Spectrophotometric Determination of Acidity Constants of 4-Dedimethylamino Sancycline (Col-3), A New Antitumor Drug

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Abstract □ A spectrophotometric technique was used to determine the acidity constants of 4-dedimethylamino sancycline (Col-3), a new antitumor drug. The apparent pK_a values of Col-3 in 0.5% methanol aqueous media at approximately 25 °C with a constant ionic strength of 0.2 were calculated manually and graphically to be 5.64 ± 0.17 (pK_{a1}) and 8.35 ± 0.07 (pK_{a2}). In addtion, the computer program SQUAD was used to confirm Col-3 pK_a values. The pK_a values obtained by SQUAD were pK_{a1} 5.63 ± 0.14 and pK_{a2} 8.39 ± 0.04. These results are in agreement with the tetracycline-like structure of Col-3.

1. Introduction

4-Dedimethylamino sancycline (Col-3) is a new synthetic derivative obtained by chemical synthesis from sancycline methiodide. Col-3 exhibits in vitro and in vivo activity as an inhibitor of matrix metalloproteinases, tumor invasion, and metastasis of a variety of tumor types. Due to its oral biovailability, Col-3 has been formulated for oral administration by the National Cancer Institute (NCI).

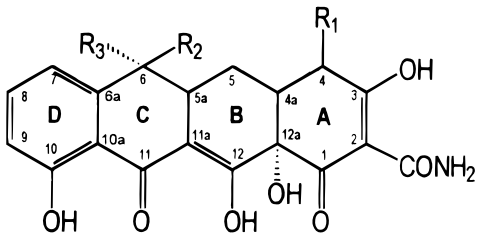
Col-3 can be classified as the simplest tetracycline. Structurally, it differs from tetracycline by the absence of the 4-dimethylamino, 6-hydroxyl, and 6-methyl groups. The chemical structures of Col-3, sancycline, and tetracycline are presented in Figure 1.

Due to the absence of the 4-dimethylamino group, Col-3 cannot exist as a zwitterion and, therefore, differs from the tetracyclines in its acid−base properties. The main goal of this study is to determine the macroscopic acid dissociation constants of Col-3. Spectrophotometry was chosen for the determination of pK_a values of Col-3 because it is too insoluble ($S_w \approx 0.01$ mg/mL) for potentiometry, and also the ionized and neutral forms of this compound absorb differently in the UV/vis regions of the spectrum.

2. Materials and Methods

2.1. Materials—Col-3 was used as received from the National Cancer Institute (Bethesda, MD). Methanol was Burdick & Jackson (Muskegon, MI) HPLC grade. Phosphoric acid, monosodium phosphate, disodium phosphate, and sodium chloride were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification.

2.2. Instrumentation—pH measurements were performed using a Corning pH meter (model no. 140) equipped with a combination glass electrode filled with potassium chloride gel. A Beckman DU-640 UV spectrophotometer was used for Spectral scan and fixed wavelength measurements.



	R_1	R_2	R_3
Tetracycline	$N(CH_3)_2$	OH	CH_3
Sancycline	$N(CH_3)_2$	H	H
Col-3	H	H	H

Figure 1—Chemical structure of Col-3, sancycline, and tetracycline.

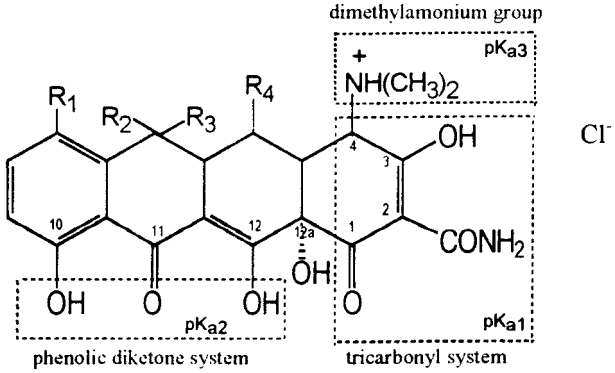


Figure 2—Structural groupings in the tetracyclines.

2.3. Methods—Aliquots of stock solution of Col-3 in methanol were added to buffers ranging from pH 2 to 11. All buffers were prepared with 0.05 M phosphate buffer and adjusted to an ionic strength of 0.2 with sodium chloride. Buffer solutions were spiked with 20 μL of the stock solution to produce 5.0 μg/mL (1.3×10^{-5} M) solutions with a final methanol concentration of 0.5%. The absorbance vs wavelength profiles of the resulting solutions were immediately obtained. All determinations were performed in duplicate at room temperature (approximately 25 °C).

3. Results and Discussion

It has been determined that the tetracycline antibiotics contain three ionizable groups[1−3] as shown in Figure 2. The first dissociation constant is due to the tricarbonyl system consisting of C1, C2, and C3 and their substitutes, the

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Table 1—The pK_a Values of the Tetracyclines in Aqueous Solution at 25 °C

	R_1	R_2	R_3	R_4	pK_{a1}	pK_{a2}	pK_{a3}
tetracycline	H	CH_3	OH	H	3.3	7.7	9.5
chlortetracycline	Cl	CH_3	OH	H	3.3	7.4	9.3
demeclocycline	Cl	H	OH	H	3.3	7.2	9.3
oxytracycline	H	CH_3	OH	OH	3.3	7.3	9.1
doxacycline	H	H	CH_3	OH	3.4	7.7	9.7
minocycline	$N(CH_3)_2$	H	H	H	2.8	7.8	9.3

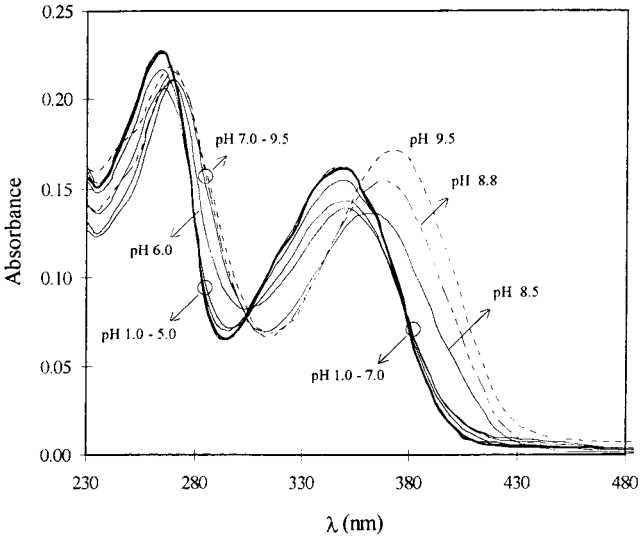

Figure 3—Absorbance vs wavelength profiles of Col-3 at different pH values.

second due to the phenolic diketone system consisting of C10, C11, C12 and their substitutes, and the third relates to the dimethylammonium functional group at C4.[2,3] The approximate pK_a values for each of these groups in some tetracycline derivatives are shown in Table 1.[1,3]

Col-3 has the first two of the acidic groupings of the tetracyclines. However, due to the absence of the dimethyammonium functional group at C4, Col-3 does not possess the third pK_a. The two pK_a values suggest that this drug can exist depending on the pH species as either the un-ionized, a monovalent anion, or a divalent anion. The UV/vis absorption spectra of Col-3 in aqueous solutions at various pH values are plotted in Figure 3.

As with most of the tetracyclines, an absorption band at about 260 nm is due to the β-tricarbonyl system, while a visible band at about 360 nm is produced by the phenolic diketone moiety.[4–7] Both anionic sites are stabilized by resonance, and they are separated from each other by carbon 12a. This sp^3 hybrid atom isolates the two light-absorbing regions and makes it possible to determine their pK_a values independently by spectrophotometry.

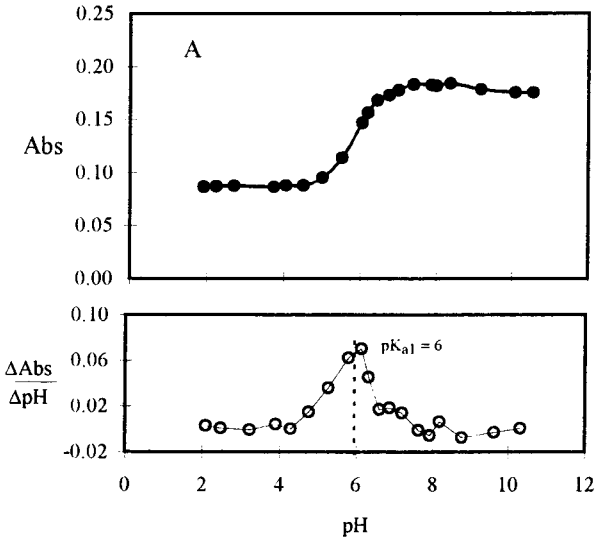

Figure 4—pH–absorbance profile of Col-3 at 286 nm (A) and the first derivative of absorbance respective to pH (B).

In this investigation, absorptions between 275 and 300 nm of the first band and between 370 and 390 nm of the second band were chosen as the analytical wavelengths for determining the first and the second pK_a values, respectively. These two wavelength intervals were chosen due to their maximum absorbance changes with pH.

The absorbance−pH curve of Col-3 at 286 nm is shown in Figure 4. The profile shows that the absorbance of the drug increases in the pH range of 4.0 to 7.0 and is constant below pH 4 and above pH 7.0. At this wavelength, the absorbance of the ionized species is higher than that of the un-ionized species, while the mono- and divalent species cannot be differentiated. The first pK_a of Col-3 was calculated at 275−300 nm using:

$$pK_a = pH + \log \frac{A_i - A}{A - A_u} \quad (1)$$

where A_i and A_u are the absorbance of the ionized and the un-ionized species, respectively. The results are shown in Table 2. The calculated pK_{a1} (5.64) is approximately equal to the pK_a determined using the derivative graph shown in Figure 4B.

Similarly, the second pK_a was calculated using the absorbance changes at 370−390 nm over the pH range of 7.39 to 8.37. At these wavelengths, the absorbance of divalent species is higher than that of the monovalent species, while the un-ionized and monovalent species cannot be differentiated. Thus, eq 1 can be used to calculate the second pK_a. The results are shown in Table 2. Both methods eq 1 and derivative graph were used to determine

Table 2—Spectroscophotometric Determined pK_a Values of Col-3 at Several Wavelengths and pH Values

	pK_{a1} at λ										pK_{a2} at λ								
pH	275	280	281	282	284	286	288	290	295	300	370	374	376	378	380	382	384	386	390
5.53	5.84	5.75	5.75	5.76	5.73	5.74	5.72	5.71	5.74	5.77									
6.06	5.69	5.74	5.73	5.71	5.71	5.72	5.73	5.74	5.73	5.76									
6.20	5.63	5.69	5.66	5.71	5.68	5.69	5.71	5.70	5.68	5.60									
6.45	6.04	6.00	5.93	5.98	5.94	5.94	5.93	5.92	5.98	6.06									
6.75	5.12	5.54	5.48	5.51	5.53	5.60	5.60	5.60	5.39	4.90									
7.39											8.26	8.24	8.23	8.23	8.23	8.23	8.24	8.24	8.24
7.86											8.35	8.35	8.35	8.35	8.35	8.35	8.35	8.36	8.36
8.00											8.42	8.41	8.41	8.41	8.42	8.42	8.42	8.42	8.42
8.37											8.39	8.39	8.40	8.39	8.40	8.39	8.39	8.39	8.39
	average pK_{a1} = 5.64 ± 0.17										average pK_{a2} = 8.35 ± 0.07								

the second pK_a as previously. The calculated value of 8.35 is again in good agreement with the value determined from the derivative graph.

From the results, the spectroscopically determined pK_a values of Col-3 are 5.64 (pK_{a1}) and 8.35 (pK_{a2}). Notice that its second pK_a value which corresponds to the loss of a proton from the phenolic diketone moiety is closed to those of the tetracyclines (~7.8). However, the first pK_a value, which is due to the loss of a proton from the tricarbonyl methane system, is shown to be over two pH units higher than that those of the tetracyclines (~3.3). This difference is due to the absence of the electron-withdrawing dimethylammonium group at the C-4. A similar increase of the pK_a value of this system due to the absence of the dimethylammonium group is found in desdedimethylaminotetracycline, which has a pK_a value of 5.94. In addition to the above methods, the computer program SQUAD (Stability QUotients from Absorbance Data) was used to confirm Col-3 pK_a values.[8] SQUAD calculates overall stability constant values such as pK_a values by means of a nonlinear least-squares approach. The data fed to SQUAD are absorption spectra, Col-3 total concentration, and a chemical model to describe the system (i.e., $H_2A \rightarrow HA^- + H^+ \rightarrow A^{2-} + 2H^+$). The p$K_a$ values obtained by SQUAD were pK_{a1} 5.63 ± 0.14 and pK_{a2} 8.39 ± 0.04. Note that the manually calculated (Table 2) and computer caluculated (SQUAD) pK_a values are in good agreement.

4. Conclusions

The macroscopic acid dissociation constants of Col-3 were determined by means of a spectrophotometric technique. The apparent pK_a values of Col-3 in 0.5% methanol aqueous media at approximately 25 °C with a constant ionic strength of 0.2 were calculated manually and graphically to be 5.64 ± 0.17 (pK_{a1}) and 8.35 ± 0.07 (pK_{a2}). The SQUAD calculated vales were pK_{a1} 5.63 ± 0.14 and pK_{a2} 8.39 ± 0.04. These results are in agreement with the tetracycline-like structure of Col-3.

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