the following conditions: The acetal (0.10 mmol) and allyltrimethylsilane (125 μL, 0.8 mmol) were dissolved in dry dichloromethane (1.0 mL, 0.1 M in acetal) was cooled to -78 °C under an atmosphere of nitrogen. The freshly prepared Lewis acid solution (659 μL, 3.6 equiv) was added via syringe (addition time 9 min) to the magnetically stirred acetal and allyltrimethylsilane solution. After complete addition of the Lewis acid solution, the resulting heterogeneous solution was stirred for an additional 36 min, quenched with 1.0 N NaOH in methanol (500 μL), and warmed to room temperature. The quenched solution was diluted with diethyl ether (2 mL), filtered through a plug of Florisil, and analyzed by gas chromatography.

Solvent Study. For each solvent the reactions were run in triplicate under the following conditions: A solution of **1** (20 mg, 0.10 mmol) and allyltrimethylsilane (64 μL, 0.40 mmol) in an appropriately dried solvent (see Tables VII and VIII) (1.0 mL, 0.1 M) was cooled to -78 °C under an atmosphere of nitrogen. Then titanium tetrachloride (13 μL, 0.12 mmol) was added to the above solution with magnetic stirring. After stirring for 1 h, the reaction mixture was quenched with 1.0 N NaOH in methanol (2 mL), and the solution was warmed to room temperature. The reaction mixture was diluted with diethyl ether (5 mL) and filtered through a plug of Florisil. The resulting solution was analyzed by gas chromatography.

Concentration Study. A magnetically stirred solution of acetal *cis*-**1a** (0.50 mmol, 0.005-0.5 M) and allyltrimethylsilane (1-20 equiv) in dry dichloromethane was cooled to -78 °C. Then titanium tetrachloride (0.5-10 equiv) was added to the above solution. After stirring (see specific substrate for reaction time), the reaction mixture was quenched with 1.0 N NaOH in methanol, and the solution was warmed to room temperature. The quenched solution was diluted with diethyl ether, filtered through a plug of Florisil, and analyzed by gas chromatography.

General Procedure for the Allylation of 3 and 5. A solution of enol ether **3** or **5** (30 mg, 0.103 mmol) and allyltrimethylsilane (32.8 μL, 0.207 mmol, 2.0 equiv) in dichloromethane (2 mL) was cooled to -78 °C. Trifluoromethanesulfonic acid (8.7 mL, 0.098 mmol, 0.95 equiv) was added neat at -78 °C, and the solution was stirred for 5 min. The reaction was quenched by the addition of 1 mL of 1 N NaOH/MeOH solution and was allowed to warm to room temperature. A 1-mL aliquot was removed, washed with water, and extracted with EtOAc. This aliquot was passed through a short plug of Florisil (3 cm) and then analyzed by capillary gas chromatography to obtain diastereomeric ratios.

General Procedure for the Allylation of 7 (TMSOTf). A solution of the acyclic acetal, **7** (50 mg, 0.103 mmol), and allyltrimethylsilane (50 μL, 0.309 mmol, 3 equiv) in dichloromethane (2 mL) was cooled to -78 °C. Trimethylsilyl trifluoromethanesulfonate (2 μL, 0.01 mmol, 0.1 equiv) was next added at -78 °C, and the solution was stirred for 2 h. The reaction was quenched by the addition of 1 mL of 1 N NaOH/MeOH solution and was allowed to warm to room temperature. A 1-mL aliquot was removed, washed with water, and extracted with EtOAc. This was passed through a short plug of Florisil (3 cm) and then analyzed by capillary gas chromatography to obtain diastereomeric ratios.

General Procedure for the Allylation of 7 ("Titanium Blend" $TiCl_4$/$Ti(Oi\text{-}Pr)_4$, 6/5). A Lewis acid solution ($TiCl_4$/$Ti(Oi\text{-}Pr)_4$, 6/5) was prepared by dissolving titanium tetrachloride (102 μL, 0.93 mmol) in dry dichloromethane (3 mL) under an atmosphere of nitrogen and then adding titanium tetraisopropoxide (231 μL, 0.78 mmol) with magnetic stirring. After complete addition of titanium tetraisopropoxide, the resulting solution was stirred for 45 min. The reactions were run in triplicate under the following conditions: The acetal **7** (0.155 mmol) and allyltrimethylsilane (197 μL, 1.24 mmol) were dissolved in dry dichloromethane (1.6 mL, 0.1 M in acetal) and cooled to -78 °C under an atmosphere of nitrogen. The freshly prepared Lewis acid solution (3.3 mL, 11 equiv) was added via syringe (addition time 2.0 h) to the magnetically stirred acetal and allyltrimethylsilane solution. After complete addition of the Lewis acid solution, the resulting heterogeneous solution was stirred for an additional 2 h followed by quenching with 1.0 N NaOH in methanol (2 mL) and warming to room temperature. The quenched solution was diluted with diethyl ether (5 mL), filtered through a plug of Florisil, and analyzed by gas chromatography.

Acknowledgment. We gratefully acknowledge financial support for this project from the National Science Foundation (NSF CHE 881847). S.E.D. acknowledges support from the National Science Foundation (Presidential Young Investigator Award, 1985-1990) and the Alexander von Humboldt Foundation for a Senior Scientist Award (1990). We are grateful to Professor Clayton H. Heathcock for providing preprints of ref 37 and for exchange of information. We also thank Jack Amburgey, Brenda Kesler, Jung-Ho Kim, Wheeseong Lee, Sunil Pansare, and Mark Schnute for the preparation of meso acetals.

Photochemical and Photophysical Studies of Tetracycline[1]

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Abstract: The photochemistry and photophysics of tetracycline (Tc) have been studied in aqueous and organic media. The primary photochemical reaction upon excitation into S_1 is conversion to lumitetracycline (LTc) and dimethylamine at pH's <7.5. Higher pH's and especially the presence of 2-mercaptoethanol favor the formation of anhydrotetracycline. Oxygen quenches LTc formation but has a minimal effect on Tc fluorescence; benzophenone sensitizes LTc formation. Triplet sensitization also produces de(dimethylamino)tetracycline (DTc), as does photolysis into S_2. It is proposed that DTc and LTc are derived from upper and lower triplets, respectively. Tc fluorescence shows a profound abnormal Stokes shift relative to absorption and a significant blue shift in this emission in basic media. The former phenomenon is attributed to an adiabatic excited-state intramolecular proton transfer (ESIPT) from the phenol functionality to the oxygen at C11. Subsequent intersystem crossing and/or an analogous ESIPT within T_1 provides a triplet species the decay of which is accompanied by intramolecular displacement of dimethylamine by C11a to give LTc. The blue-shifted emission in base is associated with a red shift in absorption; both spectral manifestations are attributed to deprotonation of the Tc ground state at C12 to form Tc^-, a species apparently unable to undergo ESIPT and photochemical conversion to LTc.

The tetracyclines represent a class of well-known phototoxic antibiotics.[2,3] The primary photochemical events and the cellular targets involved in the phototoxic response are as yet unknown, and it is also unclear as to whether the parent molecules, and/or photoproducts thereof, are responsible for the phenomenon. In fact, several major photoproducts of tetracycline (Tc) have been isolated and identified. These are shown in Scheme I and include

(1) Organic Photochemistry. 93. Part 92: Olack, G.; Morrison, H. *J. Org. Chem.* **1991**, *56*, 4969-4976. Abstracted, in part, from the doctoral dissertation of G.O., Purdue University, Dec 1990.

(2) For leading references, see: Drexel, R. E.; Olack, G. A.; Jones, C.; Chmurny, G. N.; Santini, R.; Morrison, H. *J. Org. Chem.* **1990**, *55*, 2471-2478.

(3) See also: Bjellerup, M.; Ljunggren, B. *Photodermatology* **1987**, *4*, 281-287.

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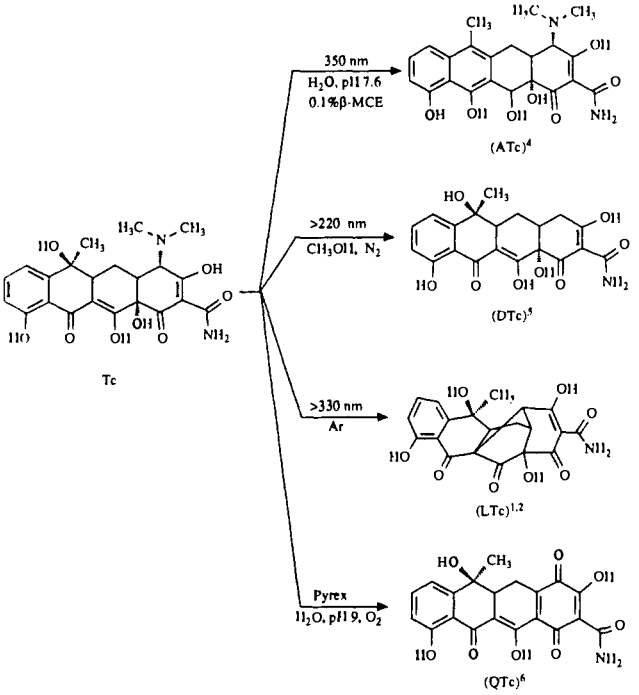
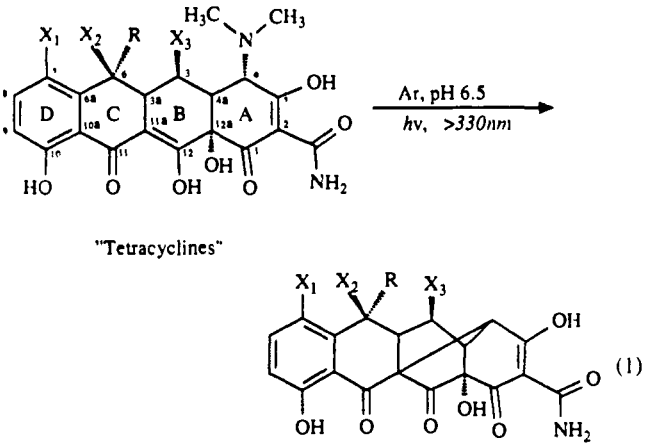
Scheme I. Major Reported Tetracycline Photoproducts



(a) lumitetracycline (LTc), formed with long-wavelength UV light in deoxygenated organic and aqueous media;[1,2] (b) anhydrotetracycline (ATc), also formed with long-wavelength UV in aqueous media, but particularly in the presence of 2-mercaptoethanol;[4] and (c) de(dimethylamino)tetracycline (DTc), formed upon irradiation with the full mercury arc in deoxygenated organic media.[5] There is also a report of the formation of a quinoid product (QTc) upon photolysis through Pyrex glass of a basic aqueous solution in the presence of oxygen, though the structure of this compound has been less well characterized.[6] Several degradation products have been isolated and identified upon the photolysis of Tc in a basic aqueous solution continuously bubbled with air, using a solar simulator, but these compounds account for a small fraction of the Tc lost under the photolysis conditions.[7] Clearly, the photochemical and photophysical properties of the tetracycline family are of interest, in fact, not only because its members are photobiologically significant but also because the tetracycline system represents a fascinating structure for excited-state studies in its own right. The compounds in this family have a basic structure which embraces two distinct chromophores, one which extends over rings B, C, and D (phenol = ring D) and a second localized in ring A. The former gives rise to long-wavelength absorption (for Tc at pH 7, λ_{max} = 360 nm) while both chromophores contribute to a second λ_{max}, for Tc, pH 7, at 270 nm.[8] The phenol-based chromophore has the potential for an appreciable differential in acidity between the ground and excited states, and this factor, when superimposed on the multiprotic character of these molecules, can be expected to lead to profound pK_a effects on both the photochemistry and the photophysics of these molecules. The observations outlined herein are consistent with this expectation.

Our studies have primarily emphasized the formation and identification of the lumitetracyclines (cf. eq 1) which are formed by every member of the tetracycline family studied in our laboratories to date.[1,2] We now report mechanistic and spectroscopic



(1)

Product		X_1	X_2	X_3	R
Lumitetracycline	(LTc)	H	OH	H	CH_3
Lumioxytetracycline	(LOTc)	H	OH	OH	CH_3
Lumidoxycycline	(LDOX)	H	H	OH	CH_3
Lumichlorotetracycline	(LCTc)	Cl	OH	H	CH_3
Lumidemeclocycline	(LDEM)	Cl	OH	H	H
Lumiminocycline	(LMIN)	NMe_2	H	H	H

results relevant to this transformation; data of interest to anhydrotetracycline formation are also included.

Results

Photochemistry. Wavelength Effects. As noted above, we have previously observed that LTc is the primary photoproduct when Tc is irradiated with long-wavelength (i.e. uranium yellow glass filtered; $\lambda > 330$ nm) light, i.e. excitation into S_1. Since the earlier report[5] of DTc formation with short-wavelength light did not have the benefit of HPLC analysis of the reaction mixture, we reexamined such a photolysis using a Vycor filter to allow for incident light of $\lambda > 220$ nm (i.e. excitation into S_2). We observed that irradiation in water at pH 7 under such conditions gives rise to both LTc and DTc, with the former disappearing upon extended photolysis. The ratio of LTc to DTc is 0.61 at modest (37%) Tc loss. *All of the results reported below are for excitation with long-wavelength light.*

Time Course Study and Formation of Dimethylamine. The conversion of Tc to LTc involves the net loss of the equivalent of dimethylamine. That this molecule is in fact extruded during the photolysis was confirmed by photolyzing Tc in an acetonitrile solution containing catalytic acetic acid and by trapping the dimethylamine formed therein with benzoyl chloride plus triethylamine. The benzamide so formed was quantified by HPLC using an internal standard. A time course study was carried out to compare the relative rates of formation of the dimethylamine and LTc and to confirm that LTc is indeed a primary photolysis product. The results are presented in Figure 1. A corresponding study in water/20% MeOH confirmed the monotonic increase of LTc and decrease of Tc in that medium as well. Ultimately, HPLC analysis showed that LTc formation accounted for ca. 65% of the Tc lost during photolysis.

Solvent Effects. In our first report[2] on the formation of LTc from Tc, we noted that this reaction is observed in acetonitrile, methanol, acetone, and water, the last buffered at pH 6.4. A more quantitative survey was carried out to compare relative rates of LTc formation in organic and aqueous media. The results are presented in Table I. Because the data in run 1 of Table I were suggestive of a pH effect on LTc formation, a pH profile was

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(5) Hlavka, J. J.; Bitha, P. *Tetrahedron Lett.* **1966**, *7*, 3843–3846.
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(7) Oka, H.; Ikai, Y.; Kawamura, N.; Yamada, M.; Harada, K.; Ito, S.; Suzuki, M. *J. Agric. Food Chem.* **1989**, *37*, 226–231.
(8) Stephens, C. R.; Murai, K.; Brunnings, K. J.; Woodward, R. B. *J. Am. Chem. Soc.* **1956**, *78*, 4155–4158.

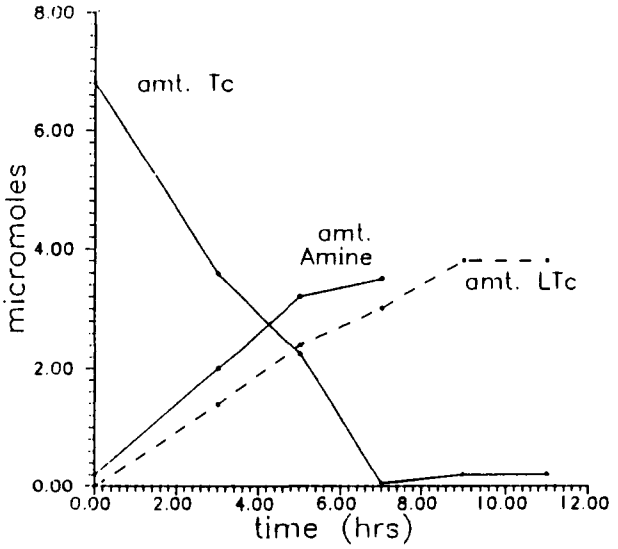


Figure 1. Time course study of the photolysis of Tc in acetonitrile using $\lambda > 330$ nm light.

Table I. Comparative Formation of LTc as a Function of Solvent

solvent	[photoproduct], mM LTc	DTc
Run 1[a]		
MeCN/HOAc	1.04	0.02
aqueous MeOH/pH 6.4[b]	0.62	0.10
MeCN	0.10	0.03
Tc·HCl/pH 3.2	0.04	
Run 2[c]		
aqueous MeOH/pH 6.4[b]	0.40	0.04
aqueous MeOH/pH 7.0[d]	0.18	0.03
MeCN	0.04	0.03
Run 3[e]		
MeOH[f]	0.08	
MeCN	0.07	
CH_2Cl_2	0.01	
DMSO[g]		

[a] Photolyses of 1 mM Tc under Ar at 8 °C for 11 h with $\lambda > 330$ nm light. [b] 20% MeOH; unbuffered. [c] Photolyses as in run 1 but for 4 h. [d] 20% MeOH; 0.10 M phosphate buffer. [e] Photolyses of 1 mM Tc under Ar at 23 °C for 4 h with $\lambda = 366$ nm light. [f] Photolyses in EtOH, *n*-PrOH, and *n*-BuOH were about 50% as efficient as that in MeOH in forming LTc; unless photolysis tubes are cleaned in a sodium bicarbonate solution prior to use, photolysis in methanol will give extensive amounts of ATc. [g] This photolysis was for 15 min and gave extensive (0.09 mM) formation of ATc.

constructed for LTc formation using acetate buffers for pH 3.5–5.5 and phosphate buffers for pH 5.5–7.5. The data are presented in Figure 2. A more extensive study of the region between pH 5.5 and 8.0 was carried out by using phosphate buffer adjusted to constant ionic strength with sodium chloride; the data are presented in Figure 3.

In a separate study, Tc was methylated to form its quaternary salt (previously shown to also give rise to LTc)[2] and the pH profile determined with phosphate buffer at constant ionic strength, as in Figure 3. A maximum in LTc formation was again observed at ca. pH 6.5; however, the value at pH 7.0 is only slightly below that for pH 6.5 and the steep decline noted at pH 7.0 in Figure 3 now begins between pH 7.0 and 7.5.

Metal Ion Effects. A solution of Tc·HCl (0.5 mM) in phosphate buffer (pH 6.5, 0.10 M) with NaCl, KCl, $CaCl_2$, or $MgCl_2$ (10 mM) or a saturated solution of $ZnCl_2$ was degassed with argon and irradiated with $\lambda > 330$ nm light for 52 min. The results are presented in Table II. Table II also includes a second study involving added $MnSO_4$. A further study of the effect of Mg^{2+} was carried out with varying salt concentrations, using added NaCl to maintain an ionic strength equivalent to 5 mM $MgCl_2$. All

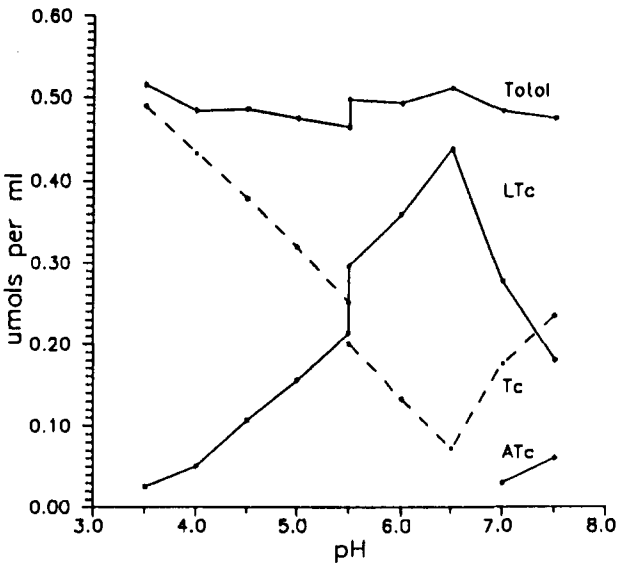


Figure 2. pH profile for conversion of 0.5 mM Tc to LTc using $\lambda > 330$ nm light. The lower point at pH 5.5 was obtained with the acetate buffer and the higher point with the phosphate buffer.

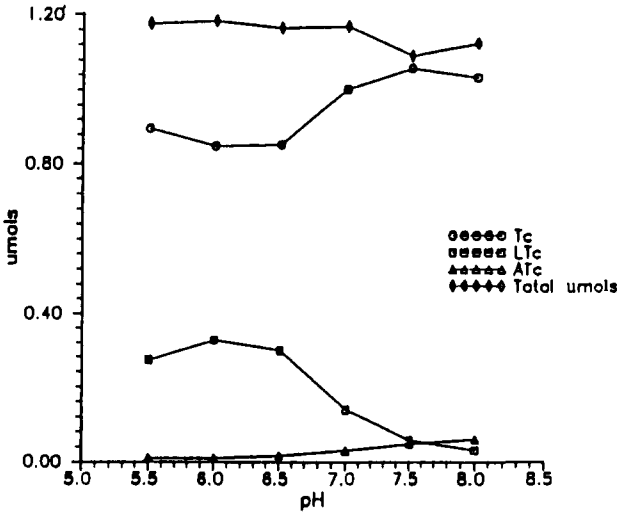


Figure 3. pH profile for the conversion of 1 mM Tc to LTc using phosphate buffer adjusted to an ionic strength of 0.15 with NaCl and light of $\lambda > 330$ nm.

Table II. Comparative Effect of Metal Ions on the Formation of LTc

metal ion	[LTc], mM	metal ion	[LTc], mM
Run 1[a]			
	0.15	Zn^{2+}	0.17
K^+	0.14	Mg^{2+}	0.20
Na^+	0.15	Ca^{2+}	0.20
Run 2[b]			
	0.17	Mg^{2+}	0.24
Na^+	0.17	Mn^{2+}	0.15[c]

[a] Photolyses with 0.5 mM Tc in 0.10 M phosphate buffer, pH 6.5, with $\lambda > 330$ nm light, 10 mM chloride salts, and saturated solution (<10 mM) of $ZnCl_2$. [b] Photolyses as in run 1, with 2.0 mM $MgSO_4$ and Na_2SO_4; ca. 2 mM $MnSO_4$ (saturated solution). [c] A separate run gave values of 0.16 and 0.14 mM for Na^+ and Mn^+, respectively.

Table III. Dependence of LTc Formation on Mg^{2+} Concentration[a]

$[Mg^{2+}]$, mM	[LTc], mM	$[Mg^{2+}]$, mM	[LTc], mM
0.00	0.27	2.5	0.50
0.25	0.33	5.0	0.47
0.50	0.37		

[a] Photolyses in phosphate buffer, pH 6.3, with NaCl added to maintain ionic strength equivalent to 5.0 mM $MgCl_2$.

Table IV. Catalysis of the Photolytic Conversion of Tc to ATc by 2-Mercaptoethanol (2-MCE)[a]

photolysis		products[c]			
2-MCE[b]	time, min	Tc	LTc	*epi*-ATc/DTc	ATc
		Run 1[d]			
	0	1628			
	20	443	62		
+	15	97		537	3011
		Run 2[e]			
	0	2952			
	30	1693	647	154	15
+	0	2624			
+	30	9	108	548	5577

[a] Photolyses of 0.1 mM Tc in TMK buffer at pH 7.6 at 5 °C with λ > 330 nm light. [b] + = 0.1% v/v 2-MCE. [c] Values are HPLC area units, uncorrected for response. [d] Without degassing. [e] After argon degassing.

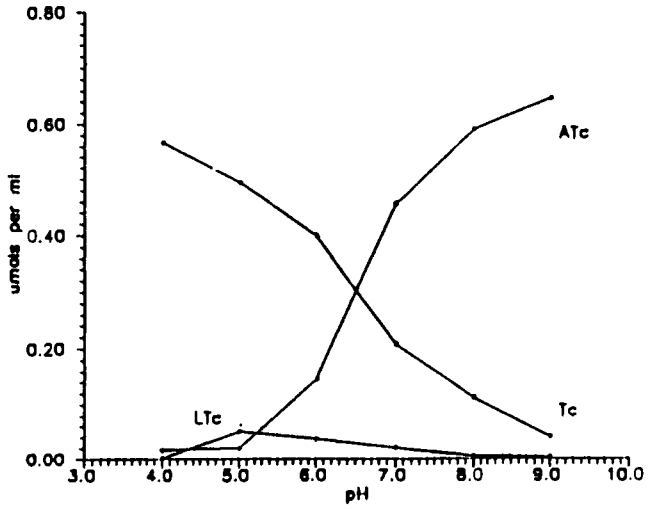


Figure 4. Photolysis of Tc in the presence of 2-MCE as a function of pH (argon-degassed BR buffer solutions).

runs were in 0.10 M, pH 6.3 phosphate buffer. The data are shown in Table III. A similar study at pH 7.5 showed no effect of Mg^{2+} on LTc formation.

Effect of 2-Mercaptoethanol (2-MCE) and Related Additives. As has been noted in the introduction, Hasan et. al[4] have observed that the presence of 2-MCE catalyzes the formation of ATc when Tc is photolyzed in pH 7.6 buffer with 350-nm light in the presence of air (cf. eq 1). We repeated this study in both nondegassed and argon-degassed solutions using our standard, uranium yellow glass filtered mercury arc (λ > 330 nm). The data are presented in Table IV and confirm that 2-MCE is a requirement for the formation of ATc in both the presence and the absence of oxygen. The effect of pH on 2-MCE catalysis in argon-degassed solutions was studied over the range pH 4–9 in Britton–Robinson (BR) buffer, and the results are presented in Figure 4.[9] Clearly, 2-MCE catalysis of ATc formation is favored by basic solutions, as was observed in the noncatalyzed runs (cf. Figure 2). When 2-MCE

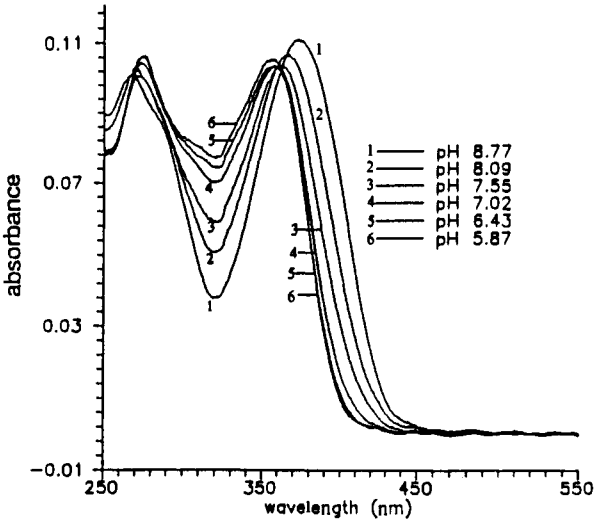


Figure 5. Absorption spectra for Tc as a function of pH.

was added to an argon-degassed, pH 6.5 phosphate buffer solution of Tc (conditions that favor LTc formation), an appreciable (ca. 50%) catalysis of conversion to this product was likewise observed. We have earlier shown (Table II) that 1.0 mM Mg^{2+} modestly (ca. 29%) catalyzes LTc formation; a similar study with 2-MCE present gave a 55% enhancement of this product.

All of the above studies were done with a large excess of 2-MCE; 0.1% v/v 2-MCE = 15 mM vs 0.5 mM Tc. A comparative study of the catalysis of ATc formation at pH 8 in BR buffer using 0.1, 0.2, and 0.5 mM 2-MCE with 0.5 mM Tc confirmed that the rate of ATc formation does increase with the 2-MCE concentration.

A series of photolyses at pH 8 in BR buffer with other potential catalysts (*N,N*-dimethylaniline, pyridine, aniline, 2-propanol, ethylene glycol, sodium azide, EDTA) showed all to be inactive. However, mercaptoacetic acid was 82% as effective as 2-MCE (0.50 mM catalyst and Tc).

Quenching and Sensitization Studies. Several experiments were run to determine the effect of oxygen on LTc formation; in all cases, virtually complete suppression of LTc formation was observed. Thus, a 4.9-h photolysis in acetonitrile gave extensive conversion of Tc to LTc under argon but just a trace of LTc detectable by HPLC when oxygen was passed through the solution. In a second study for 1.5 h, 94% quenching of LTc formation by oxygen was observed. Analogous results have been obtained in water; i.e. only a trace of LTc was detectable upon photolysis of Tc in pH 6.5 phosphate buffer under oxygen for 12.5 h, and a similar study, in which 2-MCE was present, showed LTc formation to be 97% quenched by air relative to an argon-degassed control.

The conversion of Tc to LTc can be sensitized by benzophenone. Argon-degassed acetonitrile solutions of Tc (0.6 mM), with and without benzophenone (500 mM), were irradiated in a turntable for 18 min with λ > 330 nm, conditions wherein the benzophenone absorbs virtually all of the incident light. Tetracycline is relatively unreactive in acetonitrile in the absence of constant argon sparging,[14] and minimal loss of Tc was observed in the solution lacking the sensitizer. A 60% loss of Tc and extensive conversion to LTc were observed in the tube containing benzophenone. However, there was appreciable formation of DTc in this tube as well (LTc:DTc = 3.3); DTc is formed upon direct photolysis into S_2 but is not normally seen under long-wavelength photolysis conditions (see above). In a separate experiment, LTc was found to be degraded by benzophenone sensitization (3-h photolysis), but no conversion to DTc was noted.

Spectroscopy. Ultraviolet Absorption Spectroscopy. UV spectra were obtained for Tc in buffered aqueous solution from pH 5.87 to 8.77. The spectra are presented in Figure 5. Spectra were also obtained at pH 6.5 and 7.4 over a concentration range of

(9) It should be noted that the middle pK_a for Tc is reported[10] to be 6.8 in this buffer but 7.7 in other buffers;[11-13] since the formation of ATc is favored in base, the onset of the ATc curve in Figure 4 is probably displaced slightly in lower pH's.

(10) Smyth, W. F.; Jenkins, T. C.; Smyth, M. R.; Barrett, J.; Sprake, S. *Acta Chem. Scand. Ser. B* **1978**, *32*, 131–136.

(11) Mitscher, L. A. *The Chemistry of the Tetracycline Antibodies*; Marcell Dekker: New York, 1978.

(12) Martin, B. R. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1985.

(13) Despite this fact, there is little difference observed for 2-MCE catalysis of the formation of ATc at pH 8 in BR buffer vs tris(hydroxymethyl)-aminomethane (Tris) buffer. A similar comparison of BR vs/phosphate buffer at pH 6.5 also showed BR buffer to be much more effective in generating ATc. A comparison of Tris and phosphate buffers at pH 7.2 and 7.5 showed that ATc is formed more efficiently in the Tris buffer, whereas formation of LTc is more favorable in phosphate buffer.

(14) Drexel, R. E. Doctoral Dissertation, Purdue University, May 1987.

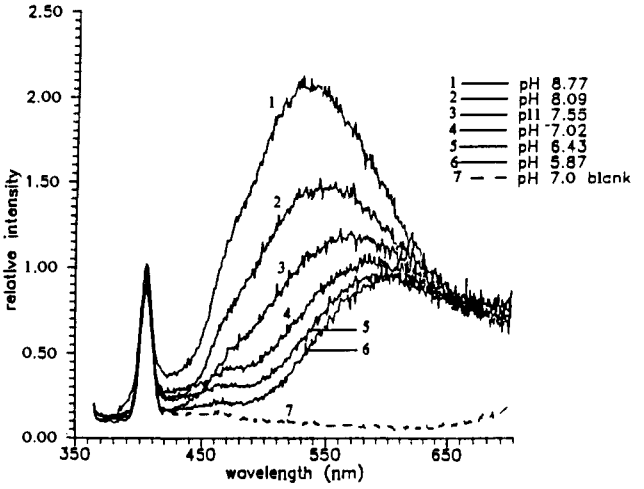


Figure 6. Emission spectra for Tc in aqueous solution as a function of pH (excitation at 355 nm).

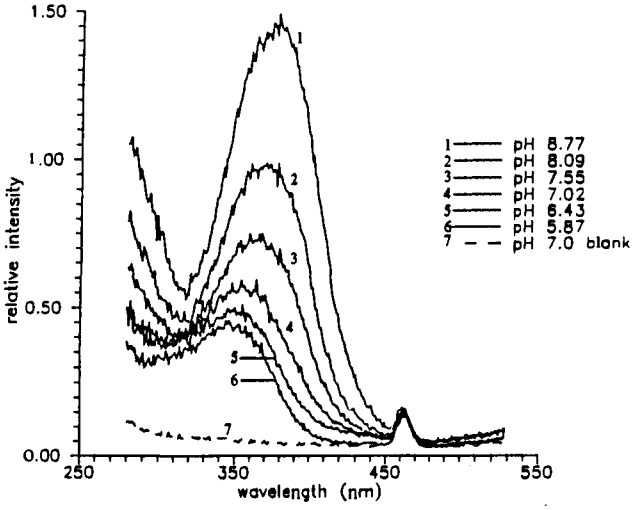


Figure 7. Excitation spectra for Tc in aqueous solution as a function of pH (emission monitored at 510 nm).

5.0–1000 μM by using 1.0- and 10-mm cells. Plots of absorbance (ca. 366 nm) versus concentration were linear at both pH values; i.e. no deviation from Beer's law was observed at these concentrations. Identical spectra were obtained for 1 μM solutions of Tc in TMK (Tris/$MgCl_2$/KCl) buffer at pH 7.6 in the presence and absence of 1% v/v 2-MCE.

Fluorescence Spectroscopy. Fluorescence emission and excitation spectra were obtained in argon-degassed phosphate buffer, over a pH range from 5.87 to 8.77, and are presented in Figures 6 and 7, respectively. The excitation and absorption spectra show reasonable overlap, both giving evidence for a small red shift at higher pH (cf. Figures 7 and 8).

Fluorescence quantum efficiencies (ϕ_f) were determined with 355-nm excitation and with quinine sulfate as a reference; values ($\times 10^{-4}$) were 3.7, 9.3, and 14 for pH 5.5, 7.0, and 8.5, respectively. These quantum efficiencies are too low, and the singlet lifetimes too short, to permit accurate measurement on our singlet lifetime fluorimeter. The fluorescence of a pH 6.5 solution bubbled with oxygen was comparable in intensity to that of an argon-deoxygenated solution.

Tc emission was also measured in organic media, and the fluorescence spectra in several organic solvents are presented in Figure 9. By contrast with what was observed in aqueous solution, significant deviations are observed between the absorption and the excitation spectra (cf. Figure 10).

Circular Dichroism Spectroscopy. Circular dichroism (CD) spectroscopy has been used by previous workers to explore conformational changes in the Tc family. We present in Figure 11 the results of a CD study on 0.05 mM Tc in 50 mM phosphate buffer at pH 5.5 and 8.0; these spectra are virtually identical with that reported for oxytetracycline (OTc) in water.[15] Tc in alcoholic solvents (MeOH, EtOH, *n*-PrOH, *n*-BuOH) also shows the strong

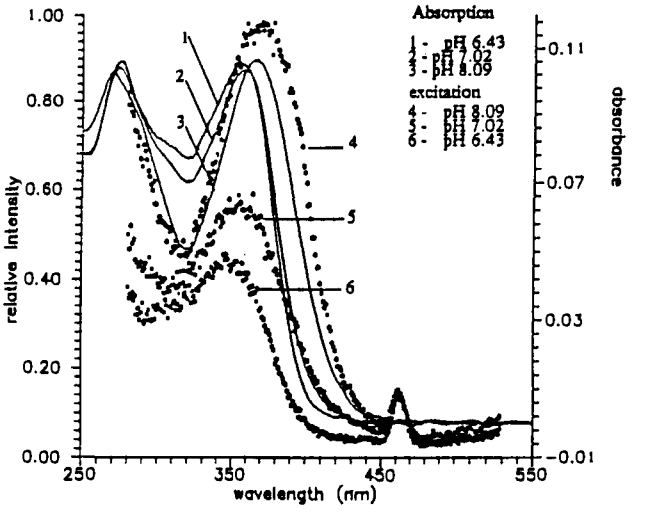


Figure 8. Comparison of absorption and excitation spectra for Tc in aqueous solution as a function of pH (emission monitored at 355 nm).

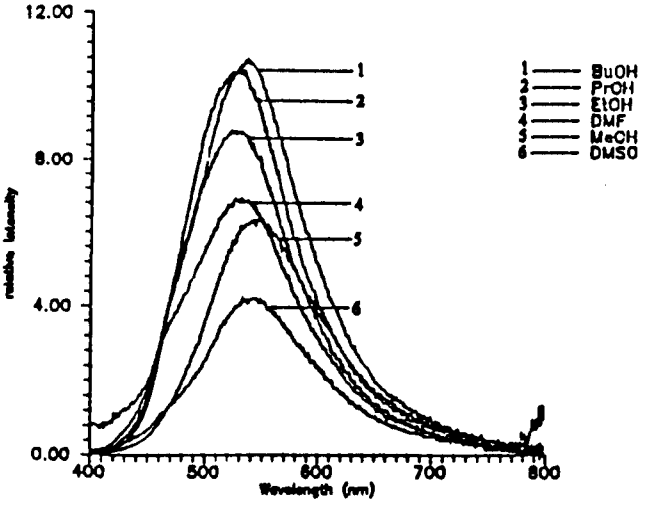


Figure 9. Fluorescence spectra for Tc in organic solvents (excitation at 355 nm).

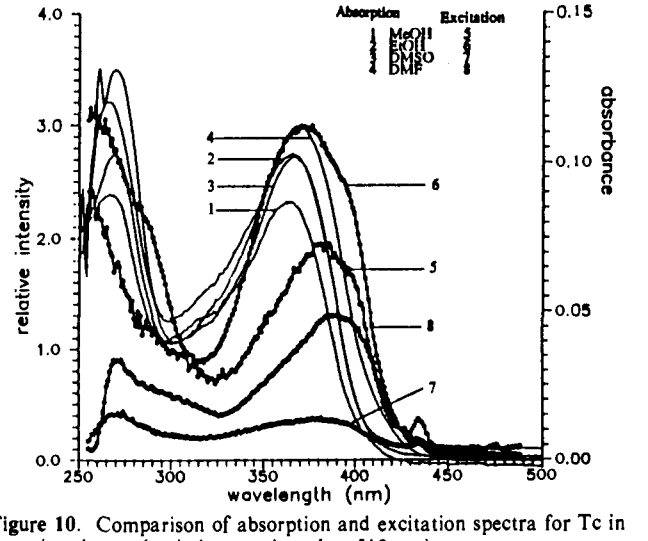


Figure 10. Comparison of absorption and excitation spectra for Tc in organic solvents (emission monitored at 510 nm).

(15) Hughes, C. J.; Stezowski, J. J.; Hughes *J. Am. Chem. Soc.* **1979**, *101*, 7655–7657.

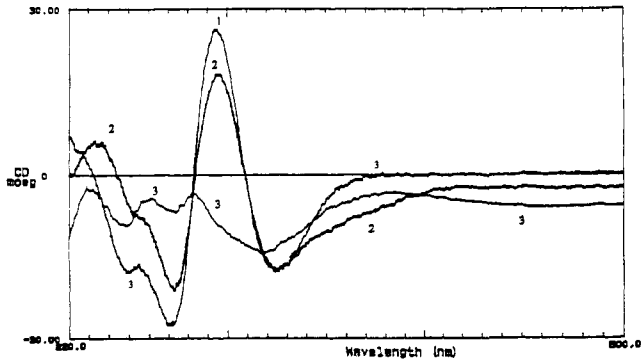


Figure 11. Circular dichroism spectra for Tc in aqueous solution and in acetonitrile: (1) pH 5.5; (2) pH 8.0; (3) acetonitrile.

Table V. Solvent and pH Effects on H-H Coupling in Tc[a]

	coupling constants, Hz				
protons	pH 6.2	pH 7.2	pH 8.3	DMSO	MeOD[b]
4-4a	<2	<2	<2	<2	2.4
4a-5α	13.5	13.5	13.3	*c*	12.5
4a-5β	3.3	2.8	2.2	*c*	3.1
5α-5β	-13.5[d]	-14.[d]	-11.6[d]	*e*	-13.0
5α-5a	11.5	11.4	11.6	7.6	10.6
5β-5a	5.3	5.3	5.3	7.6	5.8
7-8	7.6	7.6	7.5	7.6	8.4[f]
8-9	8.3	8.3	8.3	8.2	7.6[f]

[a] All solvents were >99.9% D; D_2O solutions are 1 mM Tc in 50 mM phosphate buffer, ionic strength 0.15 with the "pH" uncorrected. [b] Values from ref 2. [c] Coupling obscured by overlap of the H4a and dimethylamino resonances. [d] Assignment of this as a negative coupling by analogy with ref 2. [e] Coupling obscured by overlap of 5α and 5β resonances. [f] This assignment reverses that originally reported.[2]

negative and positive peaks seen in water at ca. 320 and 295 nm, respectively, but the strong negative peak at 270 nm is greatly diminished in the organic media. Far greater changes are reported for the OTc CD spectrum upon changing from aqueous to ethanolic media.[15] We include in Figure 11 a CD spectrum for Tc in acetonitrile which is comparable to that reported for Tc in DMSO[16] and to that which we have observed in DMF.

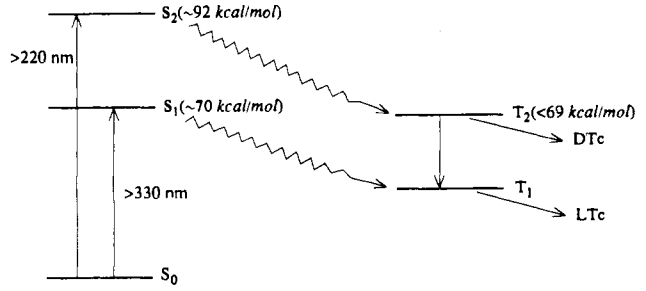
NMR Spectroscopy. ^1H-^1H coupling constants for Tc were determined in phosphate buffer at several pH values and in DMSO in order to probe for potential conformational changes in these media. The results are presented in Table V together with data for MeOD determined earlier in these laboratories.[2]

Discussion

Photochemistry. Prior studies have established that LTc is a major photoproduct of tetracycline when the antiobiotic is irradiated into S_1 (i.e. with $\lambda > 330$ nm) in water at pH 6.4. We have now extended these studies, and it is clear that LTc is the principal Tc photoproduct at physiological pH and, in fact, at all pH values from 3.5 to 7.5, at which point the formation of ATc becomes competitive (cf. Figures 2 and 3). Yields of LTc as high as 65% have been obtained. Though the stoichiometry of the Tc to LTc transformation has been suggestive of the loss of dimethylamine, this has now been confirmed by trapping the extruded amine in the form of its benzamide. A time course study has confirmed that the formation of the amine tracks the formation of LTc and that both are primary photochemical products (cf. Figure 1). In the discussion which follows, we will propose (1) that LTc forms from a triplet tetracycline excited state and (2) that excited-state intramolecular proton transfer in S_1, and possibly also in T_1, plays a prominent role in Tc photophysics and photochemistry.

There is a modest increase in the formation of LTc when the photolysis solution includes Mg^{2+} or Ca^{2+} (cf. Tables II and III). This is not surprising, since these divalent cations have long been known to enhance Tc fluorescence, presumably by increasing the excited-singlet-state lifetime of the drug (see also below).[17-19] As regards the specific excited-state precursor for LTc, there are several cogent observations: (1) the Tc to LTc reaction is virtually eliminated by the presence of oxygen, even though oxygen has no effect on Tc fluorescence; (2) LTc is formed upon excitation into both S_1 and S_2, but DTc formation is limited to excitation of Tc into S_2; (3) both LTc and DTc are formed upon benzophenone triplet sensitization of Tc. The differential effect of oxygen on fluorescence vs reaction argues against a role for the emitting species, i.e. S_1 or, more likely, deprotonated S_1 (see below), in the Tc to LTc conversion. An obvious alternative is that a triplet is involved, a possibility supported by the fact that triplet sensitization by benzophenone successfully generates LTc. However, such sensitization generates DTc as well, *a fact suggestive of multiple triplet excited states. We conclude that LTc derives from T_1 (actually, a tautomer of T_1: see below) while DTc derives from an upper triplet (e.g. T_2)*! Support for this hypothesis derives from the observation that DTc is formed upon excitation into S_2[5] but is not observed from S_1. The energy for T_2 must be <69 kcal/mol, the triplet energy of benzophenone,[20] and though this places T_2 in the same range as the ca. 70 kcal/mol estimated for S_1 from absorption data, it appears that $S_1 \rightarrow T_2$ intersystem crossing must be inefficient (perhaps due to the fact that these states are localized on different portions of the molecule). Our proposal is summarized in Scheme II.[21]

Scheme II. Proposed Jablonski Diagram for Tetracycline with Photoproducts Derived from Multiple Triplet Excited States



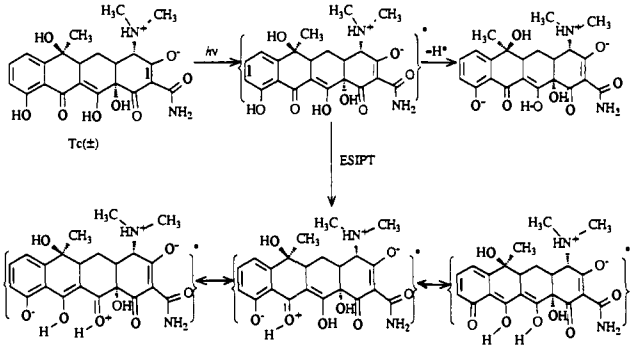
As regards ATc formation, it should be noted that the formation of this product only becomes competitive with conversion to LTc at pH's ≥7.5. The reported extraordinary catalysis of Tc to ATc by 2-MCE[4] has been confirmed (Figure 4), but the previous attribution to a protection of Tc against singlet oxygen degradation is unlikely, since 2-MCE is equally effective in argon-degassed solutions.[22]

Spectroscopic Studies. Absorption and Fluorescence Spectroscopy. The most striking aspect of Tc electronic spectroscopy is the abnormal Stokes shift evident in a comparison of the absorption and fluorescence spectra. This Stokes shift is, in turn, pH dependent with an appreciable blue shift evident in the fluorescence maximum, from 600 nm at pH 5.9 to 535 nm at pH 8.8 (cf. Figure 6). The result is a 155-240-nm Stokes shift at pH 8.8 and 5.9, respectively. The greater magnitude of the Stokes shift at the lower pH reflects the fact that the absorption spectra show a red shift in the Tc S_0 to S_1 transition on going to basic solution,[10] thus decreasing the gap between the absorption and emission maxima (cf. Figures 5 and 7). The pH dependence of the long-wavelength absorption band is reflected in the fluorescence

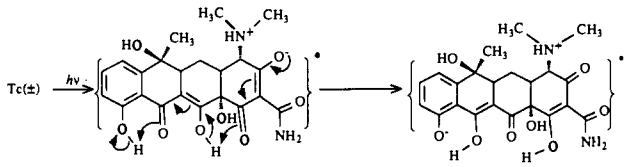
(16) Prewo, R.; Stezowski, J. J. *J. Am. Chem. Soc.* **1977**, *99*, 1117-1121.

(17) Kohn, K. *Nature* **1961**, *191*, 1156-1158.
(18) White, J. P.; Cantor, C. R. *J. Mol. Biol.* **1971**, *58*, 397-400.
(19) Mani, J. C.; Foltran, G. *Bull. Soc. Chim. Fr.* **1971**, *11*, 4141-4146.
(20) Murov, S. L. *Handbook for Photochemistry*; Marcel Dekker: New York, 1973; p 31.
(21) It is noteworthy that, in contrast with Ca^{2+} and Mg^{2+}, Mn^{2+} has a minimal quenching effect on the formation of LTc (Table II). There is a report that this ion quenches Tc fluorescence, but the effect has been attributed to heavy-atom-induced intersystem crossing (from S_1 to T_1).[18,19] Were this occurring, one would have to assume that additional heavy-atom-enhanced intersystem crossing from T_1 back to S_0 results in a slight net diminution in LTc formation.
(22) We choose not to speculate at this time on the detailed mechanism(s) for the uncatalyzed or 2-MCE-catalyzed conversion of Tc to ATc. Further studies are in progress.

Scheme III. Intermolecular and Intramolecular (ESIPT) MonoProton Transfer in the Tc(±) Excited State



Scheme IV. Diproton ESIPT for the Tc(±) Excited State



excitation spectra (which correlate well with the absorption data; cf. Figure 8). It is rather remarkable that we can find no previous notation nor discussion of the pH dependence of Tc emission nor of the abnormal Stokes shifts, presumably because prior workers have concentrated on the enhancement of Tc fluorescence with metal chelation; the metal-free molecule is very weakly emitting with ϕ_f values ranging from 3.7×10^{-4} to 14×10^{-4} at pH 5.5 and 8.5, respectively.[23]

Since the S_0 to S_1 transition embodies the chromophore-encompassing rings B, C, and D, the red shift in this transition between pH 5 and 9 may be attributed to ionization of the C10 phenol oxygen or the C12 enol in this pH regime. There have been numerous studies of the multiprotic acid properties of Tc, and the commonly accepted values for the first three macroscopic pK_a's are 3.3, 7.7,[24] and 9.8.[8,11,25] These are now assigned to the C3 enol in ring A, the C12 enol in ring B, and the protonated amino group in ring A, respectively,[11] though these assignments have not been without dispute.[10] Within the critical region where LTc formation seems to be most affected by the pH, i.e. pH 6–8, a consideration of the microscopic dissociation constants suggests that 50% of the second dissociable hydrogen is removed by pH 8, with ca. 25% each from the C12 enol and the dimethylammonium group.[25,26]

The large Stokes shift at pH 5 is strongly suggestive of an adiabatic excited-state intra- or intermolecular proton transfer and subsequent emission from the newly generated excited chromophore. This is not unexpected for Tc, since the increased acidity of e.g. phenols in the excited singlet state is well-known and the resultant emission of the phenolate anion excited state generates characteristically large Stokes shifts.[27] The presence of a proton acceptor ortho to the phenol group (e.g. the C11 keto group) makes possible excited-state intramolecular proton transfer (ESIPT)[28] which, by analogy with such well-studied systems as

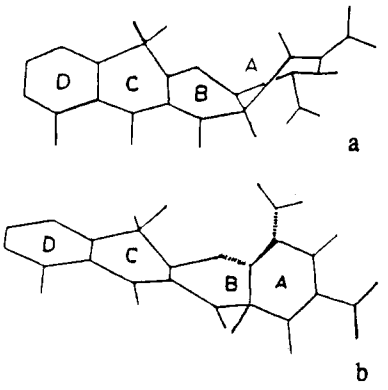
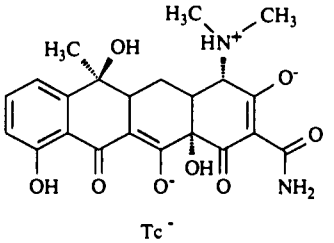


Figure 12. Conformations for Tc(±) and Tc(0).

salicyclic acid,[27a,29] can lead to an extensively resonance-stabilized phototautomer, the emission from which would also be expected to lead to a large Stokes shift (as illustrated in Scheme III for excitation of Tc(±)). A fascinating series of sequential proton and electron shifts could bring about the eventual delivery of a proton to the C1 carbonyl group (cf. Scheme IV).[14]

The red and blue shifts for Tc absorption and emission, respectively, upon changing the pH to ca. 8, occur concomitantly with the onset of deprotonation at C12 (thus generating Tc^-). The



Tc^-

red shift comes from a modest stabilization of the Tc^- ground state in the resonance-delocalized anion. The more substantial blue shift of the emission may be interpreted as an indication that the Tc^- excited state decays radiatively via a diabatic transition directly to the ground state. This hypothesis is consistent with the observation that the formation of LTc diminishes markedly within the same pH range (see below).

Tc Conformational Isomerism. Because the formation of LTc necessarily requries close spatial interaction between C4 and C11a, through an appropriate *cis*-decalin conformational reorganization of rings A and B, we carried out several studies designed to probe for a potential correlation between preferred conformational structures and the solvent and/or pH effects on the Tc to LTc interconversion. In summary, no such correlation has been observed and we have no reason to believe that the Tc conformer present at the time of electronic excitation plays any role in the ultimate photochemistry.

The conformations of the Tc family have been studied in the solid phase by X-ray crystallography[15,30] and circular dichroism spectroscopy[15,30–32] and in solution by CD and NMR spectroscopy.[33–36] Neutral tetracycline can exist as a zwitterion, Tc(±), or as the uncharged tautomer, Tc(0). Tc(±) is the form found in aqueous solution, and the primary conformation of this species is shown in Figure 12.[31,32] Tetracycline exists as Tc(0) in aprotic solvents with the conformation for this species also shown in Figure

(23) A fluorescence efficiency of 6.6×10^{-2} has been reported for oxytetracycline at pH 9: Roy, R.; Mukherjee, S. *Indian J. Chem.* **1989**, *28A*, 545–549.

(24) A value of 6.9 has been reported for the second pK_a in BR buffer; cf. ref 10.

(25) Asleson, G. L.; Frank, C. W. *J. Am. Chem. Soc.* **1976**, *98*, 4745–4749.

(26) Additional discussion of the microscopic pK_a's may be found in: (a) Leeson, J. L.; Krueger, J. E.; Nash, R. A. *Tetrahedron Lett.* **1963**, *3*, 1155–1160. (b) Rigler, N. E.; Bag, S. P.; Leyden, D. E.; Sudmeier, J. L.; Reilly, C. N. *Anal. Chem.* **1965**, *37*, 872–875.

(27) (a) For a review, see: Ireland, J. F.; Wyatt, P. A. H. *Adv. Phys. Org. Chem.* **1976**, *12*, 131–221. (b) For a recent example and leading references, see: Chow, Y. L.; Zhou, X.-M.; Gaitan, T. J.; Wu, Z.-Z. *J. Am. Chem. Soc.* **1989**, *111*, 3813–3818.

(28) For a recent discussion, see: Barbara, P. F.; Jarzeba, W. *Acc. Chem. Res.* **1988**, *21*, 195–199.

(29) Weller, A. *Z. Elektrochem.* **1956**, *60*, 1144–1177.

(30) Stezowski, J. J. *J. Am. Chem. Soc.* **1976**, *98*, 6012–6018.

(31) Mitscher, L. A.; Bonacci, A. C.; Sokoloski, T. D. *Tetrahedron Lett.* **1968**, *9*, *51*, 5361–5364.

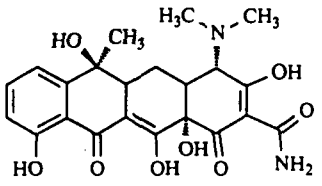
(32) Mitscher, L. A.; Bonacci, A. C.; Sokoloski, T. D. *Antimicrob. Agents Chemother.* **1968**, 78–86.

(33) Gulbis, J.; Everett, G. W. *J. Am. Chem. Soc.* **1975**, *97*, 6248–6249.

(34) Gulbis, J.; Everett, G. W.; Franck, C. W. *J. Am. Chem. Soc.* **1976**, *98*, 1280–1281.

(35) Williamson, D.; Everett, G. W. *J. Am. Chem. Soc.* **1975**, *97*, 2397–2405.

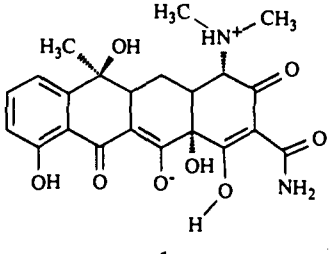
(36) Everett, G. W.; Gulbis, J.; Shaw, J. *J. Am. Chem. Soc.* **1982**, *104*, 445–447.



Tc(0)

12.[15,16,33-36] We have studied the pH dependence of the Tc CD spectra over the range pH 5.5–8.0 (cf. Figure 11) and find that the spectra correspond well to the spectrum reported[31] for Tc in 0.03 N HCl and are only slightly perturbed by pH (as has been observed by others).[32] An examination of the H–H coupling constants at pH 6.2–8.3 (Table V) confirms that there is no appreciable change in the Tc conformation in this pH range. The strong couplings seen for 4a–5α and 5α–5a are indicative of trans-diaxial relationships, while the weak couplings for 4–4a, 4a–5β, and 5β–5a suggest axial–equatorial relationships, all as expected from the proposed Tc(±) conformation.

There are several differences in the way Tc spectroscopy responds to organic media when compared to that described above for water. The fluorescence emission is similar to that seen in aqueous base, with λ_{max} at ca. 540–550 nm (Figure 9), and there is a significant red shift in the excitation spectra relative to absorption (Figure 10). This red shift is analogous to the red shift seen in both the aqueous solution absorption and excitation spectra as one makes the media more basic (Figures 7 and 8). The implication is that the species responsible for emission has a chromophore similar to Tc$^-$; a possibility is a different form of Tc(±), i.e. structure **1**. This species appears to be present in small



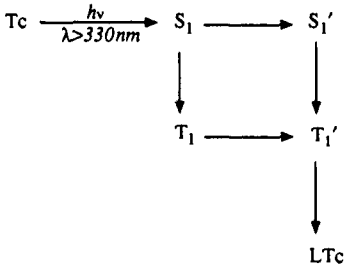
1

quantities in the organic solvents (thus, the shoulder in the excitation spectra) but must also be formed by ESIPT from Tc(0) (or Tc(±); see below), since excitation into the main absorption band gives identical emission.

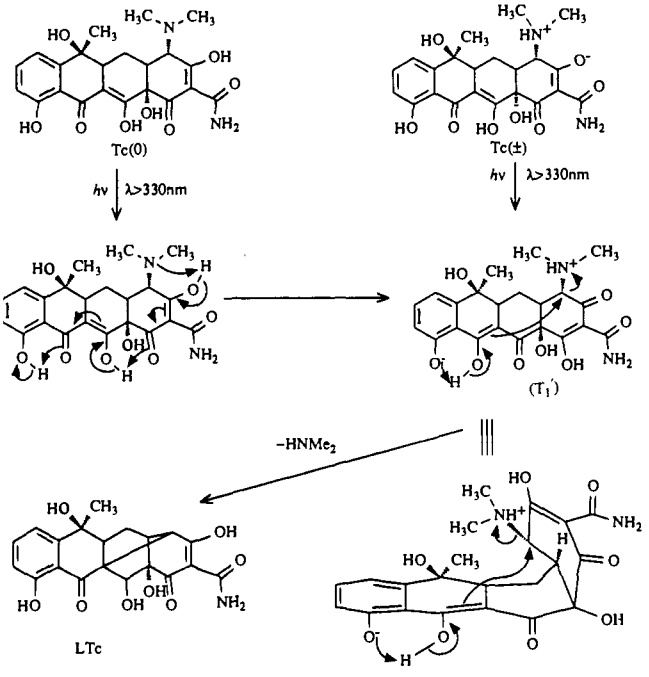
As regards conformation, we find that the Tc CD spectrum recorded in alcohol is similar to, though not identical with, the spectrum recorded in water, possibly indicative of a mixture of species (Tc(±) and Tc(0)) and conformers. In aprotic organic media, a quite different spectrum is obtained, comparable to that reported in the literature;[16] this spectrum has been attributed to the Tc(0) species (see above). The H–H coupling constants in Table V confirm these conclusions; the data in MeOD look very much like those seen in aqueous media (cf. 5α–5β, 5α–5a, and 5β–5a) whereas the coupling constants in DMSO for 5α–5a and 5β–5a are notably different. The marked reduction in the 5α–5a coupling, in particular, now is suggestive of the equatorial–equatorial relationship expected for Tc(0).

Mechanistic Discussion. Scheme II summarized our general view of the excited-state origin of LTc. In the light of the discussion above, we can elaborate the scheme further, as in Scheme V. In this scheme, we suggest that at pH 6.5 the Tc(±) excited singlet state undergoes ESIPT to form S_1'. This then undergoes intersystem crossing to T_1', the precursor to LTc. Alternatively, S_1 may, in part, undergo intersystem crossing to T_1 prior to the ESIPT step.[37] In either event, the ultimate product-forming

Scheme V. Formation of LTc from a Tautomeric Tc Triplet State



Scheme VI. Proposed Mechanism for the Conversion of Tetracycline to Lumitetracycline



nucleophilic attack by C11a or C4 must likewise be accompanied by intersystem crossing to form the LTc ground state.[38] A structural representation of Scheme V is provided in Scheme VI. The diminution in LTc formation in more acidic media can be rationalized either by inhibition of the formation of T_1' or by its rapid protonation once formed. Likewise, base should quench LTc formation, since the nucleofugal dimethylammonium group is at least partially deprotonated in Tc$^-$, and ground-state deprotonation of the 11a/12 enol would prevent ESIPT to the oxygen on C1, thus leaving an enolate anion adjacent to C4 to electrostatically inhibit nucleophilic attack. (Some role for the latter phenomenon seems assured by the fact that the Tc quaternary ammonium salt shows a similar pH profile for reactivity). Scheme VI includes an alternative route to T_1' from the species present in aprotic organic solvents, Tc(0), which involves a fascinating series of three intramolecular proton transfers.[14] It should be kept in mind that the quantum efficiencies for both emission and photochemistry in the Tc series are low; ESIPT appears to primarily provide a facile route for radiationless decay.

Conclusions. The primary photochemical reaction of Tc at physiological pH is the formation of LTc. The reaction appears to involve a low-lying triplet state, whereas T_2 has been implicated in an alternative deamination reaction which produces de(dimethylamino)tetracycline. T_2 is generated when S_2 is populated by direct photolysis or by triplet sensitization with benzophenone. A mechanism for converting Tc to LTc is proposed which involves excited-state intramolecular multiproton transfer, a phenomenon that also brings about a classic abnormal Stokes (red) shift in Tc

(37) The increase in acidity exhibited by phenol triplet excited states is typically much more modest than for excited singlet states (cf. ref 27a). However, the presence of an adjacent basic site can greatly exaggerate the effect: Werner, T. *J. Phys. Chem.* **1979**, *83*, 320–325.

(38) Precedent for bonding between these centers in the Tc series may be found in: Barton, D.; Ley, S. V.; Meguro, R.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1977**, 790–791.

fluorescence. Both the conversion of Tc to LTc and the abnormally shifted emission become insignificant in basic media.

Experimental Section

The experimental procedures have been detailed in previous papers in this series[1,2] or in the doctoral dissertation of G.O.

Solvents were MeOH and MeCN (Burdick and Jackson, high purity), DMSO, DMF, *n*-BuOH, *n*-PrOH, and anhydrous EtOH (Aldrich, high purity), and deionized water distilled through a Corning Megapure 3MP system. Tc and Tc·HCl were from Sigma and used as received. DTc was a gift from Dr. J. Hlavka, and ATc was previously prepared in these laboratories by Dr. R. E. Drexel. TcMeI was prepared by the literature procedure.[39] 2-Mercaptoethanol was purchased from Aldrich and used as received. All photolyses employed 7-mm quartz tubes with a Hanovia medium-pressure 450-W Hg lamp filtered by a uranium yellow glass sleeve (Houde glass; $\lambda > 330$ nm) as the light source unless otherwise noted. Solutions were degassed with argon for 20 min, sealed, and irradiated in a turnable. HPLC utilized a Varian Vista 5000 series chromatograph with a Varian UV-100 detector, a Hewlett-Packard HP-3376 recording integrator, and a 10-μm Alltech C-18 4.6 mm × 25 cm column. Acetophenone, *p*-methoxyacetophenone, or propiophenone was utilized as an internal standard for Tc, LTc, DTc, and ATc analysis. Analysis for *N,N*-dimethylbenzamide was carried out on a 5-μm Varian C-18 Micropak 4.6 mm × 20 cm column with acetophenone as the internal standard. CD data were collected on a Jasco J-600 polarimeter using a 1 cm path length quartz cell. Fluorescence and excitation spectra were obtained on an SLM-Aminco SPF-500 spectrofluorimeter operated in corrected mode using a 1-cm² quartz fluorescence cell with a filter passing light with $\lambda > 400$ nm on the emission side. Samples were deoxygenated with Ar for 20–30 min prior to study. Fluorescence data were corrected for absorbance and refractive index differences, with quinine sulfate in 0.1 N H_2SO_4 (absorbance at 355 nm ca. 0.1) utilized as a standard ($\phi = 0.55$)[40] for fluorescence quantum efficiencies. Solution pH values were determined with a Corning 125 pH meter, calibrated with Fisher pH 4.00, 7.00, and 10.0 standards prior to use.

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Registry No. Tc, 60-54-8; LTc, 115747-16-5.

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(40) Parker, C. A. *Photoluminescence of Solutions*; Elsevier Publishing Co.: New York, 1968.

Pendant-Capped Porphyrins. 1. The Synthesis of a Biphenyl Pendant-Capped Iron(III) Porphyrin Model of Catalase

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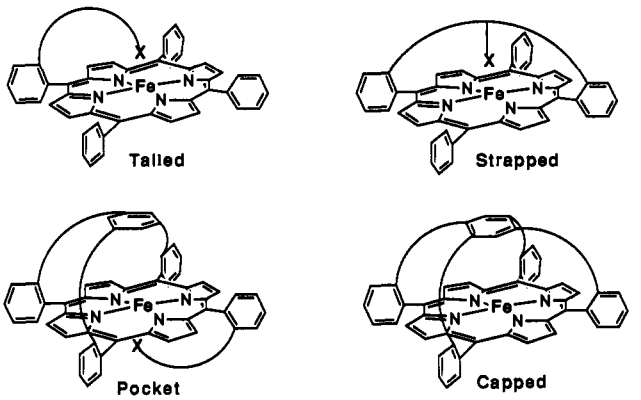
Abstract: A phenol pendant-capped porphyrin ((PHPCP)H_2) and its iron(III) complex ((PPCP)Fe^{III}) have been synthesized. ^1H and ^{13}C NMR were used to characterize all intermediates leading to the final product. In addition, the heteronuclear ^{13}C-^1H shift correlation experiment was used to further characterize the tetraaldehyde precursor of the pendant-capped porphyrin. Proton and carbon chemical shifts for the free base porphyrin (PHPCP)H_2 were assigned by using one-dimensional NMR experiments (including ^{13}C APT) and two-dimensional homonuclear COSY and ROESY experiments. In (PHPCP)H_2, the phenol that provides the ligand is hung from the ceiling of a vaulted dome or tepee with protected walls supported by four legs that are anchored to the 2-position of the phenyl rings at the 5,10,15,20-*meso*-carbons of (TPP)H_2. In the ground-state structure of (PPCP)Fe^{III}, the phenol is dissociated such that the resultant phenolate species is ligated to the high-spin ($S = {}^5/_2$) iron(III). Ligation of phenolate and iron moieties is established by (i) comparison of ^1H NMR to the ^1H NMR of the phenolate complex of $[(TPP)Fe^{III}]^+$; (ii) comparison of the UV/vis spectrum to that for the phenolate complex of $[(TPP)Fe^{III}]^+$; (iii) disappearance of the O–H stretch in the IR accompanying complexation of (PHPCP)H_2 with Fe(III); and (iv) laser desorption mass spectroscopy. At the active site of catalase, the protoporphyrin-IX iron(III) is ligated to the phenolate form of a tyrosine residue.

Introduction

The structures in Chart I depict the general structural characteristics of a number of modified 5,10,15,20-tetraphenylporphyrins $[(TPP)M^{n+}(X)_y]$. A tailed $(TPP)M^{n+}(X)_y$ possesses a chain of atoms extending from the 2-position of a single *meso*-phenyl substituent that terminates in a ligand species. The chain of atoms is of such a length that the ligand substituent may interact with the central M^{n+} moiety.[2] A strapped $(TPP)M^{n+}(X)_y$ possesses a chain of atoms (which holds a ligand in the vicinity of the M^{n+} moiety) extending from the 2-position of a phenyl substituted on the 5-*meso*-carbon of TPP to the 2-position of a phenyl on the 15-*meso*-carbon of TPP.[3] Pocket $(TPP)M^{n+}(X)_y$ structures possess three chains emanating from a common atom

Chart I



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