Photophysics of 7-Hydroxytetrahydroisoquinoline-3-carboxylic Acid and Its Derivatives

Wiesław Wiczk,* Krystyna Stachowiak, Piotr Skurski, Leszek Łankiewicz, Alicja Michniewicz, and Anna Rój

Contribution from the Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland

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Abstract: The following derivatives of 7-hydroxytetrahydroisoquinoline-3-carboxylic acid {Tic(OH) [**I**]}, a conformationally restricted analogue of tyrosine, were synthesized for the purpose of photophysical studies and in order to elucidate the nature of tyrosine fluorescence and its decay: Ac-Tic(OH) [**II**], Ac-Tic(OH)-NHMe [**III**], Tic(OH)-NHMe [**IV**], Ala-Tic(OH) [**V**], Ac-Ala-Tic(OH) [**VI**], and Tic(OH)-Gly-NH_2 [**VII**]. For the simple Tic(OH) derivatives **I**−**IV**, the *N*-methylamide was found to be a more effective quencher than the acetyl group. For the peptidic derivatives **V**−**VII** the highest quenching of the fluorescence of the phenolic chromophore was observed in the case of Ala-Tic(OH). The simple Tic(OH) derivatives **I**−**IV** were also the subject of theoretical studies (MOPAC 93). The obtained thermodynamic parameters (MOPAC calculations) and the fluorescence components were discussed on the basis of the rotamer theory in order to explain the participation of an individual rotamer in the complex process of the fluorescence decay of tyrosine.

Introduction

The fluorescence of tyrosine, tyrosine derivatives, and tyrosine residue in peptides and proteins is the subject of extensive investigations. The tyrosine zwitterion and derivatives with an ionized α-carboxyl group exhibit monoexponential decay kinetics. Conversion of the α-carboxyl group to the corresponding amide or its protonation results in a complex fluorescence decay.[1−4] Several explanations for the complex fluorescence kinetics of tryptophan and tyrosine have been forwarded, including the involvement of the ^1L_a and ^1L_b states,[5−7] an excited state reaction,[8−10] and different lifetimes for the side-chain rotamers around the $C^\alpha-C^\beta$ bond.[3,11−22] In the rotamer model, multiexponential decay kinetics are proposed to be the result of the presence of a number of ground-state rotamers, some of which do not interconvert within the fluorescence time scale (typically 3−5 ns). Individual rotamers are assumed to exhibit monoexponential decay kinetics. This model, introduced by Gauduchon and Wahl,[3] suggests a charge transfer quenching between the excited aromatic chromophore (indole or phenol ring) as a donor and electrophilic units in the amino acid backbone (carbonyl or protonated amino group[15−17,20]) as the acceptor. As shown by Laws et al.,[1,21] the shorter fluorescence decay lifetime was associated with the protonated carboxyl group, while the longer lifetime was associated with ionized carboxylate. The experimental basis of the rotamer theory was the observation of Cowgill[23] that the peptide carbonyl, or the amide group is responsible for the quenching of tyrosine fluorescence in proteins and the suggestion of Tournon et al.[24] that the carbonyl groups can quench the fluorescence of aromatic rings efficiently by a charge transfer mechanism. Each rotamer has a different distance between the phenolic ring and the quenching groups (amide and/or carboxyl), which explains the differentiation in the photophysical behavior of different rotamers. Direct interaction between the peptide carbonyl or amide group and the phenol ring is responsible for the quenching of the tyrosine fluorescence in peptides and proteins as was previously suggested by Cowgill[23,25,26] and Feitelson.[27,28] By

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the use of the global and linked-function analysis Laws et al. were able to show that the rotamer model can explain the complex fluorescence decay of tyrosine analogues,[1,21] tyrosine,[20,21] and tryptophan residue[17] in small peptides. The rotamer model showed in the case of tyrosine analogues and peptides containing tyrosine or tryptophan residue that the rotamer in which the phenol or indole ring can come into closest contact with the carbonyl group has the shortest fluorescence lifetime. The analysis of these results makes it clear that the rotamer interconversion around the $C^{\alpha}-C^{\beta}$ bond in tyrosine is slower than the lifetime of the excited state. Unfortunately for tyrosine components exhibiting a single-exponential decay, Laws et al.[2] were unable to establish whether (i) the slow-exchange rotamer model is the accurate description, but the three rotamers have similar unresolved fluorescence lifetimes, or (ii) the rotamer interconversion is fast enough to average the emission. The rotamer model has also been used to explain the acrylamide quenching of tyrosine amide.[22] The charge-transfer reaction between the electrophilic unit in the amino acid backbone and the excited aromatic phenol subunit leads to a biexponential fluorescence decay of tyrosine in an acidic aqueous solution. It is also reasonable that quenching is more efficient with protonated carboxyl groups since ionization would diminish the electron-accepting capability of the carbonyl function. This phenomenon was investigated by Kungl.[29] Based on the dynamics of tyrosine and the peptide Gly-Tyr-Gly *in vacuo*, and in water, calculated with classical molecular dynamics and with stochastic computer simulations, he concluded that, since the rotamers frequently interconvert within the fluorescence lifetime of tyrosine, their contribution to the non-exponential fluorescence decay should be negligible.

Molecular dynamics simulations of the conformational dynamics of tryptophan were performed by Gordon et al.[30] They have obtained different results depending on the model used for hydrogen representation in the simulation. The rotamer model for the tryptophan zwitterion can be supported using the CHARM intramolecular potential on condition that hydrogen atoms are explicitly included in the model of tryptophan. Gordon et al.[30] have also shown that the predicted relative populations of tryptophan rotamers are not consistent with the experimental data. On the other hand, the rotamer model is not supported by the results obtained by James and Ware for homo-tryptophan.[31] In homo-tryptophan (an analogue of tryptophan with an additional methylene group separating the indole ring from the α-carbon) the $\alpha-\beta$ conformers play a less significant role by virtue of the $\beta-\gamma$ and $\gamma-\delta$ conformers. Homo-tryptophan, with a much larger number of conformations, exhibits the same qualitative behavior as tryptophan, but with quenching lifetimes somewhat larger. On the other hand, Barkley et al.,[18,19] by a global analysis of time-resolved fluorescence data of a rotationally constrained tryptophan derivative, 3-carboxy-1,2,3,4-tetrahydro-2-carboline (Tcc), revealed biexponential decay for a zwitterion and an anion. The relative amplitudes match the relative populations of the two conformers. Consequently, one lifetime was assigned to each conformer. The shorter lifetime component was associated with the conformer having the carboxylate closest to the indole ring. The ethyl ester of Tcc, the derivative with better electron acceptor properties, has both lifetimes shorter.[18,19] This suggests that the intramolecular electron transfer may be an important mode of fluorescence quenching.

The question remains unanswered as to whether the two fluorescence lifetimes of simple tyrosine derivatives in a solution are due to ground-state rotamers. Accordinglly, we decided to synthesize a tyrosine derivative—7-hydroxytetrahydro-isoquinoline-3-carboxylic acid (Tic(OH))—in which rotations about the $C^{\alpha}-C^{\beta}$ and $C^{\beta}-C^{\gamma}$ bonds are restricted by incorporating the amino group into the six-membered isoquinoline ring. This constraint limits the number of conformers available in the molecule greatly affecting the electronic structure of the chromophore. In this paper we present the fluorescence decay results for derivatives of the modified tyrosine—Tic(OH).

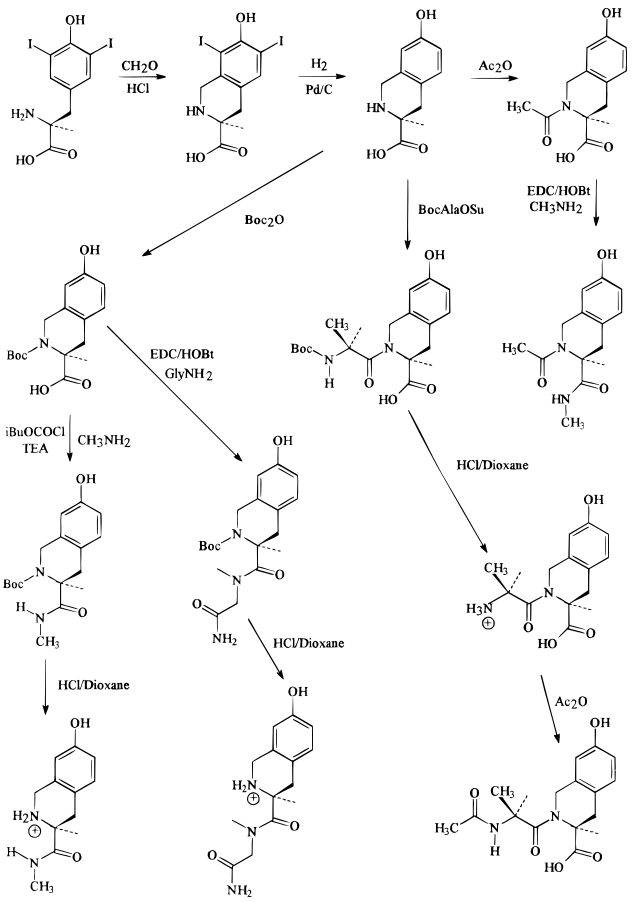

Figure 1. Synthetic scheme.

Experimental Methods

Synthetic Methods. The following derivatives of 7-hydroxytetrahydroisoquinoline-3-carboxylic acid (H-Tic(OH)-OH) were obtained: Ac-Tic(OH)-OH [**II**], Ac-Tic(OH)-NHMe [**III**], H-Tic(OH)-NHMe [**IV**], H-Ala-Tic(OH)-OH [**V**], Ac-Ala-Tic(OH)-OH [**VI**], H-Tic(OH)-GlyNH$_2$ [**VII**]. The methodology of Tic(OH) and preparation of its derivatives are presented in the scheme in Figure 1. Here we describe only briefly the major steps. 1,2,3,4-Tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid [H-Tic(OH)-OH (**I**)] was synthesized by a Pictet-Spengler reaction of 3,5-diiodo-L-tyrosine with formaldehyde, followed by catalytic hydrogenation (removal of iodine).[32] 3,5-Diiodo-L-tyrosine was purchased from Fluka AG. The parent compound [**I**] was *N*-acylated by di-*tert*-butyl dicarbonate, *N*-*tert*-butyloxycarbonylalanine *N*-hydroxysuccinimide ester, or acetic anhydride giving Boc-Tic(OH)-OH, Boc-Ala-Tic-(OH)-OH, or Ac-Tic(OH)-OH [**II**], respectively. Boc-Tic(OH)-OH was used as a substrate for the synthesis of H-Tic(OH)-NHMe [**IV**] and H-Tic(OH)-Gly-NH$_2$ [**VII**]. For this purpose Boc-Tic(OH)-OH was coupled with methylamine (33% CH_3NH_2 in EtOH) in the presence of isobutyl chloroformate or with glycine amide using the 1-ethyl-3-(3-(dimethylamino)propyl)carbodimide hydrochloride/*N*-hydroxybenzotriazole [EDC/HOBT] method fol-

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lowed by acidolitic removal of Boc protection. Compounds **V** [H-Ala-Tic-(OH)-OH] and **VI** [Ac-Ala-Tic(OH)-OH] were obtained from Boc-Ala-Tic-(OH)-OH after deprotection of the *tert*-butyloxycarbonyl group and *N*-acetylation of the alanyl residue. The *tert*-butyloxycarbonyl group as a temporary protection was removed in each of the cases mentioned by the action of a solution of 4 N HCl in dioxane. Ac-Tic(OH)-NHMe [**III**] was obtained by the reaction of Ac-Tic(OH)-OH [**II**] with methylamine using EDC/HOBT as the coupling reagent.

After purification by means of column chromatography, the chemical homogeneity of synthesized compounds was checked by ^{1}H-NMR spectra, analytical reversed HPLC (a linear 60 min gradient from 0 to 80% CH_3CN in 0.1% TFA in H_2O at a flow rate of 1 mL/min on a Kromasil C-8 column, 4.6 × 250 mm), 5 μm and mass spectrometry (FDMS, FAB MS).

Spectroscopic Measurements. Fluorescence decays were collected by the time-resolved single photon counting technique on an Edinburgh Analytical Instrument type CD-900 fluorimeter interfaced with an IBM PC AT. The excitation source was a flash lamp filled with 0.5 atm of hydrogen operated at 40 kHz with about 6.5 kV across a 1 mm electrode gap. The half width of the instrument response was 1.2 ns. The excitation (270 nm) and emission wavelength (310 nm) were selected by means of monochromators (about 10 nm band width).

Fluorescence decays from sample and the reference (Ludox, observation wavelength 310 nm) were measured to 4×10^4 counts in the peak. The counting rate did not exceed 2% of the lamp repetition rate. The decay curves were stored in 1024 channels at 0.054 ns/channel. Fluorescence decay data were fitted by iterative convolution to the sum of exponents:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \qquad (1)$$

where τ_i is the decay time of the ith component and α_i is its pre-exponential factor.

The adequacy of the exponential decay fitting was judged by inspection of the plots of weighted residuals, the statistical parameters χ^2, and the shape of the autocorrelation function of the weighted residuals and serial variance ratio (SVR).

The steady-state spectra were obtained on a Perkin-Elmer LS-50B spectrofluorimeter with a 2.5 nm band width for excitation and emission. The excitation wavelength was 270 nm. The quantum yields were measured relative to the value of 0.14 for tyrosine in water at room temperature.[33] A sample concentration was about 5×10^{-5} M in the steady-state measurement and 1×10^{-4} M in the time-resolved experiments. All measurements were made in double-deionized water, pH 7.0, at room temperature. The pH of the solutions was adjusted to 7.0, directly in cuvette, with sodium hydroxide solution (Suprapur, Merck) using combination pH electrode (ultra-thin, Aldrich) and pHM-52 apparatus (Radiometer) just before measurements. Then the cuvette was closed with a cap to avoid diffusion of CO_2 to the solution. We did not use any buffer because of a possibility of an additional fluorescence quenching related to a hydrogen bond formation between the phenolic hydroxyl group and a buffer.[34−36]

Absorption spectra were measured on a Beckman model DU-600 spectrophotometer.

Computational Details. Quantum mechanics calculations were carried out at the semiempirical PM3[37] level employing the MOPAC 93[38] package.

Unconstrained geometry optimization of the molecules studied was performed using the EF[39] optimization procedure. The final norm of the energy gradient was always less than 0.1 kcal/mol. After each optimization was completed, energy Hessian was calculated and checked for positive definiteness, in order to assess whether the structures were true minima. The geometries and energies of the molecules in the transition states were optimized in two consecutive steps. First, the SADDLE calculations[40,41] led to the approximation of the saddle point structure. Next, the gradient minimization by the TS procedure[39] for this structure was carried out. After the transition state was optimized, the corresponding energy Hessian always had one and only one negative eigenvalue that indicated a first order transition state.

In order to study the solvent effect, the Conductor-Like Screening Model (COSMO)[42] incorporated into the MOPAC program package was applied both for minimum structures and saddle point configurations. The dielectric constant of water was taken as 78.4 (at 298 K). It should be noted that such formalism provides only electrostatic contribution to the free enthalpy of solvation. One should note that the same ideal gas expressions for the transitional and rotational terms in thermal correction for both gaseous and aqueous phase were used. In the past the approximation turned out to be sufficient.[43] The only difference is in the vibrational term which results from different frequencies for gaseous and aqueous systems.

All calculations were carried out using a Hewett-Packard 735 Apollo workstation.

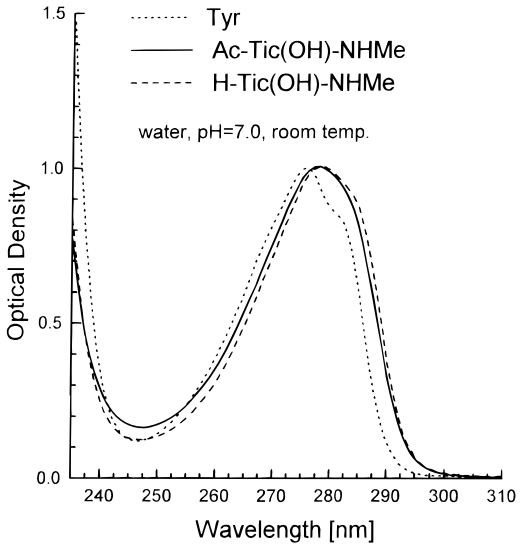

Figure 2. Absorption spectra of Tyr, Ac-Tic(OH)-NHMe, and H-Tic (OH)-NHMe measured in water at room temperature, at pH 7.0.

Results and Discussion

Absorbance. The absorption spectra of Tic(OH) and its derivatives are similar to the spectra of the appropiate derivatives of tyrosine by means of the shape and position of characteristic bands (Figure 2). The respective spectra of the derivatives of Tic(OH) are only somewhat broader and slightly red-shifted. The maximum of the longwave absorption spectrum of Tyr in water is around 275 nm, whereas the respective absorption spectrum of the Tic(OH) derivative with an unblocked amino group has its maximum around 279 nm. Moreover the absorption spectra of Tic(OH) derivatives with the blocked amino group displayed an additional red-shift (1 nm) of the maximum. The modification of the carboxylic group did not influence the position of the maximum of the absorption spectra of derivatives of the cyclic analogue of tyrosine or the shape of the band. In the case of Tic(OH) and its derivatives less structured absorption spectra than for tyrosine were observed. The red-shift observed for the absorption spectra of *N*-acetylated

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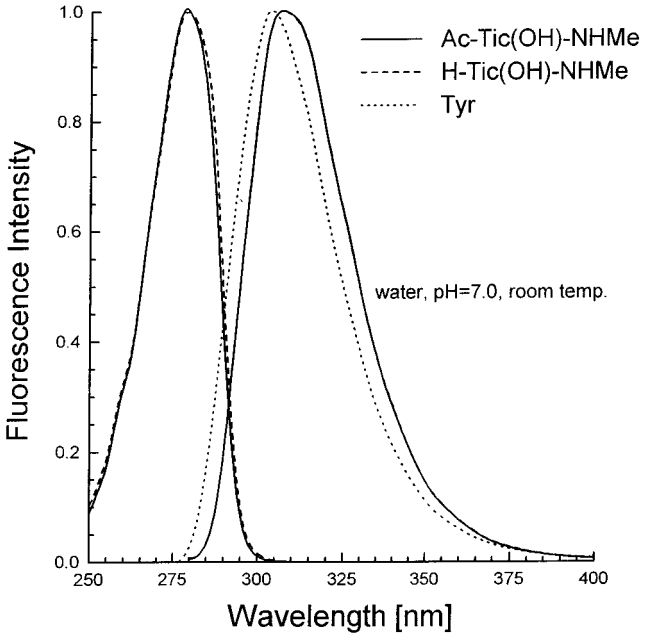

Figure 3. Emission and fluorescence excitation spectra of Tyr, Ac-Tic(OH)-NHMe, and H-Tic (OH)-NHMe measured in water at room temperature, at pH 7.0.

derivatives of Tic(OH) in comparison with that of the non-acetylated derivatives indicates a certain interaction of the acetyl group with the phenolic chromophore (hyperresonance), despite the separation of these two moieties by one methylene group ($-CH_2-$). The shift of the maximum of the absorption spectra of the cyclic analogue of tyrosine is probably due to a change in the conformation of a semirigid six-membered ring which is the effect of a different characteristic of isoquinoline nitrogen (isoquinoline contains a secondary amino group and its acetylation results in a more rigid flat conformation of the ring). Similar changes and relations were observed for fluorescence excitation spectra of Tic(OH) derivatives (Figure 3).

Steady-State Fluorescence. The steady-state emission spectra of the zwitterion of Tic(OH) and its derivatives are broad and featureless (Figure 3). The emission maximum occurs at 307 nm for Tic(OH) and all other studied derivatives. All emission spectra of Tic(OH) derivatives are red-shifted as compared with the tyrosine spectra (λ_{max} = 303 nm) and possesses a greater half-width.

Contrary to the increase of the quantum yield observed for the rigid analogue of Trp (3-carboxy-1,2,3,4-tetrahydro-2-carboline),[18,19] the fluorescence quantum yield of the Tic(OH) zwitterion in water at pH 7.0 is slightly lower ($\phi = 0.096$) than that of tyrosine in water ($\phi = 0.14$). The fluorescence quantum yields of Tic(OH) derivatives are in the range of 0.095 for Ac-Tic(OH)-OH to 0.023 for the most quenched derivative, H-Ala-Tic(OH)-OH (Table 1).

Analyzing the data collected in Table 1, it is clear that *N*-acetylation did not change the fluorescence of parent compounds significantly. The same phenomenon was observed for tyrosine and acetyltyrosine. The decay times (τ) obtained by Laws et al.[2] for Ac-Tyr and Tyr were 3.6 and 3.76 ns, respectively, whereas Gauduchon and Wahl[4] obtained shorter ones values—3.2 ns for Ac-Tyr and 3.38 ns for Tyr. The fluorescence quenching increases a little when *N*-acetylation is combined with modification of the carboxylate (amidation). On the other hand, introduction of the carboxyamide to the compound without *N*-protection gave a high, about 20%, increase of the quenching. The amidation of the peptide Tic-(OH)-Gly-OH also improved the fluorescence quenching (the quantum yield for Tic(OH)-Gly-NH_2, $\phi = 0.065$), but the effect of this transformation is not as strong as observed for Tic(OH)-NHMe. This change in the quenching efficiency is distinctly an effect of the distance between the chromophores (the phenol and the amide)—the separation of the chromophores by the one additional $-CH_2-$ group (as in Tic(OH)-Gly-NH_2) decreased the quenching efficiency. A similar dependence of the fluorescence quenching from the distance between chromophores was observed by Cowgill[44] for derivatives of tyrosine (Tyr-Gly and Tyr-Gly-Gly).

Table 1. Fluorescence Quantum Yields (ϕ) and Lifetimes (τ) of 7-Hydroxytetrahydroisoquinoline-3-carboxylic Acid (H-Tic(OH)-OH) Derivatives in Water (pH 7.0 at Room Temperature)

no.[a]	compd	ϕ	τ [ns]	χ^2_R [b]
I	H-Tic(OH)-OH	0.096	2.92	1.04
II	Ac-Tic(OH)-OH	0.095	2.88	1.03
III	Ac-Tic(OH)-NHMe	0.091	2.82	0.99
IV	H-Tic(OH)-NHMe	0.077	2.23	1.12
V	H-Ala-Tic(OH)-OH	0.023	0.82	1.10
VI	Ac-Ala-Tic(OH)-OH	0.060	1.87	1.11
VI	H-Tic(OH)-Gly-NH_2	0.065	2.10	1.10
	H-Tyr-OH	0.14	3.42	0.99

[a] Compound number in the reaction scheme. [b] Goodness of fit.

A significant influence on the fluorescence quenching was observed for the *N*-acylation of the cyclic analogue of tyrosine (Tic(OH)) by alanine and acetylalanine . The quantum yield for Ac-Ala-Tic(OH)-OH, the compound containing two amide groups, $\phi = 0.060$, is comparable with the data for Tic(OH)-Gly-NH_2 ($\phi = 0.065$)—the compound containing also two amide moieties. The quenching efficiency of the fluorescence of Ala-Tic(OH)-OH is about 75% of that observed for the *N*-acylated derivative (Ac-Ala-Tic(OH)-OH). The influence of the protonation of the amino group on the quenching efficiency of the fluorescence of the phenolic chromophore by carbonyl group can be analyzed by comparing the fluorescence data (quantum yield) for Tic(OH)-NHMe ($\phi = 0.077$) and Ac-Tic(OH)-NHMe ($\phi = 0.091$). These parameters explicitly indicate the important role of the amino group in the neighborhood of the quencher (carbonyl) in the quenching process of the fluorescence of the aromatic amino acids. The same direction in the effect of the acetylation of the amino group was observed for tyrosine amide, e.g. the longer fluorescence lifetime for Ac-Tyr-NH_2 than for Tyr-NH_2 (studies performed by Laws et al.[2] and by Gauduchon and Wahl[4]).

The quenching efficiency of the ammonium group in tryptophan and tyrosine has been discussed in terms of the electrostatic effect on the quenching efficiency of the carbonyl group and not attributed directly to a straight quenching process.[3,5,23,44] The electrostatic field produced by the ammonium group in the derivatives of tyrosine with protected carboxylate can change the polarization of the surrounding water molecules, which will lead to higher polarization of the carbonyl group and increasing electron—acceptor property of the amide group or can strongly increase hydration of the amide bond, facilitating electron transfer. Low quantum yield of the fluorescence of tyrosine with ionized hydroxyl and its appearance at a longer wavelength (λ_{max} = 345 nm)[45] can cause an apparent quenching effect of tyrosine fluorescence. The data of the fluorescence quenching obtained for Tic(OH)-NHMe and Ala-Tic(OH)-NHMe and Tic(OH) derivatives with the acety-

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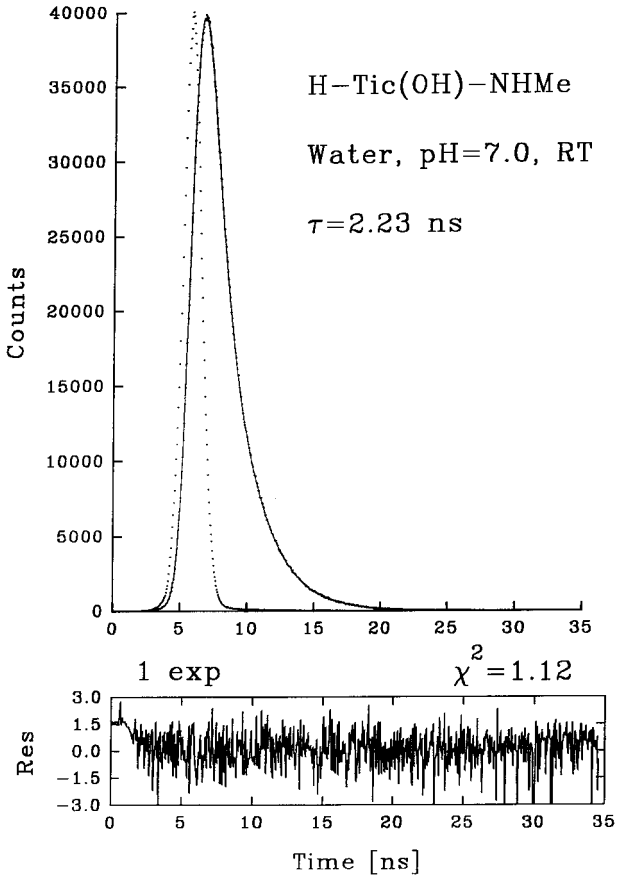

Figure 4. Measured (doted line) and fitted decay curve (solid line) vs time (left set of points—lamp profile) for H-Tic(OH)-NHMe. The weighted residuals are plotted at the lower part of the figure.

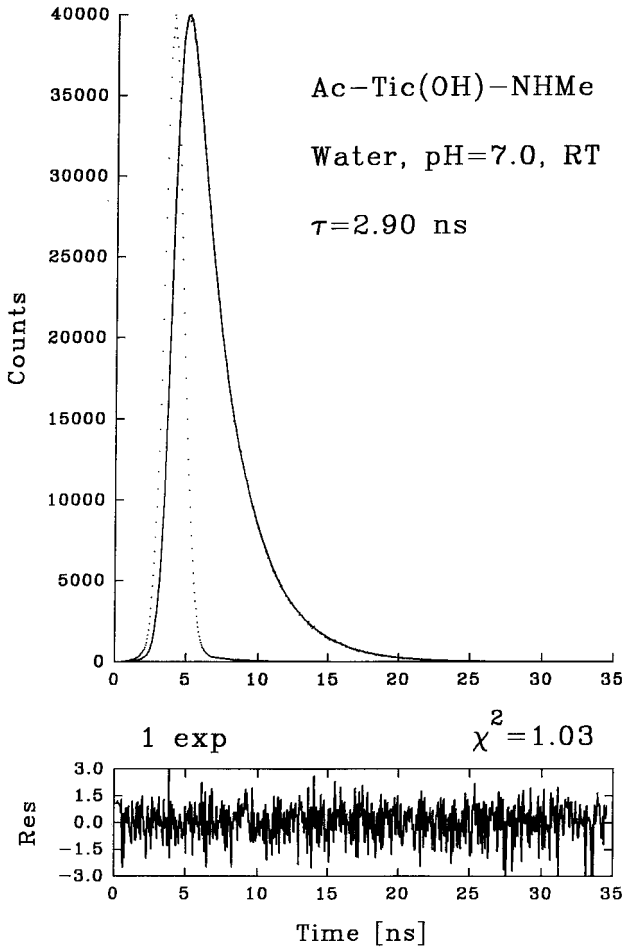

Figure 5. Measured (doted line) and fitted decay curve (solid line) vs time (left set of points—lamp profile) for Ac-Tic(OH)-NHMe. The weighted residuals are plotted at the lower part of the figure.

lated amino group proved the strong influence of the protonated amino group on the quenching properties of the amide group.

Fluorescence Decays. Fluorescence decays of all derivatives of Tic(OH) were measured up to 4×10^4 counts per peak to obtain the highest possible accuracy of the parameters. All measured decays are monoexponential (Table 1, Figures 4 and 5) with the statistical parameter χ^2_R in the range 0.99−1.12 and did not improve even when biexponential decay fitting was applied. The values of weighted residuals and an autocorrelation function do not display any systematic deviations and they are around zero. There is a direct correlation between the fluorescence decay times and the quantum yields for all compounds studied (Table 1)—the derivatives with shorter decay times possess lower quantum yields. Fluorescence rate constants (k_f) calculated using the equation $k_f = \phi/\tau$ are in the range of from 3.3×10^7 s^{-1} for Ac-Tic(OH)-OH to 2.8×10^7 s^{-1} for H-Ala-Tic(OH)-OH, and these values are close to the rate constant calculated for tyrosine (3.8×10^7 s^{-1}).[2,32] The incorporation of the substituents of the amino group and carboxylate does not change the fluorescence rate constants in the cyclic tyrosine derivatives significantly and the lower values of the quantum yields and the shorter decay times are obviously the result of the fluorescence quenching caused by the substituents.

Theoretical Calculations. Theoretical calculations of the lowest energy conformers of Tic(OH) and derivatives of Tic-(OH) with the modified amino group and/or carboxylate were performed using the PM3 method (the derivatives containing the peptide bond were not subjects of these calculations). The calculations were carried out in (i) a gaseous phase and (ii) including water as a solvent. The calculated thermodynamic quantities of the different conformations of Tic(OH) derivatives and the transition states between the conformations are given in Tables 2 and 3. Although only two minimum energy structures could be found, Figures 6 and 7 illustrate the two conformers viewed down the plane of the phenol ring. For compounds with a free amino group, these structures correspond to the well-characterized half-chair forms of cyclohexane. Similar structures were found by Barkley et al.[18,19] for the rotationally constrained tryptophan derivatives. For the compounds with blocked amino groups the lowest energy conformation differs from the characteristic half-chair form because of the planarity of the amide bond formed after acetylation (see Figure 7).

The T type conformation, named according to the paper by Barkley et al.,[18] always has a lower gaseous-phase energy than the T′ type, with the exception of the cation for which the T′ type is the lowest energy conformation. The heat of formation of both conformers and the transition state for all studied derivatives of Tic(OH) are also presented in Tables 2 and 3. These data were used to calculate the population of the particular conformers based on the stationary-state theory using the following equations:

$$\Delta G = \Delta H - T\Delta S \tag{2}$$

$$\Delta G = -RT \ln K \tag{3}$$

and the assumption that the sum of all conformers is normalized to 1 ($p_T + p_{T'} = 1$).

The differences in the heat formation for both conformers of all studied Tic(OH) derivatives possessing a charge are small (about 1 kcal/mol) except for the disubstituted derivative (Ac-

Table 2. Thermodynamic Parameters and Equilibrium Constants between T and T′ conformers for Tic(OH) Derivatives in the Gas Phase

thermodynamic parameter	conformer	$^{+}H_2$TicNHMe	AcTicNHMe	AcTicCOO^{-}	$^{+}H_2$TicCOO^{-}
HOF[a] (kcal/mol)	T	−90.377	−105.828	−186.255	−82.097
	T′	−88.094	−105.459	−185.325	−77.783
	TS[c]	96.276	−99.293	−180.304	−71.348
S[b] (cal/(mol·K))	T	166.098	136.115	139.959	107.018
	T′	166.028	133.509	140.104	109.402
	TS	118.202	135.632	123.433	107.991
population	T	0.020	0.876	0.184	0.998
	T′	0.980	0.124	0.816	0.002
$K_{T \leftrightharpoons T'}$		0.021	6.91	0.225	435.30

[a] HOF = heat of formation. [b] S = entropy. [c] Transition state.

Table 3. Thermodynamic Parameters and Equilibrium Constants between T and T′ Conformers for Tic(OH) Derivatives with Solvation

thermodynamic parameter	conformer	$^{+}H_2$TicNHMe	AcTicNHMe	AcTicCOO^{-}	$^{+}H_2$TicCOO^{-}
HOF[a] (kcal/mol)	T	20.468	−130.750	−282.896	−136.609
	T′	20.016	−127.221	−281.083	−137.320
	TS[c]	23.469	−125.282	−278.013	−133.109
S[b] (cal/(mol·K))	T	100.514	109.032	100.274	95.254
	T′	102.895	111.397	104.844	98.964
	TS	102.010	114.748	104.913	97.84
population	T	0.124	0.9915	0.681	0.0446
	T′	0.876	0.0085	0.319	0.9554
$K_{T \leftrightharpoons T'}$		0.141	116.96	2.13	0.0467

[a] HOF = heat of formation. [b] S = entropy. [c] Transition state.

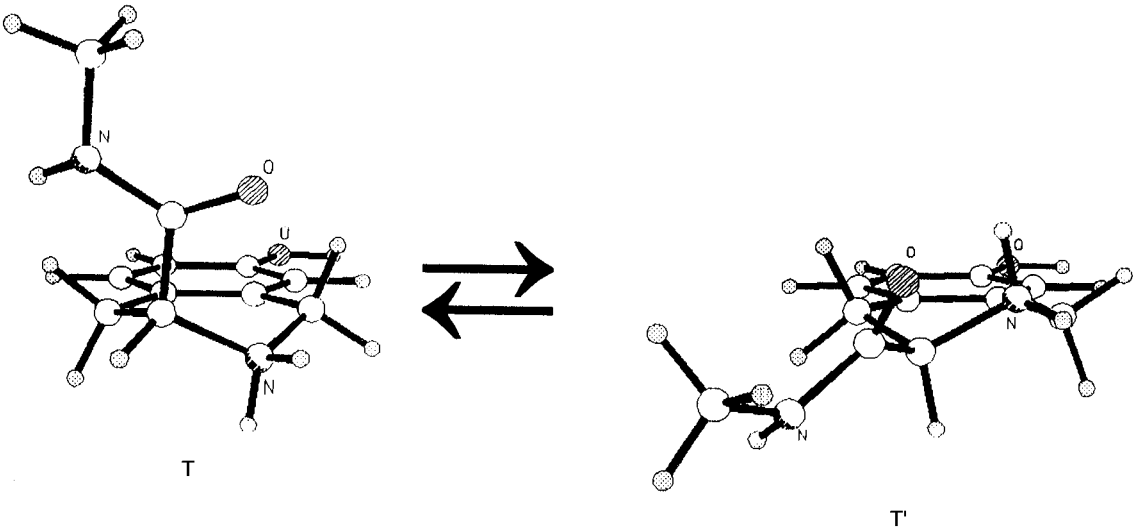

Figure 6. T and T′ conformers of the protonated form of Tic(OH)-NHMe.

Tic(OH)-NHMe) for which the difference is higher: 4.3 kcal/mol. We also found small differences between entropies of the individual conformers—about 0.5 kcal/mol. Again, the higher difference was found in the case of Ac-Tic(OH)-NHMe—about 2 kcal/mol. The value of the energy barrier of the conformers' conversion, calculated as the difference between the formation heat of the saddle point and the energy of the lower energy conformer, is about 6 kcal/mol which is comparable with the data obtained by Barkley et al.[18,19,46] for the rotationally constrained tryptophan derivatives and that found for the inversion of 1,4−disubstituted cyclohexanes.[47] The parent compound, Tic(OH), was exceptional, in terms of the energy barrier of the conformers' interconversion. The energy barrier was about 11 kcal/mol. The equilibrium constants and populations of the particular conformers collected in Table 2 (calculated on the basis of the obtained thermodynamical parameters) revealed that the derivatives with the free amino group exist as practically one conformer (contribution of the second is less than 2%)—it is the T′ type for Tic(OH)-NHMe and the T type for Tic(OH) (zwitterion). On the other hand, the derivatives with the acetylated amino group exist predominantly in the T type conformation (more than 80% of the whole population of conformers).

A different situation occurs when the solvation is included in our calculations. The lowest energy conformers for the derivatives with the free amino group are the T′ type regardless of the status of the carboxylate (free or amidated). On the contrary, the lowest energy conformers found for the acetylated derivatives exist in the T type conformation, but similar to the derivatives with the free amino group the state of the carboxylic group does not influence the ring conformation. The enthalpies of the formation calculated for individual conformers (solvation included) differ significantly less than the ones obtained in the gaseous phase, especially for the compounds possessing charges. The compounds with the free amino group (positively charged) have a difference in the enthalpies of around 0.7 kcal/mol,

(46) Yu, H-T.; Vela, M. A.; Fronczek, F. R.; McLaughlin, M. L.; Barkley, M. D. *J. Amer. Chem. Soc.* **1995**, *117*, 348−357.

(47) Jesen, F. R.; Bushweller, C. H. *J. Amer. Chem. Soc.* **1969**, *91*, 5774−5782.

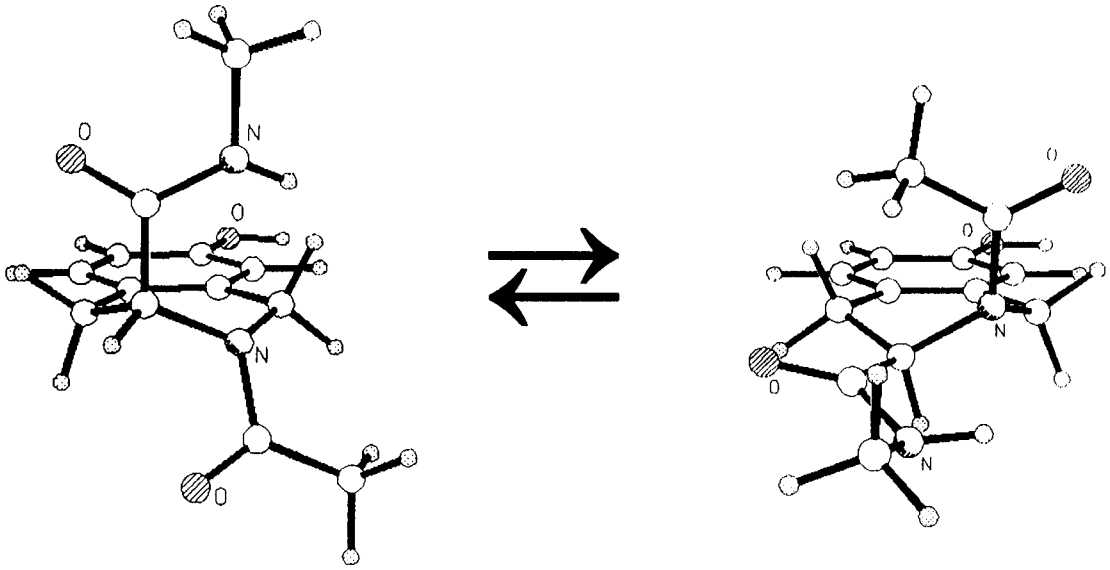

Figure 7. T and T′ conformers of Ac-Tic(OH)-NHMe.

whereas for the compounds with free carboxylate (negatively charged) the difference in the entalpies is slightly higher (1.2 kcal/mol). A more significant difference in the enthalpies of conformer formation than in the gaseous phase (almost 1 order of magnitude) was observed for Ac-Tic(OH)-NHMe ($\Delta HOF_{pg} = 0.37$ kcal/mol, $\Delta HOF_{sol} = 3.2$ kcal/mol). A more significant difference was also found for the entropy of both conformers when the solvation was taken into account during the calculations of all Tic(OH) derivatives (2.5−4.5 kcal/(mol K)). On the other hand, the significant decrease in the enthalpy of activation of the ring inversion was discovered for the derivatives with the free amino group ($\Delta H^{\#} \sim 3$ kcal/mol), while for the compounds with the acetylated amino group the decrease was smaller ($\Delta H^{\#} \sim 5.5$ kcal/mol).

The decrease of the enthalpy of activation of the ring inversion and a higher difference in entropy of the particular conformers and the transition state obtained in the experiments, taking into account the solvation effect, do not permit neglect of the entropy component in the calculations of the equilibrium constant and rate of ring inversion possible in the gaseous phase (especially at room or higher temperature). The free energy of activation of the ring inversion, calculated with the solvation effect included, for all studied derivatives was in the range 3.2−3.8 kcal/mol except for the parent compound, Tic(OH), for which the energy is higher (4.5 kcal/mol). These values are generally lower than the energy calculated in the gaseous phase. The rate constants of the conversion through the energy barrier during the ring inversion are calculated on the basis of the transition-state theory using the equation

$$k_{T\rightarrow T} = \kappa\left(\frac{kT}{h}\right)\exp\left(-\frac{\Delta G^{\#}}{RT}\right) \qquad (4)$$

where k is Boltzmann constant, T is the absolute temperature, h is Planck's constant, and $G^{\#}$ are the free energies of activation, these being collected in Table 4. It is difficult to assign a value to the transmission coefficient, κ, which incorporates all correction factors and uncertainties. We chose, as Barkley et al.[19] did, $\kappa = 0.4$, and for this value the calculated average lifetimes of T and T′ conformers are tens of picoseconds. The T type conformer of Ac-Tic(OH)-NHMe and the T′ type of Tic(OH) have the longest lifetimes (200 and 860 ps, respectively). The rate constant of the ring conversion obtained for the parent

Table 4. Ring-Inversion Rate Constants of the T ⇆ T′ interconversion in Water Calculated Based on the Data of Tables 2 and 3

rate constant (lifetime)	$^{+}H_2$TicNHMe	AcTicNHMe	AcTicCOO^{-}	$^{+}H_2$TicCOO^{-}
$10^{-9}k_{T\rightarrow T'}$ [s^{-1}]	53.9	4.44	6.80	25.0
($10^{10}\tau$ [s])	(0.19)	(2.25)	(1.47)	(0.40)
$10^{-9}k_{T'\rightarrow T}$ [s^{-1}]	11.5	506	14.5	1.16
($10^{10}\tau$ [s])	(0.87)	(0.02)	(0.69)	(8.57)

Tic(OH) is almost 2 times bigger than that found by Yu et al.[46] for 3-carboxy-1,2,3,4-tetrahydro-2-carboline, whereas the rate constants for all other studied Tic(OH) derivatives are even bigger—more than 1 order of magnitude. According to Yu et al.[46] the energy barrier of the ring inversion in the excited state does not change very much—it increases by 0.4−0.9 kcal/mol. Because of the dynamic equilibrium in the excited state, the ring inversion can occur several times during the fluorescence lifetime (around 3 ns) of the excited state of the Tic(OH) derivatives. For these reasons, the measured fluorescence decay is the averaged decays of both conformers.

Conclusions

In the rotamer model, three chemically distinct environments exist for the phenol ring about the $C^{\alpha}-C^{\beta}$ bond and each rotamer has its own associated decay constant, and the relative weighing of the amplitudes is set by the ground-state rotamer populations. The rotamers having different lifetimes have also been suggested as the cause of multiexponential decay of tryptophan,[11−16] rotationally constrained tryptophan,[18,19,46] and tryptophan in protein crystals.[48] The described data of the fluorescence decay measurements for Tic(OH) derivatives have confirmed only partially the rotamer model of quenching. Theoretical calculations showed that Tic(OH) can adopt two half-chair conformations in solution. The lowest energy conformation for Tic(OH) derivatives with the free amino group is the T′ type, whereas for the derivatives with the acetylated amino group it is the T type. Including hydration (within the framework of the COSMO approach) this results in lowering the barrier to ring inversion to about 3.5 kcal/mol. The contribution of the second conformer (the T type for the derivatives with the free amino group and

(48) Dahms, T. E. S.; Wilis, K. J.; Szabo, A. G. *J. Amer. Chem. Soc.* **1995**, *117*, 2321−2326.

T′ for the acetylated group) in the whole population of rotamers is lower than 20%, except Ac-Tic(OH)-NHMe for which the contribution of the lower populated rotamer is less than 1%. The obtained rate constant of the ring conversion for the parent Tic(OH) is almost two times bigger than that found by Barkley et al.[19,46] for 3-carboxy-1,2,3,4−tetrahydro-2-carboline and by Yu et al.[46] for 1,2,3,4-tetrahydro-2-carboline, whereas the rate constants for all other studied Tic(OH) derivatives are even greater—more than one order of magnitude. So, the average fluorescence lifetimes of all conformers in the solution are in the range of hundred of picoseconds. The apparent monoexponential decays imply that either the conformers interconvert in the excited state rapidly, compared to the fluorescence time scale (about 3 ns), or the lifetimes of the two conformers are similar.

The results of the fluorescence decays obtained for Tic(OH) derivatives provide new suggestions regarding the quenching mechanism of the fluorescence of tyrosine analogues. The fluorescence quenching by *N*-terminal or *C*-terminal protecting groups depends on the distance between the chromophore (phenolic ring) and a quenching moiety (acetyl, *N*-methylamide) and their direct surroundings. A thorough analysis of the dependence of the effectiveness of the quenching groups from a distance between the chromophore and the quencher led us to the conclusion that the previously suggested charge-transfer mechanism[3,24] is highly probable (the weak quenching of tyrosine fluorescence by the acetyl in Ac-Tyr and the strong quenching by the acetyl in the case of Ac-βTyr(Me)[49]). The results obtained in our investigations partially explained the influence of the NH_3^+ group on the quenching of tyrosine fluorescence. This group is not directly involved in the quenching process, which is consistent with previous suggestions,[3,44] but it influences the quenching ability of the amide group through its close space location, *via* enhancement of hydration of the carbonyl group, and has strict spatial requirements. On the basis of the above-mentioned statement about the role of the amino group in the quenching, one can easily explain the more efficient quenching of the fluorescence of the amides: Tic(OH)-NHMe and Ala-Tic(OH)-NHMe, compounds containing the quencher and the free amino group (see Table 1). However, on the basis of these data no satisfactory explanation for the mechanism of the interaction between the NH_3^+ group and the amide bond could be found. The effect of the charge field of the NH_3^+ group and related to this better hydration of the amide bond suggested by Cowgill[44] seems to be a reasonably good explanation for the mechanism of the NH_3^+ group action. A full explanation of the fluorescence decay mechanism for tyrosine analogues requires further study, especially studies of model compounds with different conformational freedom of the chromophore and protecting groups at *N*- and *C*-termini.

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