Excited-State Proton Transfer from Solvent to Aromatic Carbons in Tomaymycin

Qi Chen, Wanda J. Walczak, and Mary D. Barkley*

Chemistry Department, Louisiana State University
Baton Rouge, Louisiana 70803

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Excited-state proton transfer reactions are ubiquitous in the photophysics of aromatic compounds. The pK_a^* value in the excited state often differs greatly from the ground-state value, leading to protonation or deprotonation of the excited species. In addition, the aromatic ring may become more basic in the excited state, rendering ring carbon atoms susceptible to electrophilic protonation and culminating in proton or isotope exchange.[1] This so-called proton-induced fluorescence quenching or photoprotonation is an important nonradiative decay process. The H−D exchange reaction is readily monitored by NMR and mass spectrometry. Photochemical isotope exchange reactions have been extensively characterized in 1-methoxynapthalene,[2] 1-naphthol,[3] and dimethoxybenzenes[4] under acidic conditions and in indole derivatives with ammonium as proton donor.[5−8] Recently, H−D exchange in the excited aromatic ring of dimethoxybenzenes was observed in hexafluoroisopropyl alcohol.[9] This solvent is formally neutral, but more acidic than water.[10] Here we report photochemical H−D exchange at aromatic carbons of tomaymycin in methanol and provide evidence for proton transfer as a major fluorescence quenching process in water. To the best of our knowledge, this is the first example of proton transfer from solvent to aromatic carbons at neutral pH.

Tomaymycin, a pyrrolo[1,4]benzodiazepine antibiotic, undergoes facile epimerization at C11 in protic solvents.[11] The 11-hydroxy form exists in water mostly as the (11*S*,11a*S*) diastereomer, whereas the 11-methyl ether in methanol is a mixture of (11*R*,11a*S*) and (11*S*,11a*S*) diastereomers. The two diastereomers have different fluorescence lifetimes.[11] The fluorescence quantum yield and lifetime increase about 4-fold in going from water to methanol. Figure 1 shows that most of the fluorescence intensity change upon transferring tomaymycin between water and methanol is instantaneous. The absorbance changes due to methylation of tomaymycin and hydrolysis of tomaymycin methyl ether take several hours. The epimerization of *R* and *S* diastereomers at C11 is also slow.[11] Therefore, substitution at C11 does not greatly affect the fluorescence.

The fluorescence quantum yield Φ_F and average lifetime $\bar{\tau}$ of tomaymycin are about 50% higher in D_2O than in H_2O (Table 1). A deuterium isotope effect on quantum yield and lifetime suggests that excited-state proton transfer is an important nonradiative process.[12] A similar solvent isotope effect in 8-methoxytomaymycin rules out proton transfer reactions involving the 8-phenolic proton. Both fluorescence intensity and solvent isotope effects in tomaymycin are independent of pH in the range pH 3−7. The 8-phenolic proton has a pK_a = 8, and the phenolate form is nonfluorescent.[13]

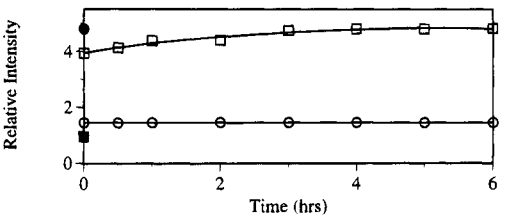

Figure 1. Time-dependent solvent effects on fluorescence intensity. At time = 0, tomaymycin solutions were diluted 10-fold. TME in methanol diluted into (●) methanol and (○) water. TOM in water diluted into (■) water and (□) methanol. Excitation wavelength λ = 320 nm, emission wavelength λ = 400 nm.

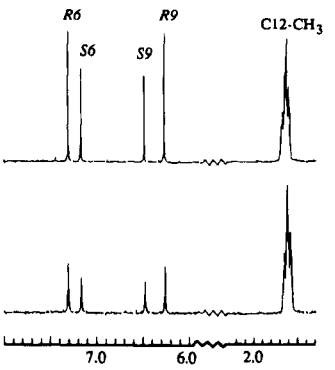

Figure 2. Partial 200 MHz ^{1}H-NMR spectra of TME in CD_3OD: (top) no irradiation; (bottom) 2 h irradiation.

Table 1. Deuterium Isotope Effects

compound	solvent[a]	λ (nm) abs$_{max}$	λ (nm) em$_{max}$	Φ_F	$\bar{\tau}$[b] (ns)
tomaymycin	H_2O	311	410	0.07 ± 0.01	1.0 ± 0.1
	D_2O	311	410	0.10 ± 0.01	1.6 ± 0.1
	CH_3OH	320	400	0.34 ± 0.02	4.3 ± 0.1[c]
	CH_3OD	320	400	0.34 ± 0.02	4.5 ± 0.1[d]
8-methoxyto-	H_2O	311	411	0.10 ± 0.01	1.4 ± 0.1
maymycin	D_2O	311	411	0.14 ± 0.01	2.0 ± 0.1

[a] Aqueous buffers are 0.01 M cacodylic acid, 0.1 M NaCl, 0.1 mM EDTA adjusted to pH (pD) 5.1. [b] Average lifetime $\bar{\tau} = \sum_i \alpha_i \tau_i$ of the biexponential fluorescence decay with amplitudes α_i and lifetimes τ_i (ref 11), excitation wavelength λ = 332 nm, emission wavelength λ = 400 nm. [c] (11*S*,11a*S*) diastereomer: $\alpha_1 = 0.36$, $\tau_1 = 3.5$ ns. (11*R*,11a*S*) diastereomer: $\alpha_2 = 0.64$, $\tau_2 = 4.9$ ns. [d] (11*S*,11a*S*) diastereomer: $\alpha_1 = 0.33$, $\tau_1 = 3.7$ ns. (11*R*,11a*S*) diastereomer: $\alpha_2 = 0.67$, $\tau_2 = 5.0$ ns.

Photochemical isotope exchange was monitored by ^{1}H NMR (200 and 400 MHz) in CD_3OD (Sigma, 99.9%) due to low solubility in water. Tomaymycin methyl ether was irradiated with a 200 W Xe−Hg lamp using a Corning 0-53 UV (≤300 nm) cutoff filter. Extent of H−D exchange of C6 and C9 aromatic protons was determined from peak areas relative to C12 methyl protons (Figure 2). After 2 h of irradiation, the *R* diastereomer showed 44% H−D exchange at C6 and 64% at C9, whereas the *S* diastereomer showed 60% exchange at C6 and 72% at C9. Thus exchange appears to be faster in the *S* diastereomer than in the *R* diastereomer and faster at C9 than at C6 in both diastereomers. No ground-state H−D exchange was detected in unirradiated sample. 2-Methoxyphenol did not

(1) Shizuka, H. *Acc. Chem. Res.* **1985**, *18*, 141−147.
(2) Shizuka, H.; Tobita, S. *J. Am. Chem. Soc.* **1982**, *104*, 6919−6927.
(3) Webb, S. P.; Phillps, L. A.; Yeh, S. W.; Tolbert, L. M.; Clark, J. H. *J. Phys. Chem.* **1986**, *90*, 5154−5164.
(4) Pollard, R.; Wu, S.; Zhang, G.; Wan, P. *J. Org. Chem.* **1993**, *58*, 2605−2613.
(5) Saito, I.; Suglyama, H.; Yamamoto, A.; Muramatsu, S.; Matsuura, T. *J. Am. Chem. Soc.* **1984**, *106*, 4286−4287.
(6) Shizuka, H.; Serizawa, M.; Kobayashi, H.; Kameta, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *J. Am. Chem. Soc.* **1988**, *110*, 1726−1732.
(7) Shizuka, H.; Serizawa, M.; Shimo, T.; Saito, I.; Matsuura, T. *J. Am. Chem. Soc.* **1988**, *110*, 1930−1934.
(8) Yu, H.-T.; Colucci, W. J.; McLaughlin, M. L.; Barkley, M. D. *J. Am. Chem. Soc.* **1992**, *114*, 8449−8454.
(9) Mathivanan, N.; Cozens, F.; McClelland, R. A.; Steeken, S. *J. Am. Chem. Soc.* **1992**, *114*, 2198−2203.
(10) Filler, R. S.; Ralph M. *J. Org. Chem.* **1967**, *32*, 1217−1219.
(11) Barkley, M. D.; Cheatham, S.; Thurston, D. E.; Hurley, L. H. *Biochemistry* **1986**, *25*, 3021−3031.
(12) Stryer, L. *J. Am. Chem. Soc.* **1966**, *88*, 5708−5712.
(13) Barkley, M. D.; Thomas, T. J.; Maskos, K.; Remers, W. A. *Biochemistry* **1991**, *30*, 4421−4431.

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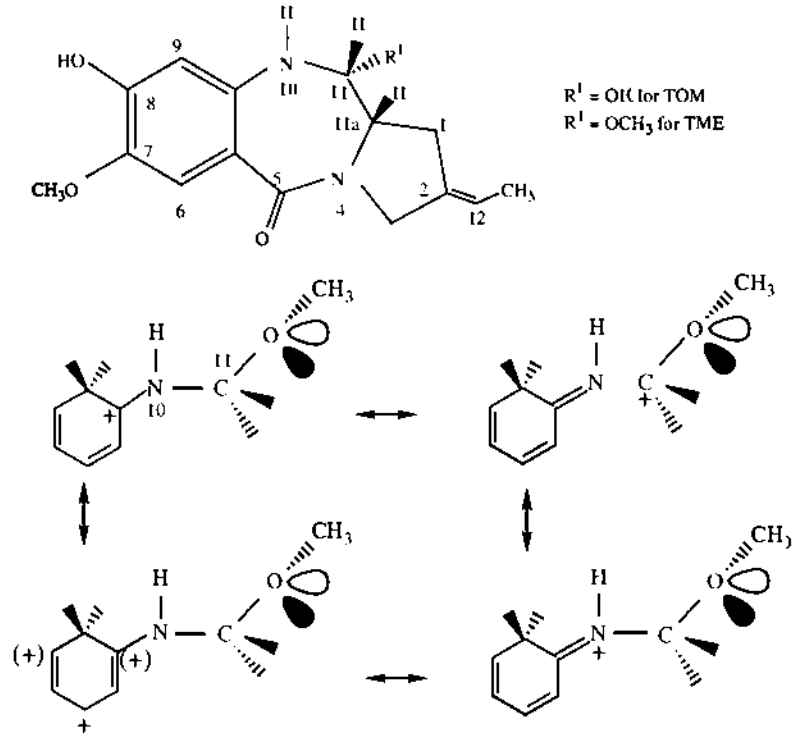

Figure 3. Numbering scheme for TOM and TME. Partial resonance structures of predominant conformer in (11*S*,11a*S*) TME showing the antiperiplanar overlap between oxygen lone pair and N10−C11 bond.

show H−D exchange in CD_3OD after 5 h irradiation without the filter, suggesting that hydroxy and methoxy substituents alone do not account for the photochemical isotope exchange of tomaymycin in neutral solution. The H−D exchange reaction was quenched by acrylamide with about the same Stern−Volmer constant, $K_{sv,R}^{D} = 12 \pm 1\ M^{-1}$, as the fluorescence quenching constant, $K_{sv,F}^{D} = 10.2 \pm 0.3\ M^{-1}$. This indicates that H−D exchange occurs in the singlet excited state.

The reaction quantum yield $\Phi_R^{D} = 0.03 \pm 0.02$ for H−D exchange in CD_3OD was determined by chemical actinometry with ferrioxalate using a 313 nm interference filter (microCoatings).[14] The proton transfer rate $k_{pt}^{D} = (1.1 \pm 0.7) \times 10^7\ s^{-1}$ was calculated from $\Phi_R^{D} = 0.63\ k_{pt}^{D}\bar{\tau}$, where $\bar{\tau}$ is the average fluorescence lifetime (Table 1). The factor 0.63 is an estimate of $k_h/(k_h + k_d)$, where k_h and k_d are the rates of proton and deuteron loss in the cationic aryl intermediate.[2,6] If excited-state proton transfer and intersystem crossing were the only major nonradiative channels, the proton transfer rate estimated from the fluorescence lifetime (*vide infra*) would be $(6.1 \pm 1.4) \times 10^7\ s^{-1}$. Silent proton transfer at the benzodiazepine ring juncture as well as at C7 and C8 or an unknown nonradiative process could explain the discrepancy between these two values for the proton transfer rate. The lifetime difference between the (11*R*,11a*S*) and (11*S*,11a*S*) diastereomers in methanol may be due to the anomeric effect, which enhances proton transfer in the *S* diastereomer by increasing electron density in the aromatic ring. Energy minimization of tomaymycin (Sybyl, Tripos Associates) for different N10−C11−O−methyl torsions indicates that the 180° torsion is predominant in the *R* diastereomer, while the 60° torsion is most populated in the *S* diastereomer. The antiperiplanar overlap between one of the oxygen lone pairs and the N10−C11 bond in the *S* diastereomer lengthens the N10−C11 bond by 0.005 Å compared to the *R* diastereomer and allows resonance structures that enhance proton transfer at the benzene moiety. Figure 3 presents the case for attack at C9. Analogous resonance structures for attack at C6 show that the electron-withdrawing effect of the carbonyl makes one of the resonance structures less favorable and therefore proton exchange at C6 less likely.

Proton transfer rates in aqueous solutions were estimated from $\bar{\tau}^{-1} = k_r + k_{isc} + k_{pt}$ and $\Phi_F = k_r\bar{\tau}$. Here $k_{isc} = (8.5 \pm 1.0) \times 10^7\ s^{-1}$ is assumed to be independent of solvent.[15] In H_2O and D_2O, $k_{pt}^{H} = (8.4 \pm 0.7) \times 10^8\ s^{-1}$ and $k_{pt}^{D} = (4.8 \pm 0.2) \times 10^8\ s^{-1}$. A linear fit of the proton transfer rates for H_2O and D_2O mixtures gives a correlation coefficient of 0.992. A proton inventory plot is linear if one proton is involved in the proton transfer reaction.[16] The autoprotolysis constant for water, 1.0×10^{-14}, is $>5.8 \times 10^{-16}$ for methanol, indicating that water is a better proton donor.[17] Proton transfer will be faster, and thus the fluorescence quantum yield and lifetime will be lower in water than in methanol. This is consistent with the large isotope effect observed for the fluorescence quantum yield and lifetime in water compared to methanol. The isotope effect on the proton transfer rate in water estimated from $k_{pt}^{H}/k_{pt}^{D} = 1.75$. Assuming 1.75 for the isotope effect in methanol and the low value of the proton transfer rate based on Φ_R^{D}, we estimate a difference in average decay rate of about $1 \times 10^7\ s^{-1}$ between CH_3OH and CH_3OD, in agreement with the difference calculated from the lifetime data in Table 1. Alternatively, assuming the high value of the proton transfer rate based on the lifetime data, we estimate an isotope effect $k_{pt}^{H}/k_{pt}^{D} = 1.1$ in methanol.

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(14) Calvert, J. G.; Pitts, J. N. *Photochemistry*; John Wiley and Sons, Inc.: New York, 1966; pp 783−786.

(15) Barkley, M. D.; Chen, Q.; Maskos, K.; Remers, W. R.; manuscript in preparation.

(16) Kresge, A. *Pure Appl. Chem.* **1964**, *8*, 243−258.

(17) Coetzee, J. F. R.; Calvin D. *Solute−Solvent Interactions*; Marcell Dekker: New York, 1969; p 199.