Conformations and Tautomers of Tetracycline

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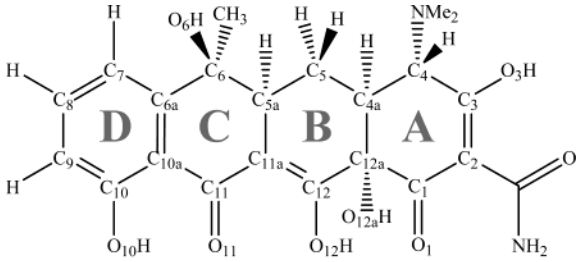
Density functional theory (DFT) has been used to investigate the conformations and tautomeric forms of neutral tetracycline in aqueous solution. The results suggest that the extended conformation is 3−3.5 kcal mol^{-1} more stable than the twisted one for equivalent tautomers and that as many as six different tautomeric forms lie within 10 kcal mol^{-1} of the most stable one. The energetic preference for the extended conformation is a solvent effect. Calculated infrared, NMR, and UV/vis spectra are used to suggest ways of differentiating between tautomers and conformations experimentally. It is shown that the ^{13}C chemical shifts of C4a, C5, and C6 can be used to distinguish between the twisted and extended conformations.

Introduction

The tetracycline class of antibiotics[1] has achieved importance beyond their considerable therapeutic usefulness as inducers of the Tet-Repressor protein, which has been used as an experimental switch to regulate gene expression.[2] However, many fundamental aspects of the chemistry of the tetracyclines remain unclear. One such aspect is the exact nature of both the conformations in solution and the tautomeric forms of neutral tetracycline, **1**. Schneider[3] has reviewed the recent literature on the protonation states of tetracyclines and has concluded that they are chameleon-like because they can adopt many different structures (both conformations and tautomeric forms) with relatively small differences in energy. Thus, the presence of a metal ion, for instance, can change the preferred tautomeric form.



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Although there have been many X-ray crystal structure determinations,[4−7] circular dichroism,[8−11] and NMR[12,13] studies of tetracycline and its derivatives, no clear systematic picture of the conformational and tautomeric equilibria of tetracycline and its derivatives has emerged. Our earlier calculational study[14] of the conformations of the fully neutral form and one of the possible zwitterions suggested that three types of conformations exist for neutral tetracycline. In this study, which concentrated on searching the conformational potential energy hypersurface of tetracycline as fully as possible, only the generally accepted tautomeric form of the zwitterions was investigated. We now report a study using density functional theory in order to determine the relative importance of different tautomers of neutral tetracycline in which we have used the results of our previous work[14] to limit the extent of the conformational search. A further aim of this work is to define some spectroscopic characteristics of the species calculated in order to aid their experimental identification.

Methods

Density functional calculations all used the Becke 3-parameter hybrid functional[15] in conjunction with the Lee−Yang−Parr correlation functional[16] (B3LYP) with the 6-31G(d) basis set.[17] Geometries were optimized fully in vacuo using Gaussian 98.[18] The structures obtained were confirmed as local minima by calculating their normal vibrations within the harmonic approximation. Single-point calculations on the optimized geometries using the self-consistent reaction field (SCRF) technique in a simulated water continuum were used to obtain energies "in solution". The SCRF calculations used the standard polarizable continuum model (PCM) with a cavity generated using the united-atom topological model.[19] Single-point calculations with the same basis set were also performed with a second-order Møller−Plesset (MP2)[20] correction for electron correlation. Corrected MP2 relative energies were calculated using the gas-phase Born−Oppenheimer relative energies from these calculations and applying the vibrational and solvation corrections calculated with B3LYP. NMR chemical shifts were calculated using the gauge-independent atomic orbital (GIAO) approach.[21] The calculated shieldings, S, for carbon atoms were converted to ^{13}C chemical shifts relative to tetramethylsilane, δ, using the regression formula[22]

$$\delta = 208.65 - (1.0653S) \qquad (1)$$

Finally, the absorption and fluorescence spectra of the B3LYP/6-31G(d) structures were calculated using a singles-plus-pair-doubles configuration interaction (CI)[23] within the program VAMP 8.1[24] using the AM1 Hamiltonian[25] and our SCRF-model for excited states[26] as extended by Gedeck and Schneider to treat nonequilibrium solvation.[27] Sixteen occupied and sixteen

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TABLE 1: Calculated Relative Energies (kcal mol^{-1}) for Neutral Tetracycline Conformations and Tautomers[a]

species	conformation	O12a	protonation state						relative energy			MP2−corrected relative energy (PCM, 298 K)
			O1	O3	O11	O12	amine	amide O	0 K	298 K	PCM, 298 K	
N1	1	N		A		11			2.9	3.0	0.0	0.0
N2	1	N		A		12a			9.4	9.2	4.1	2.0
N3	2	1		A		11			0.0	0.0	2.8	3.7
N4	1	12		A		11			3.8	3.3	3.2	5.6
N5	1	12		A		1			15.6	14.9	9.2	11.7
N6	2	1		A		1			12.5	12.4	11.7	12.0
Z1	1	12				11	12a		20.4	24.4	7.9	2.6
Z2	1	12	12				12a		23.1	33.3	*(10.9)*[b]	*(3.9)*[b]
Z3	1	12		A			12a		27.0	29.9	8.4	4.3
Z4	1	12				11	3		28.2	26.3	7.0	4.4
Z5	1	1,12				1	12a		27.8	29.7	11.4	4.8
Z6	1	12					12a	3	33.6	28.5	8.2	5.2
Z7	2	1				11	6		29.8	25.5	9.7	5.8
Z8	1	12	A				12a		26.6	27.9	8.2	6.0
Z9	2	1				11	3		30.2	20.6	8.0	6.1
Z10	1	12		A			3		24.5	39.0	9.3	6.2
Z11	1	12					3	3	32.2	35.8	8.2	6.2
Z12	1	12			12		12a		31.9	26.4	12.3	6.4
Z13	2	1					6	3	28.4	32.6	10.9	7.1
Z14	2	1			12		3		30.8	23.3	10.5	7.6
Z15	2	1			12		6		25.2	28.7	13.6	8.4
Z16	2	1	A				6		35.9	32.2	12.1	8.7
Z17	2	1				1	6		37.4	30.8	14.3	9.3
Z18	2	1					3	3	36.3	37.9	13.6	10.1
Z19	2	1	A				3		39.4	36.0	13.2	10.7
Z20	1	12				1	3		36.0	34.9	14.0	11.6

[a] Conformation **1** is that usually known as the extended conformation[28,29] and conformation **2** is the twisted one. The column headed O12a defines the atom to which the proton bonded to O12a (always protonated) makes a hydrogen bond. The columns headed protonation state indicate that the atom given is protonated if an entry is present. The entries for the individual conformations indicate the atom to which the relevant proton makes a hydrogen bond. The Me_2N nitrogen is indicated by N and the amide oxygen atom by A. The MP2−corrected relative energy was calculated as described in the Methods section. [b] The UAHF−PCM calculation failed consistently for structure **Z2**. A PCM-calculation using the default isodensity surface (IEPCM) was therefore compared with an analogous calculation for the most stable species, **N1**.

virtual orbitals were included in the CI. Tests with larger and smaller numbers of active orbitals suggested that the results are converged for all calculations with more than 24 orbitals in the active space.

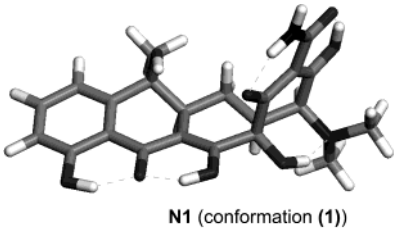
Starting structures for the geometry optimizations were selected from the three conformations found in our earlier AM1 study[14] and the X-ray structure of tetracycline hexahydrate.[6] The most likely tautomers were constructed for each of the conformations by shifting protons without changing the geometries of the non-hydrogen atoms. This gave a total of 48 starting structures, 22 of which optimized to local minima found for other starting geometries. This procedure resulted in 26 unique combinations of the ring conformation and tautomeric form. These 26 structures were then characterized in terms of their calculated energies at 298 K in aqueous solution and their spectroscopic properties. During the optimization, the two most similar conformations used as starting geometries converged to one larger cluster, which we have designated conformation **(1)** below. This conformation corresponds to that known as the A[28] or extended[29] conformation. This conformational search procedure is not exhaustive, but should give a good representation of possible tetracycline conformations and tautomers.

Results

Conformations Obtained. Table 1 shows the calculated relative energies for the 26 minima obtained for neutral tetracycline. Table S1 of the Supporting Information gives the calculated total and zero-point vibrational energies.

The completely neutral (i.e., nonzwitterionic) form of tetracycline, designated **N1**-6 in Table 1, forms six different conformers in two different ring conformations, each of which can exist in the form in which O12H is hydrogen bonded to O1 or O11. Conformation **2** corresponds to the so-called twisted conformation[29] and is found to be most stable in the gas phase, but conformation **1** becomes most stable in solution. This observation explains the discrepancy among previous AM1 studies on tetracyclines. We earlier[14] found the twisted conformation to be the more stable in the gas phase, whereas Zerner et al.[30] studied anhydrotetracycline and Duarte et al.[31] studied tetracycline, both using a continuum solvent simulation, and concluded that the extended conformation was the more stable. The conformations are distinguished by the oxygen to which O12aH makes a hydrogen bond. In conformation **1**, it bridges to O12 or to the dimethylamino group, and in conformation **2** to O1. This results in the structures shown in Figure 1 for the conformations in which the hydrogen on O12 forms a hydrogen bond to O11. These conformations are always more stable than their counterparts in which O12H builds hydrogen bonds to O1 or O12a. The hydrogen-bonding distances are summarized in Table S2 of the Supporting Information.

The **N1**, **N2**, **N4**, and **N5** structures differ essentially only in the hydrogen-bond network. The ring conformations are very similar (the heavy atom root-mean-square deviations (RMSDs) between the four structures are all less than 0.6 Å). However, the other two structures, **N3** and **N6** differ considerably, especially in the C and B rings, where C6 is bent strongly upward (toward the A ring) and C5 strongly downward. This results in a more globular structure than conformation **1** that is held fixed by the trifurcated hydrogen bond network between O1 and the hydrogens on O12, O12a, and the NH_2 group of the amide. This compact structure with its very effective intramolecular hydrogen bonds is found to be most stable in



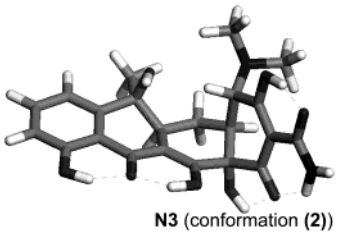


Figure 1. B3LYP/6-31G(d) optimized structures for the two ring conformations of completely neutral tetracycline. Only the conformers in which O12H is hydrogen bonded to O11 are shown. These conformations are found to be more stable in aqueous solution than their equivalents in which O12H is hydrogen bonded to O1 or O12a. The conformation typified by **N1** is labeled **1** throughout and corresponds to the extended conformation.[29] **N3** exemplifies the twisted[29] conformation and is labeled **2** throughout.

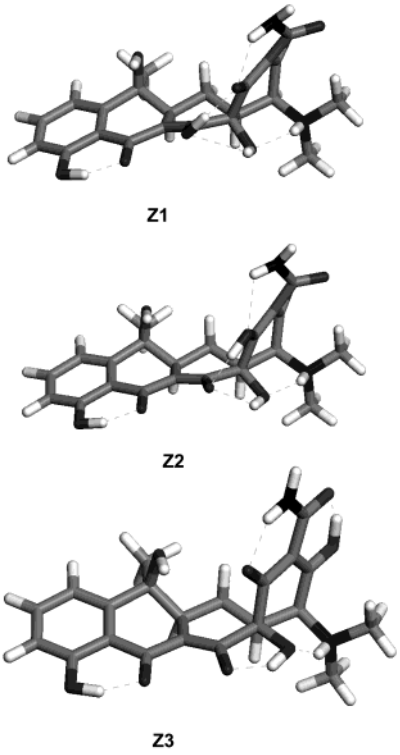


Figure 2. Three most stable zwitterionic structures of neutral tetracycline in aqueous solution (all conformation **1**).

the gas phase, **N3** both with B3LYP and MP2, but the more open and solvent-accessible structures **N1** and **N2** are calculated to be more stable in aqueous solution.

The most stable zwitterionic tautomers are also found to adopt conformation **1** (**Z1**−**Z6**). The lowest energy structure with conformation **2** is found to be **Z7**, 3.2 kcal mol^{-1} less stable than **Z1** in aqueous solution at 298 K. The three most stable zwitterionic structures in aqueous solution (**Z1**−**Z3**) are shown in Figure 2. Their ring conformations all bear a strong resemblance to that of **N1**. This conformation is held relatively firmly by the hydrogen bond between the Me_2NH^+ group and O12a, which is found in six of the seven lowest-energy structures in conformation **1**. The only exception is **Z4**, which has instead an $Me_2NH^+\cdots$O3 hydrogen bond. This structure was obtained by using the X-ray structure of tetracycline hexahydrate.[6] The RMSD between the X-ray and optimized structures for **Z4** (all non-hydrogen atoms) is 0.29 Å. The other constant feature of the structures in conformation **1** is the hydrogen bond between O12a−H and O12.

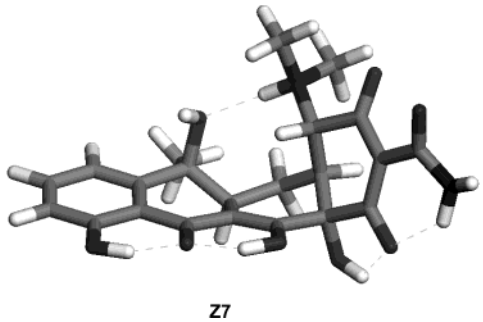


Figure 3. Lowest energy zwitterionic tetracycline structure found for conformation **2**.

The lowest energy zwitterionic structure, **Z7**, that adopts conformation **2** is shown in Figure 3. Once again, the hydrogen bond formed by the Me_2NH^+ group is characteristic. The most stable arrangement appears to be the hydrogen bond to O6 with a second set of less stable structures in which the bonding partner is O3. Conformation **2** also appears to favor tautomers in which O12 is not protonated.

All 26 structures were superimposed, and the RMSDs for the non-hydrogen atoms were calculated.[32] Table S3 of the Supporting Information shows the RMSD matrix thus obtained. These RMSD values were used to assign the conformations given in Table 1. The 26 conformations obtained divide cleanly into two groups: those that we have designated conformation **1** (extended) form a homogeneous set of scaffold conformations and conformation **2** (twisted) constitutes a second. All RMSDs within either group are found to be less than 1.0 Å, and those between groups are typically 1.5−2.3 Å. Our results thus suggest that only two conformations, **1** and **2**, are important in solution for neutral tetracycline.

The calculated energies (Table 1) suggest that the neutral species are slightly preferred (by 2−2.5 kcal mol^{-1}) over corresponding zwitterionic structures in aqueous solution. However, this result should be treated with caution because DFT calculations are known[33] to disfavor zwitterions energetically and to underestimate the polarity and stability of charge-separated species in general. This effect disfavors the zwitterions in two ways. First, their gas phase energies are slightly higher than they should be. We have taken this effect into account by calculating the MP2/6-31G(d)[20] energies of the B3LYP/6-31G(d) optimized structures (see Table 1). Using these energies with the B3LYP/6-31G(d) zero-point vibrational energies and the UAHF−PCM B3LYP/6-31G(d) solvation energies gives the MP2-corrected relative free energies shown in Table 1. These are our best estimates of the relative energies in solution. However, the fact that DFT underestimates the charge separation probably also results in the DFT-calculated solvation energies being slightly too low, so that we expect the zwitterions still to be disfavored slightly relative to the totally neutral structures. This is borne out by comparison with experimental results (see below).

The UAHF−PCM solvation calculation failed for structure **Z2**, so that in this case, an isodensity-PCM calculation[34] was performed. The energy obtained from this calculation was

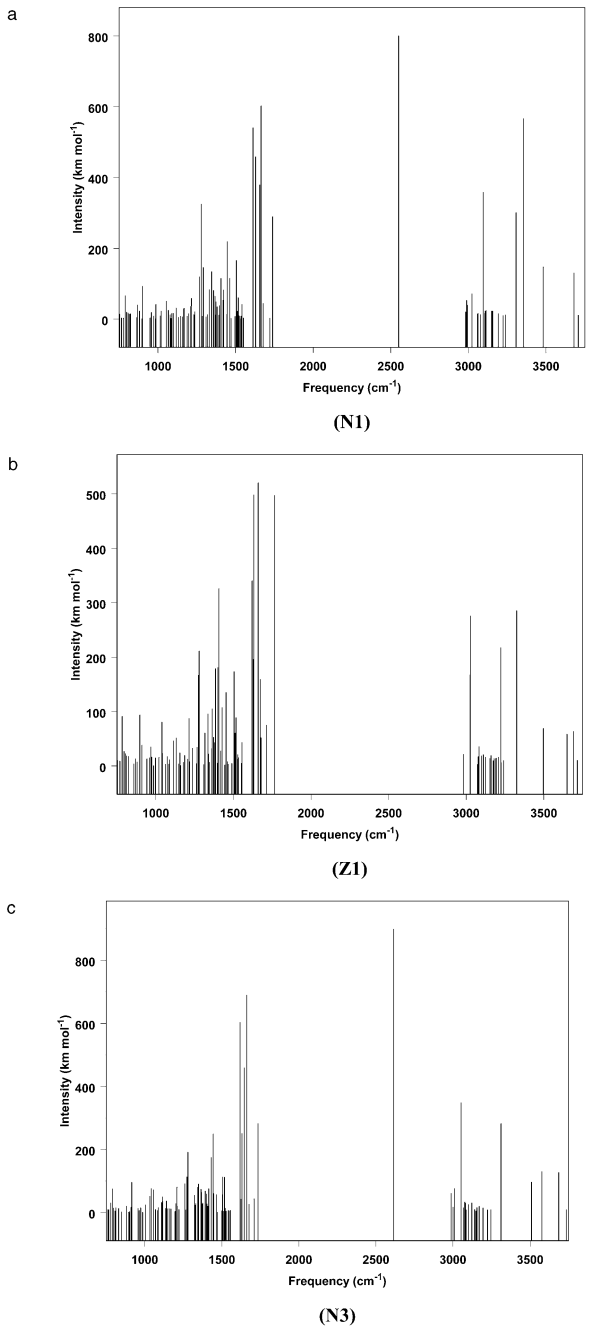


Figure 4. Calculated (B3LYP/6-31G(d)) infrared frequencies and intensities for the most stable neutral structure, **N1**, the most stable zwitterion, **Z1**, and the most stable structure with conformation **2**, **N3**. The frequencies are unscaled and only those between 750 and 3750 cm^{-1} are shown.

compared with that obtained for **Z1** using the same technique, and a relative energy was estimated from these calculations (italics in Table 1). From this calculation, **Z2** is the second most stable zwitterionic structure in solution, but this estimate is uncertain. Unfortunately, no single continuum-model solvation technique available in Gaussian 98 was successful for all 26 structures.

Vibrational Spectra. The B3LYP/6-31G(d) calculated vibrational frequencies and infrared intensities for the most stable neutral structure, **N1**, zwitterion, **Z1**, and the lowest energy structure with conformation **2**, **N3**, are shown as line diagrams in Figure 4. The calculated frequencies and intensities for all 26 structures are available as Table S4 of the Supporting Information.

The frequency range between 2750 and 3000 cm^{-1} is suggested by the calculations to be most characteristic for distinguishing these three structures. Intense peaks in this range are due to essentially localized O3H stretch/bend vibrations (2550.7 and 2615.6 cm^{-1} for **N1** and **N3**, respectively). For all of the structures calculated, peaks occur in this general region for O3H···O$_{amide}$, Me$_2$NH···O12a, O1H···O$_{amide}$, O1H···O11, O11H···O12, and O10H···O11 localized vibrations. However, for the calculated structures within 5.0 kcal mol^{-1} of the most stable in solution, a strong peak in this region is completely diagnostic for protonation on O3.

To judge the discriminating power of vibrational spectroscopy between the 26 structures, we calculated the principal components (factors)[35,36] of the calculated (unscaled) frequencies arranged as a vector of increasing values for each structure. However, none of the calculated principal components correlates significantly with either protonation states or hydrogen-bond linkages. Simple correlation of binary indicators of these structural features with the individual vibrational frequencies also yields no useful information. As our analysis treats the vibrational frequencies in ascending order for each compound, significant shifts in individual frequencies that correspond to each other in different structures would not be detected. We also tried ordering the absorptions according to their intensities in order to differentiate between structural features using recursive partitioning, cluster analysis, and principal components. However, none of these approaches proved to be predictive, so that we are making the calculated data available via the Supporting Information for others to make more detailed analyses.

Nuclear Magnetic Resonance Spectra. The calculated NMR chemical shifts (GIAO−B3LYP/6-31G(d)//6-31G(d)) for all 26 structures are given in Tables S4 (^{13}C) and S5 (^{15}N and ^{17}O) of the supporting material. The NMR data are inherently more useful for analysis of the conformation and tautomeric form of tetracycline simply because each chemical shift can be assigned to an atom, whereas vibrational modes are only characterized by their frequency and intensity. We therefore carried out recursive partitioning (FIRM[37]) and correlation analyses in order to determine the relationship between the number of the conformation (**1** or **2**) and the ^{13}C chemical shifts. These analyses revealed the peaks for C4a, C5, and C6 as being diagnostic for the ring conformation. Figure 5 shows scatter plots of the calculated chemical shifts for pairs of these carbons with the points color coded according to the conformation. In all three cases, the combination of two chemical shifts allows a clear assignment of the conformation.

Tables S4 and S5 of the Supporting Information also allow the assignment of protonation states for individual atoms. These assignments are, however, relatively straightforward and need not be discussed here, especially as tautomeric equilibria are fast on the NMR time scale. ^{15}N chemical shifts have, for instance, been used to identify tetracycline tautomers.[39]

UV/Visible Spectra. The calculated excitation energies (vertical excitation, nonrelaxed solvent) for the 26 tetracycline structures are shown in Table 2. The calculated spectra agree well with the experimental observations.[3] At neutral pH in water, tetracycline gives a broad absorption peak at about 355 nm that is separated from a second absorption around 275 nm by a broad valley.

Statistical analysis of the results reveals two features that may be useful in interpreting spectra. The first is that the tautomers in which O11 is protonated absorb at significantly lower energies than the others. This observation suggests that the large Stokes

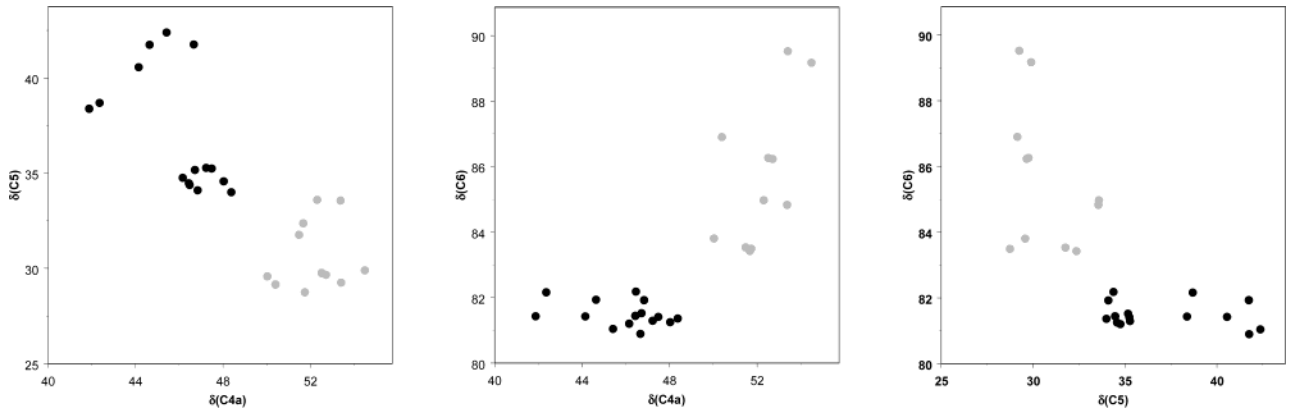


Figure 5. Discrimination between the extended **1** and twisted **2** conformations of neutral tetracycline using the ^{13}C chemical shifts of carbons 4a, 5, and 6. Conformation **1** is denoted by black circles, and **2** is denoted by gray circles.

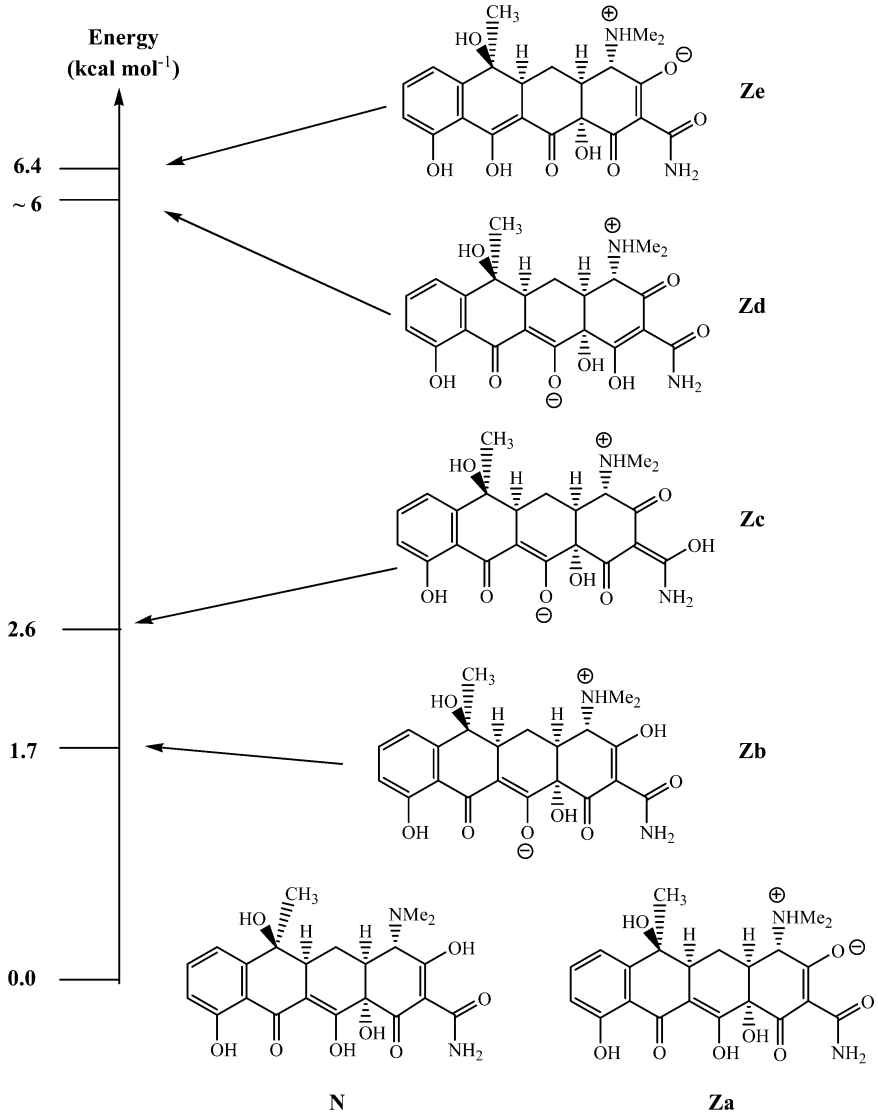


Figure 6. Our best estimate of the relative stabilities of neutral tetracycline tautomers in aqueous solution at 298 K. The relative energies of all zwitterionic structures have been lowered to make the results compatible with experiment and that of **Zd** raised by about 2 kcal mol^{-1}, as discussed in the text.

shift observed for tetracycline[3] may be due to an excited-state proton shift from O12 to O11. More detailed calculations with geometry optimization for the excited states support this hypothesis.[39] The Stokes shift due to solvent relaxation is calculated to be only about 3−5 nm for the S^0 to S^1 transition, so that solvent effects cannot be responsible for the experimental observation. At neutral pH in water, tetracycline gives a fluorescence band with a maximum at about 560 nm and a long tail to beyond 700 nm.[3]

The second observation is that a moderately intense (oscillator strength ≥ 0.1) S^0 to S^2 transition roughly 20 nm higher in energy than S^0 to S^1 is diagnostic for an O12−H···O11 hydrogen bond. The experimental spectrum of tetracycline at neutral pH appears to contain such an absorption as a broad shoulder.[3] As it has

TABLE 2: Calculated (AM1, Singles Plus Pair Excitations CI, 32 Active MOs) Absorption Spectra (Wavelength in nm, Oscillator Strength in Parentheses) for the 26 Tetracycline Structures[a]

	peak 1		valley		peak 2
N1	336 (0.302)	318 (0.146)	2 states	292 (0.110)	274 (0.270)
N2	327 (0.168)		3 states	288 (0.335)	275 (0.287)
N3	331 (0.264)		4 states	282 (0.171)	278 (0.175)
N4	332 (0.281)	310 (0.161)	2 states	287 (0.179)	279 (0.232)
N5	324 (0.173)		4 states	282 (0.167)	270 (0.471)
N6	326 (0.158)		5 states	276 (0.482)	
Z1	341 (0.345)	319 (0.129)	2 states	291 (0.100)	273 (0.181)
Z2	343 (0.465)		5 states		
Z3	338 (0.371)		4 states	269 (0.299)	
Z4	339 (0.299)	320 (0.130)	2 states	296 (0.196)	269 (0.301)
Z5	333 (0.239)		3 states	294 (0.179)	282 (0.114)
Z6	348 (0.362)		5 states	279 (0.280)	
Z7	343 (0.303)	321 (0.111)	3 states	267 (0.304)	
Z8	345 (0.425)		4 states	277 (0.269)	251 (0.131)
Z9	338 (0.296)	316 (0.113)	3 states	266 (0.423)	
Z10	349 (0.352)		5 states	260 (0.332)	
Z11	359 (0.344)		4 states	278 (0.342)	
Z12	360 (0.442)		3 states	285 (0.142)	282 (0.158) 273 (0.135)
Z13	341 (0.337)		4 states	270 (0.267)	
Z14	357 (0.4019		4 states	268 (0.413)	
Z15	359 (0.362)		4 states	265 (0.369)	
Z16	345 (0.357)		4 states	269 (0.162)	
Z17	336 (0.205)	312 (0.151) 298 (0.145)	2 states	270 (0.363)	
Z18	345 (0.357)		4 states	268 (0.313)	
Z19	344 (0.397)		5 states	274 (0.123)	
Z20	333 (0.192)		4 states	285 (0.384)	269 (0.376)

[a] The data are given for absorption in aqueous solution. Only peaks with an oscillator strength greater than 0.1 are shown.

been suggested[3] that the presence of the O12−H···O11 hydrogen bond decreases fluorescence yield, there should be some correlation between the occurrence of this absorption and the fluorescence yield.

Discussion

Our calculations confirm that two ring conformations are important for tetracycline in solution and that these correspond to the extended **1** and twisted **2** conformations described previously.[3,28,29] The presence of an O12a−H···O1 hydrogen bond is prerequisite for the formation of the twisted **2** conformation (see Table 1). This hydrogen bond is present in all of the structures calculated to be an energy minimum in the twisted **2** conformation. Note that the twisted conformation has been proposed to be stabilized by a strong O3−H···O_{amide} hydrogen bond.[40,41] We find this hydrogen bond in all neutral structures investigated (of both conformations) and in none of the twisted zwitterionic conformations. The extended **1** conformation is the more stable, both for the neutral structure and for the zwitterions. The most stable structures for the twisted **2** conformation in aqueous solution at room temperature are found to be 3.7 and 3.2 kcal mol^{-1} less stable than the most stable neutral and zwitterionic structures, respectively. As in both cases, the most stable structures of the two conformations exist in the same protonation state. This calculated energy difference should be reliable, so that we can conclude that the twisted **2** conformation is inherently 3−4 kcal mol^{-1} less stable than the extended **1** conformation for neutral tetracycline in water at 298 K.

Our calculations suggest that the most stable zwitterionic structure is 2.6 kcal mol^{-1} less stable than the best neutral structure in water at 298 K. Comparison with the experimental results[8] suggests that the calculations disfavor the zwitterions by about 2.5 kcal mol^{-1} as the two tautomeric forms must be about equally stable in aqueous solution.[8] The calculations, however, reveal that five different zwitterionic tautomers can exist with 9 kcal mol^{-1} of the most stable structure. Either O3 or O12 or both can be deprotonated with an energetic preference for deprotonation at O3. If both O3 and O12 are deprotonated, O1, the amide oxygen, or O11 may be protonated, in that energetic order. Table 1 suggests that the energy calculated for structure **Z2**, for which we could not obtain UAHF−PCM results and therefore had to use the IEPCM technique, may be artificially too stable by about 2 kcal mol^{-1}. Unfortunately, we were unable to find a single SCRF technique that was successful for all 26 structures (UAHF−PCM was the most reliable with only one failure), so that we are unable to use a consistent solvation energy treatment for the entire series of molecules. If we assume that the true relative energy of **Z2** is about 2 kcal mol^{-1} higher than that given in Table 1 and that the most stable zwitterion is roughly as stable as the most stable conformation of the neutral form, we can deduce a rough scheme of the order of stability of the tautomers as shown in Figure 6.

Figure 6 suggests that, when docked into a receptor or complexed with a metal ion, at least four tautomers are energetically accessible at room temperature and probably six. Tautomer **Zb**, for instance, will be strongly stabilized by complexation of a metal ion to O11 and O12, as found in the X-ray structure of the tetracycline:Mg^{2+} complex docked into the Tet-repressor receptor,[42] but **Zc** is also a candidate for such stabilization. Given that the tautomeric forms of docked ligands are often assumed in resolving X-ray structures, there clearly remains some uncertainty as to which tautomeric form of tetracycline is present under which circumstances.

Of the spectroscopic methods simulated in this work, vibrational spectroscopy is the least promising for determining the conformation and structure of tetracycline in solution, although some diagnostic peaks could be identified. ^{13}C NMR spectroscopy allows the determination of the conformation from the observed chemical shifts of C4a, C5, and C6 (provided that the conformational equilibrium is slow enough), and absorption spectroscopy can give useful information about the tautomeric form. We note that our data suggest that the first excited-state exists in a different tautomeric form to the ground state and

that an excited-state rearrangement can occur. Our results for this process are in accord with experimental observations.[3]

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Supporting Information Available: Table S1 shows the calculated total and zero-point energies for neutral tetracycline conformations and tautomers, Table S2 the hydrogen-bonding distances, and Table S3 the root-mean-square deviations between the non-hydrogen-atom skeletons of the minimum-energy structures found for neutral tetracycline. Table S4 shows the calculated infrared frequencies, Table S5 the calculated ^{13}C chemical shifts, and Table S6 the calculated ^{15}N and ^{17}O isotropic magnetic shieldings for the 26 tetracycline structures. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Chopra, I.; Roberts, M. *Microbiol. Mol. Biol. Rev.* **2001**, *65*, 232.

(2) Hillen, W.; Berens, C. *BIOspektrum* **2002**, *8*, 355. Hinrichs, W.; Orth, P.; Kisker, C.; Schnappinger, D.; Hillen, W.; Saenger, W. *NATO Science Series, Series C: Mathematical and Physical Sciences*; Kluwer Academic: Dordrecht, The Netherlands, 1999; pp 349−365.

(3) Schneider, S. In *Tetracyclines in Biology, Chemistry and Medicine*; Nelson, M., Hillen, W., Greenwald, R. A., Eds.; Birkhäuser: Zürich, 2001; pp 65−104.

(4) Stezowski, J. J. *J. Am. Chem. Soc.* **1976**, *98*, 6012.

(5) Palenik, G. J.; Mathew, M.; Restivo, R. *J. Am. Chem. Soc.* **1978**, *100*, 4458. Palenik, G. J.; Bentley, J. A. *J. Am. Chem. Soc.* **1978**, *100*, 2863.

(6) Caira, M. R.; Nassimbeni, L. R.; Russell, J. C. *Acta Crystallogr. B* **1977**, *33*, 1171.

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

(8) Hughes, L. J.; Stezowski, J. J.; Hughes, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 7655.

(9) Mitscher, L. A.; Bonacci, A. C.; Sokoloski, T. D. *Antimicrob. Ag. Chemother.* **1970**, 78.

(10) Mitscher, L. A.; Bonacci, A. C.; Sokoloski, T. D., *Tetrahedron Lett.* **1968**, *51*, 5361.

(11) Shaw, J.; Everett, G. W., Jr. *J. Inorg. Biochem.* **1982**, *17*, 305.

(12) Casy, A. F.; Yasin, A. *Magn. Reson. Chem.* **1985**, *23*, 767.

(13) Celotti, M.; Fazakerley, G. V. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1319.

(14) Lanig, H.; Gottschalk, M.; Schneider, S.; Clark, T. *J. Mol. Model.* **1999**, *5*, 46.

(15) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372, 5648; Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623.

(16) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev.* **1988**, *37*, 785. Miehlich, B.; Savin, A.; Stoll, H.; Preuss, H. *Chem. Phys. Lett.* **1989**, *157*, 200.

(17) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724. Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257. Hariharan, P. C.; Pople, J. A. *Mol. Phys.* **1974**, *27*, 209. Gordon, M. S. *Chem. Phys. Lett.* **1980**, *76*, 163. Hariharan, P. C.; Pople, J. A. *Theo. Chim. Acta* **1973**, *28*, 213.

(18) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11.3; Gaussian, Inc.: Pittsburgh, PA, 1998.

(19) Barone, V.; Cossi, M.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3210.

(20) Frisch, M. J.; Head-Gordon, M.; Pople, J. A. *Chem. Phys. Lett.* **1990**, *166*, 275. Frisch, M. J.; Head-Gordon, M.; Pople, J. A. *Chem. Phys. Lett*. **1990**, *166*, 281. Pople, J. A.; Krishnan, R.; Schlegel, H. B.; Binkley, J. S. *Int. J. Quantum Chem., Quantum Chem. Symp.* **1975**, *13*, 325. Handy, N. C.; Schaefer, H. F., III. *J. Chem. Phys.* **1984**, *81*, 5031.

(21) Wolinski, K.; Hilton, J. F.; Pulay, P. *J. Am. Chem. Soc.* **1990**, *112*, 8251. Dodds, J. L.; McWeeny, R.; Sadlej, A. J. *Mol. Phys.* **1980**, *41*, 1419. Ditchfield, R. *Mol. Phys.* **1974,** *27*, 789.

(22) van Eikema Hommes, N. J. R.; Clark, T. *J. Mol. Model*. to be submitted.

(23) *(23)* Clark, T.; Chandrasekhar, J. *Israel J. Chem.* **1993**, *33*, 435.

(24) Clark, T.; Alex, A.; Beck, B.; Burkhardt, F.; Chandrasekhar, J.; Gedeck, P.; Horn, A. H. C.; Hutter, M.; Martin, B.; Rauhut, G.; Sauer, W.; Schindler, T.; Steinke, T. *VAMP 8.1*; Universität Erlangen-Nürnberg, 2002.

(25) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902. Holder, A. J. In *Encyclopedia of Computational Chemistry*; Schleyer, P. v. R., Allinger, N. L., Clark, T., Gasteiger, J., Kollman, P. A., Schaefer, H. F., III., Schreiner, P. R., Eds.; Wiley: Chichester, U.K., 1998; Vol. 1 p 8.

(26) *(26)* Rauhut, G.; Clark, T.; Steinke, T. *J. Am. Chem. Soc*. **1993**, 115, 9174.

(27) Gedeck, P.; Schneider, S. *J. Photochem. Photobiol. A: Chem.* **1997**, *105*, 165.

(28) Mitscher, L. A.; Bonacci, A. C.; Sokolowski, T. D. In *Antimicrobial Agents and Chemotherapy*; Hobby, G. L., Ed.; American Society for Microbiology: Bethesda, MD, 1968; pp 78−86.

(29) Gulbis, J.; Everett, G. W. *Tetrahedron Lett.* **1976**, *32*, 913.

(30) Dos Santos, H. F.; De Almeida, W. B.; Zerner, M. C. *J. Pharm. Sci*. **1998**, *87*, 190.

(31) Duarte, H. A.; Carvalho, S.; Paniago, E. B.; Simas, A. M. *J. Pharm. Sci.* **1999**, *88*, 111.

(32) Kabsch, W. *Acta Crystallogr. A* **1978**, *34*, 827.

(33) Szilagyi, R. K.; Metz, M.; Solomon, E. I.; *J. Phys. Chem. A* **2002**, *106*, 2994.

(34) Foresman, J. B.; Keith, T. A.; Wiberg, K. B.; Snoonian, J.; Frisch M. J. *J. Phys. Chem.* **1996**, *100*, 16098.

(35) *Factor Analysis in Chemistry*; Malinowski, E. R., Ed.; Wiley: New York, 2002.

(36) Principal components were calculated using Tsar 3.3 (Oxford Molecular, 2000). The data were standardized by mean and standard deviation before calculating the principal components.

(37) Hawkins, D. M. *FIRM: University of Minnesota, School of Statistics*, http://www.stat.umn.edu/users/FIRM/

(38) Curtis, R.; Wasylischen, R. E. *Can. J. Chem.* **1991**, *69*, 834.

(39) Schneider, S.; Clark, T. manuscript in preparation.

(40) Cioni, P.; Strambini, G. B. *J. Mol. Biol.* **1996**, *29*, 789.

(41) Ricci, R. W.; Nesta, J. M. *J. Phys. Chem.* **1976**, *80*, 974.

(42) Hinrichs, W.; Kisker, C.; Duvel, M.; Muller, A.; Tovar, K.; Hillen, W.; Saenger, W. *Science* **1994**, *264*, 418.