removal of the t-Boc group from **1c** at pH values <3. The saturating relationship observed above pH 4 was attributed to reversible general acid/base opening of the A-ring by chloride with eventual substitution at the chloromethyl center by water (Scheme 2).

Scheme 1. Mechanism of Specific Acid-Catalyzed Cyclopropyl Ring Opening



Scheme 2. Mechanism of Reversible Cyclopropyl Ring Opening

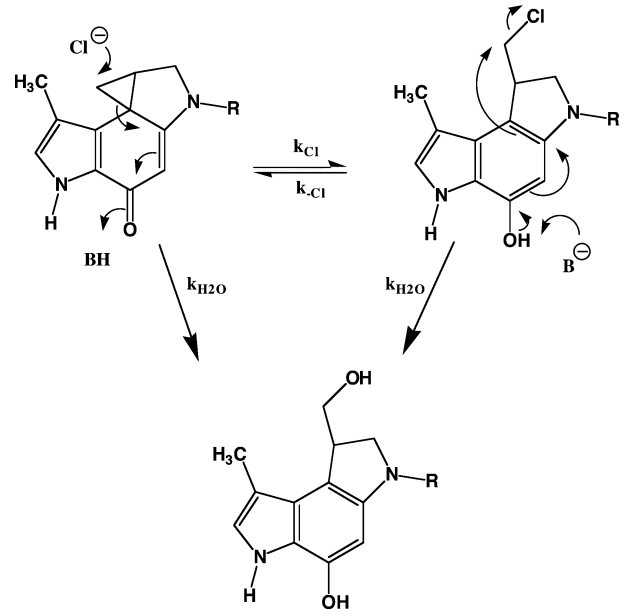


Table 1. Rate Law Used To Fit log Chloride Second-Order Rate Constants and log Water First-Order Rate Constants vs pH Data for **1a**−**c**[a]

$$k\ (M^{-1}\ min^{-1}) = k\ (M^{-2}\ min^{-1})a_H/(a_H + K_a)$$

compd	k_{Cl}, M^{-2} min^{-1}	k_{H_2O}, M^{-1} min^{-1}	pK_a
1a	22.4 ± 0.6	1.14 ± 0.04	0.5, 1.0
1b	38.5 ± 1.3	0.94 ± 0.01	1.04, 0.97
1c	23.2	not determined	1.01

[a] The third-order chloride trapping rate constants (k_{Cl}), second-order water-trapping rate constants (k_{H_2O}), and protonated carbonyl pK_a values are tabulated for **1a**−**c**.

Shown in the inset of Figure 1 are examples of the linear chloride-dependent plots for **1a**. The slopes of these plots were plotted as the log vs pH (Figure 1), and these data were computer fit to a single pK_a rate law that required product formation from the protonated species (Table 1). The *y*-intercepts were likewise plotted as the log vs pH (Figure 2) and computer fit to the same

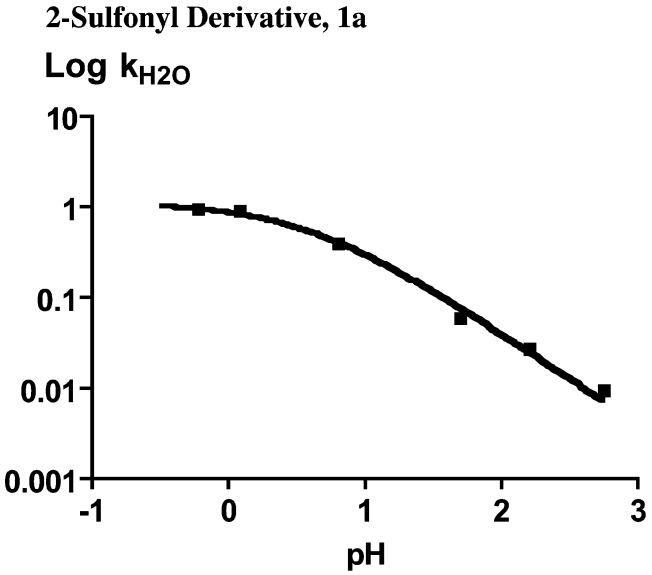


Figure 2. Plot of the log first-order rate constant for water-mediated cyclopropyl ring opening of **1a** vs pH. These first-order rate constants were determined from *y*-intercepts of chloride dilution plots (see inset of Figure 1), obtained at constant pH values. The solid line was computer-generated with the equation shown in Table 1.

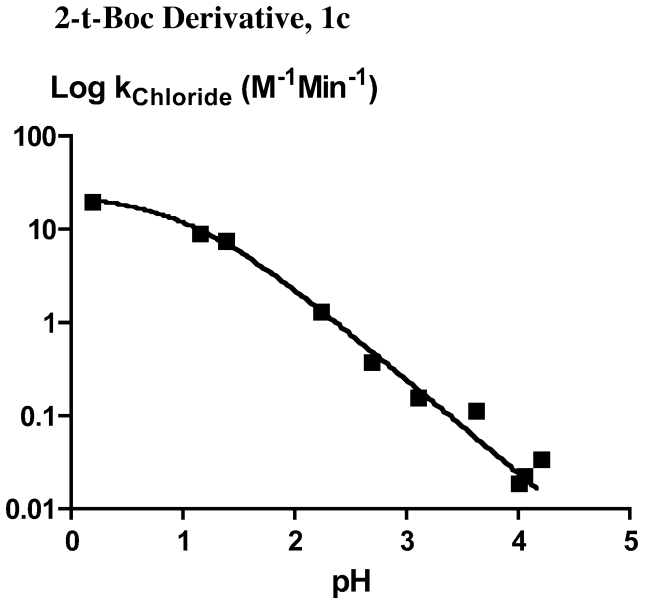


Figure 3. Plot of the log second-order rate constant for chloride-mediated cyclopropyl ring opening of **1c** vs pH.

rate law shown in Table 1. The pH−rate profiles in Figures 1 and 2 each provided values for the protonated carbonyl pK_a for **1a** as well as the third-order rate constant (M^{-2} min^{-1}) for chloride trapping and the rate constant (min^{-1}) for water trapping. Shown in Figures 3 and 4 are examples of pH−rate data obtained for **1b** and **1c**. Kinetic and pK_a data obtained from pH−rate profiles are summarized in Table 1. Since *y*-intercept values included rates for removal of the t-Boc group from **1c** as well as water trapping, a water-trapping rate could not be determined for **1c**.

The presence of general-acid-catalyzed opening of the cyclopropyl ring was not observed directly in these studies. Thus, buffer dilutions did not result in rate changes at various pH values. A key observation was that saturation kinetics with respect to chloride was observed at the higher end of the pH

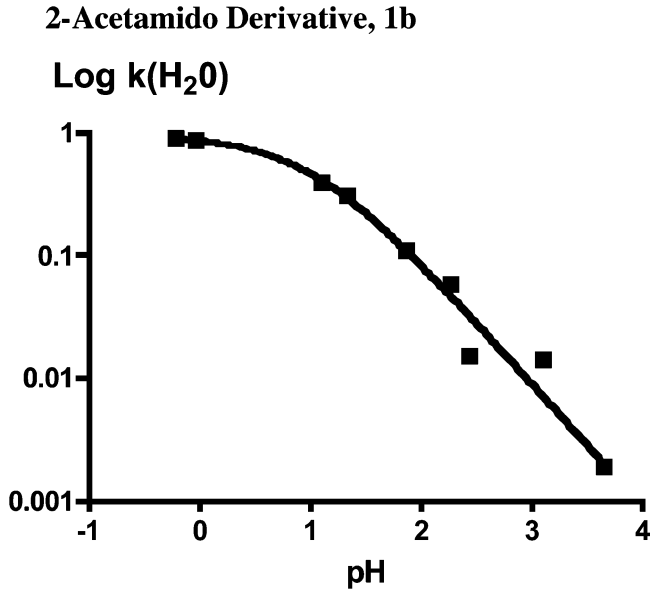


Figure 4. Plot of the log first-order rate constant for water-mediated cyclopropyl ring opening of **1b** vs pH. These first-order rate constants were determined from *y*-intercepts of chloride dilution plots (not shown). The solid line was computer-generated with the equation shown in Table 1.

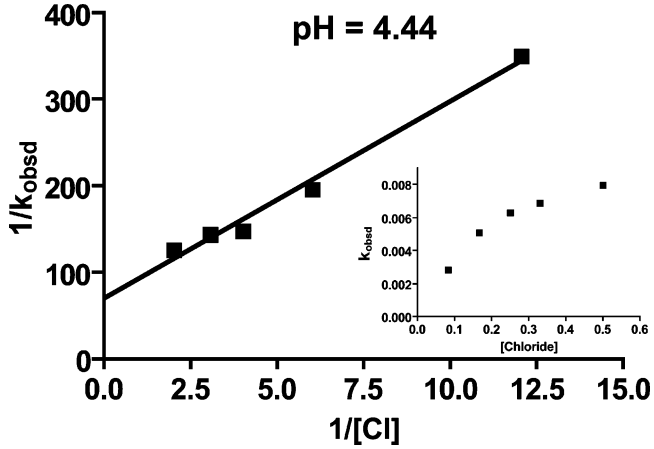


Figure 5. Reciprocal plot of k_{obsd} vs chloride concentration for cyclopropyl ring opening at pH 4.44. The inset shows the saturation evident in a direct plot of these data.

range of this study. The mechanism change responsible for saturation is likely responsible for the deviation of pH−rate data from the rate law in Table 1 at the high pH end of the profile (see Figure 1). Saturation became obvious in reaction pHs >4 (Figure 5) and was attributed to the process outlined in Scheme 2, where general-acid-catalyzed opening of the cyclopropyl ring by chloride is in rapid equilibrium with general-base-catalyzed cyclopropyl ring closure. Analysis of the saturation kinetics with reciprocal plots afforded the constants shown in Table 2. The linear reciprocal equation shown in Table 2 was obtained using material balance with respect to the equilibrating open and closed cyclopropyl forms of the A-ring (Scheme 2). For an example of material balance, see the derivation of the Michaelis−Menten equation.[15] The equilibrium constants in Table 2 show that the fraction of chloride trapping product increases with decreasing pH of the acetate buffer, consistent with acetic acid-catalyzed cyclopropyl ring opening.

(15) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman: San Francisco, CA, 1977; pp 84−89.

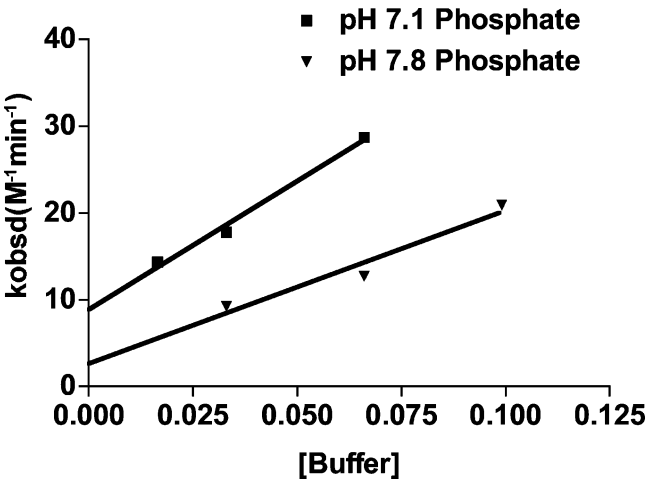


Figure 6. Plots of the second-order rate constants for thiolate opening of the cyclopropyl ring of **1a** versus phosphate buffer concentration at pH 7.1 and 7.8.

Table 2. Reciprocal Relationship Derived for the Saturating Reaction Shown in Scheme 2 Using Material Balance and Equilibrium (k_{Cl}/k_{-Cl}) and Rate (k_{H_2O}) Data for Cyclopropyl Ring Opening of 1a in Acetate Buffers

$$1/v = k_{-Cl}/k_{Cl}k_{H_2O} \times 1/[Cl] + 1/k_{H_2O};$$
$$\text{slope} = k_{-Cl}/k_{Cl}k_{H_2O};\ \gamma_{intercept} = 1/k_{H_2O}$$

pH	k_{Cl}/k_{-Cl}	k_{H_2O}
4.20	8.10	0.011
4.44	4.36	0.014
4.7	1.64	0.013

Reversible chloride opening of the cyclopropyl ring was not observed above pH 5. To demonstrate general-acid-catalyzed cyclopropyl ring opening at pH values closer to neutrality, we employed the more reactive hydroxyethyl thiolate nucleophile in reaction mixtures buffered to pH 7.1 and 7.8 with phosphate. Global fitting was carried out from 280 to 400 nm on buffered reaction mixtures (phosphate buffer concentration from 0.01 to 0.1 M) consisting of **1a** and 2-hydroxyethylthiol (concentrations from 0.019 to 0.19 M). Plots of the second-order rate constants for thiolate opening of the cyclopropyl ring versus buffer concentration at pH 7.1 and 7.8 are shown in Figure 6. These plots reveal that buffer catalysis is involved in nucleophile-mediated opening of the cyclopropyl ring near neutrality. The steeper slope of the pH 7.1 curve indicates the presence of general-acid catalysis. Monobasic phosphate is the general acid at pH 7.1, while the weaker general acid, dibasic phosphate, is more prevalent at pH 7.8. The most noteworthy aspect of the data in Figure 6 is the nonzero intercepts that indicate the presence of catalysis by lyate species (largely water at neutrality). Thus, general-acid catalysis by water at neutrality could play a role in CC-1065 alkylation of the DNA major groove.

Discussion

Protonated Carbonyl pK_a Values. The pK_a value measured for the protonated A-ring carbonyl is ~1, which is consistent with the value of 1.2 previously reported for a synthetic analogue of CC-1065.[6] We consider this pK_a value typical of a protonated carbonyl group that is conjugated with an electron-rich ring. Previously, we determined that the pK_a of an *O*-protonated indoloquinone had a value of 1.9 and showed by means of a Bronsted plot that electron-rich protonated quinones can have

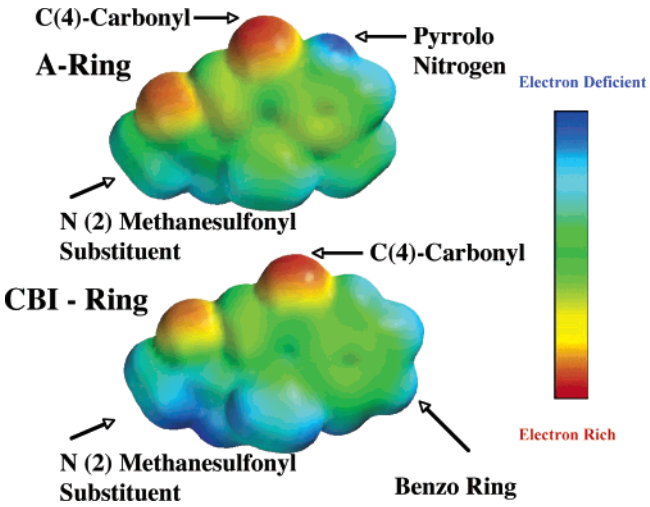


Figure 7. Electrostatic potential maps for the A-ring of CC-1065 and for the CBI analogue of the A-ring.

pK_a values as high as 5.5.[10] Table 1 reveals that the protonated carbonyl pK_a values are independent of the electronic character of the N-2 substituent. This observation suggests that the fused pyrrolo ring rather than the N-2 center supplies electron density to the carbonyl oxygen. The independence of the rates of cyclopropyl ring opening on the electronic character of the N-2 substituent is likewise attributed to the predominant electron-releasing influence of the fused pyrrolo ring.

Substituent Effects on Cyclopropyl Ring Opening Rates and Protonated Carbonyl pK_a Values. Evidence that the fused pyrrolo ring releases electron density to the carbonyl oxygen and controls cyclopropyl ring opening is supported by the electrostatic potential surface for the CC-1065 A-ring shown in Figure 7. The indole nitrogen releases electrons to the carbonyl oxygen, as indicated by the high positive charge density at the indole nitrogen (intense blue color). In contrast, the N-2 nitrogen releases electrons to a lesser degree and therefore has less positive charge density (light blue color). This observation is validated by the absence of a significant effect of N-2 substituents on protonated carbonyl pK_a values and cyclopropyl ring-opening rates (Table 1). Replacing the pyrrolo ring with a benzo ring to afford the CBIs[16,17] changes the electrostatic potential map such that the N-2 center (now blue in color) predominantly releases electron density to the carbonyl oxygen. In contrast to the pyrrolo ring, the benzo ring (now light blue in color) releases much less electron density to the carbonyl center. Substituent effects on CBI reactivity reported by the Boger group support this assessment. Hammett plots revealed that N-2 substituents had a profound effect on cyclopropyl ring solvolysis ($\rho = -3$), while substituents on the benzo ring had only a small effect on solvolysis ($\rho = -0.3$).[8,18] The susceptibility of the CBI to shape catalysis, wherein conjugation between the N-2 substituent and the carbonyl is lost due to distortion of the vinylogous amide, is evidence of stabilization by the N-2 center. [8] In contrast, the present study supports stabilization of the A-ring of CC-1065 by conjugation of the pyrrolo ring with the carbonyl rather than the N-2 center. This result suggests

(16) Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 5823−32.
(17) Boger, D. L.; McKie, J. A.; Nishi, T.; Ogiku, T. *J. Am. Chem. Soc.* **1997**, *119*, 311−325.
(18) Boger, D. L.; McKie, J. A.; Han, N.; Tarby, C. M.; Riggs, H. W.; Kitos, P. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 659−64.

that shape catalysis will be less important for the A-ring (CPI ring) of CC-1065 than for the CBI A-ring analogues.

Saturation Kinetics and General-Acid-Catalyzed Cyclopropyl Ring Opening. The present report asserts that reversible cyclopropyl ring opening masks the direct observation of general-acid-catalyzed A-ring cyclopropyl ring opening at pH values from 4 to 5. At this pH range, the chloride-mediated opening of the cyclopropyl ring was independent of pH and exhibited no general-acid catalysis. However, the presence of Michaelis−Menten or saturation kinetics indicated that chloride trapping was reversible. Measurement of equilibrium constants for chloride trapping as a function of pH revealed the presence of general-acid ring opening (acetic acid) and general-base (acetate) ring closure (Table 2). Raising the pH to values >5 with phosphate buffers containing chloride resulted in the absence of discernible cyclopropyl ring opening. Similarily, Boger and Garbaccio observed that CC-1065 was stable toward solvolysis in the pH range.[19] Our explanation for this observation is that the presence of relatively weak general acids (water and monobasic/dibasic phosphate) preclude cyclopropyl ring opening with the weak nucleophiles present (chloride and water). Consistent with this explanation, we observed the general-acid-catalyzed opening of the A-ring when a hydroxyethyl mercaptide nucleophile was added to reaction mixtures held at pH values near neutrality. Unlike chloride-mediated ring opening, the mercaptide-mediated ring opening is irreversible, thereby permitting the direct observation of general-acid catalysis. The observation that the water-catalyzed opening of the CC-1065 A-ring can occur at neutrality supports the role of ordered water in formation of the CC-1065 DNA adducts.[4,20] The absence of observable general-acid-catalyzed DNA alkylations by CC-1065 and related analogues[9] could be explained by the presence of equilibrium A-ring opening and closing, as observed with chloride in the present study. Significantly, CC-1065 alkylations of DNA exhibit saturation kinetics with respect to DNA, although this phenomenon has been attributed to noncovalent binding.[21] In conclusion, our results support the mechanism for alkylation by the CC-1065 A-ring cyclopropyl group wherein water acts as a general acid.

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(19) Boger, D. L.; Garbaccio, R. M. *J. Org. Chem.* **1999**, *64*, 5666−5669.

(20) Park, H.-J. *Arch. Pharm. Res.* **2002**, *25*, 11−24.

(21) Warpehoski, M. A.; Harper, D. E. *J. Am. Chem. Soc.* **1995**, *117*, 2951−2952.