HYPERICIN AND ITS PERYLENE QUINONE ANALOGS: PROBING STRUCTURE, DYNAMICS, AND INTERACTIONS WITH THE ENVIRONMENT

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I. INTRODUCTION: EXCITED-STATE INTRAMOLECULAR H-ATOM TRANSFER IN HYPERICIN-
LIKE PERYLENE QUINONES

Hypericin and hypocrellin (Fig. 1.1.) are naturally occurring perylene quinones that have generated great interest recently owing to their biological activity [3–22], in particular, their light-induced biological activity [23–27]. The importance of light for their function has motivated our study of the photophysics of hypericin and its analogs [28–38]. By means of H/D substitution, investigation of O-methylated analogs, and complementary studies using both transient absorption and fluorescence upconversion spectroscopies, we have argued that the major primary photophysical process in hypericin and hypocrellin A in organic solvents is excited-state hydrogen atom transfer. We have suggested that the labile protons resulting from the intramolecular hydrogen-atom transfer reactions may be important for understanding the light-induced biological activity of hypericin and hypocrellin A. Notably, hypericin and hypocrellin A acidify their surroundings upon light absorption [39–41]. The role of photogenerated protons takes on significance in the context of the growing body of literature implicating changes in pH with inhibition of virus replication [42], antitumor activity [43, 44], and apoptosis (programmed cell death) [45–47].

Our argument for intramolecular excited-state H-Atom transfer in hypericin is as follows. The deshydroxy analog of hypericin, mesonaphthobianthrone (Fig. 1.1), is nonfluorescent, except in strong acids [29, 38] (e.g., sulfuric acid), where it produces a fluorescence spectrum that has nearly the same shape as that of hypericin in alcohols (Fig. 1.2). These results demonstrate the importance of a “protonated” carbonyl group for producing hypericin-like fluorescence. The hypericin emission spectrum grows in on a 6–12-ps time scale in all solvents, except in sulfuric acid where it is instantaneous. Thus the rise time for the appearance of the hypericin emission is taken as evidence for an excited-state H-atom transfer [29, 48]. Confirming this interpretation are the fluorescence upconversion measurements of hypericin and O-methyl hypericin analogs [38, 48, 49], which are incapable of executing intramolecular excited-state
Figure 1.1. Hypericin (normal form, double tautomer, and monomutomer), mesonaphthobianthrone, hypocrellin A (normal form, double tautomer, and monomutomer), hypocrellin B, hypomycin B [1], and calphostin C [2].
H-atom-transfer reactions. Hypericin exhibits a rise in its fluorescence signal, whereas the methylated derivatives do not. Rising components of \(/C_{24}10\) ps, attributable to intramolecular H-atom transfer are clearly observed in the fluorescence upconversion traces of both hypericin and hypocrellin A (Figs. 1.3 and 1.4, respectively). For simplicity, in the rest of our discussion, we refer to this shorter-lived component as the ‘10-ps component.’ The amplitude of the rising component is emission wavelength dependent and occurs on the blue edge of the emission spectra. The clear and complementary observation in fluorescence of the \(~10\)-ps component in both hypericin and hypocrellin A is a crucial link in providing a unified model of the hypericin and

Figure 1.2. Comparison of the fluorescence excitation and emission spectra of hypericin (a) and hypocrellin (b) in a 1:1 ethanol/methanol mixture at room temperature. The excitation spectra were measured at 295 K (solid line) and at 77 K (dashed line). The excitation spectra were monitored at 650 nm at 295 K and at 620 nm at 77 K.
hypocrellin photophysics [34]. In addition to the 10-ps component in hypocrellin A, there is a longer-lived transient, also assigned to H-atom transfer, whose time constant ranges from 50 to 250 ps in the solvents we have studied [33].

The H-atom transfer rate in hypericin has no significant solvent dependence [29]. The H-atom transfer rate for the longer component in hypocrellin A has a strong dependence on the bulk viscosity [33]. The time constant for H-atom

**Figure 1.3.** (Top) Fluorescence upconversion transient for hypericin in ethanol at $\lambda_{em} = 576$ nm. The fit curve is described by the following equation (with background subtracted): $F(t) = -0.21 \exp(-t/6.5 \text{ ps}) + 1.00 \exp(-t/\infty)$. (Bottom) At $\lambda_{em} = 653$ nm, however, there is no rising component in the fluorescence trace. Similar behavior is observed for hypocrellin A (Fig. 1.4). The excitation wavelength was the second harmonic of our unamplified Ti:sapphire oscillator, 414 nm. The panel below the kinetic trace displays the residuals between the fit and the data.
transfer in hypocrellin ranges from 50 to 250 ps in the solvents we have studied. The viscosity dependence is remarkable not only because it is absent in hypericin but also because it is exceedingly well described by a bulk effect and does not require specific consideration of the structural aspects of the solvents, which vary considerably. It is often the case that trends are followed only for solvents of a given kind, for example, alkane or alcohols, primary alcohol or higher degree alcohol, and hydrogen bonding or nonhydrogen bonding. NMR results cited below lead us to suggest that the viscosity dependence on the excited-state transfer process is a consequence of the coupling of the H-atom transfer to conformational changes of the seven-membered ring in hypocrellin [50]. Hypericin does not have such a ring and does not exhibit such viscosity-dependent effects.

We now consider two objections that may be raised to the assignment of the excited-state processes in hypericin and hypocrellin as H-atom transfer. These are the absence of a deuterium isotope effect for the 10-ps component of hypericin and hypocrellin and the mirror image symmetry between the absorption and the emission spectra in hypericin and hypocrellin.

Figure 1.4. A series of upconversion traces for hypocrellin A in octanol collected at different emission wavelengths. $F(t) = -0.10 \exp (-t/4.1 \text{ ps}) + 1.00 \exp (-t/\infty)$; $\lambda_{em} = 591 \text{ nm}$. Note that at redder emission wavelengths, the amplitude of the rising component is negligible.
The lack of a deuterium isotope effect [29, 32, 34, 38, 51] may be attributed to the reaction coordinate not being identified with the proton coordinate. There is precedent for this in other systems [52–54].

Requiring the absence of mirror image symmetry between the absorption and the emission spectra assumes that the potential energy surface of the emitting species is significantly different from that of the absorbing species. Such a displacement in the coordinate of the emitting species is clearly evident in the most commonly studied proton transfer systems (Fig. 1.5): methyl salicylate [55], 7-azaindole dimer [56, 57], 2-phenyl-benzotriazole [58], and 3-hydroxyflavone [59–63]. If, however, we consider systems in which the normal and tautomer species are symmetric, or nearly so, this disparity no longer exists or is significantly minimized. 5-Hydroxytropolone [64, 65] presents an excellent example of such a case. Other examples are the double H-atom transfer in naphthazarin [66] and in the 4,9-dihydroxyperylene-3,10-quinone subunit of hypocrellin, producing entirely symmetric structures (Fig. 1.5).

We argue that hypericin and hypocrellin A have very similarly symmetric normal and tautomeric forms, as indicated by the highlighted bond systems in Fig. 1.1. That is, regardless of the tautomeric form in which the molecule finds itself, there is always conserved an aromatic core to which is attached a hydroxyl group peri to a carbonyl group. Even in the case of the monotautomerized species, it is possible to draw resonance forms that, upon superposition, restore the aromatic character of the substructure involved in the H-atom-transfer reaction. Consequently, we conclude that the mirror image symmetry observed in hypericin and hypocrellin is not at all surprising. If, on the other hand, the excited-state reaction were a genuine proton transfer, then the resulting charge-separated species would be expected to exhibit an emission spectrum significantly different from that of the absorption spectrum, as in 3-hydroxyflavone, and the rate of reaction should be very sensitive to solvent polarity, which is not the case for hypericin or hypocrellin.

### II. OUTSTANDING QUESTIONS REGARDING HYPERICIN-LIKE PERYLENE QUINONES

#### A. Ground-State Heterogeneity

As noted above, a possible objection to our assignment of the excited-state reaction to H-atom transfer in these perylene quinone systems is the observation of mirror image symmetry between the absorption and the emission spectra, which indicates minimal structural changes between the absorbing and the emitting species. Our first attempt to explain this symmetry was to suggest that the
Figure 1.5. Proton-transfer and H-atom-transfer species. In the upper half of the figure, the product of tautomerization is *structurally and electronically* different from the reactant. In contrast, H-atom transfer in 5-Hydroxytropolone results in nearly identical species, and double-tautomers of naphthazarin and 4,9-dihydroxyperylene-3,10-quinone are the “mirror” images of “normal” structures.
Ground state of hypericin was populated with at least one other species, for example, a monotautomer [29]. This seemed to be reasonable, especially given the breadth of the visible absorption spectrum: There are no “gaps” of zero absorbance anywhere between the ultraviolet and ~600 nm.

Temperature-dependant $^1$H NMR and 2D-ROESY studies of hypericin, however, indicate that there is only one conformer/tautomer for hypericin in the ground state [50]. On the other hand, the NMR measurements indicate that three ground-state species are significantly populated for hypocrellin A [48, 50], owing largely to the flexibility of the seven-membered ring. That the seven-membered ring plays an important role in determining the populations of conformers and tautomers in the ground state is demonstrated by the NMR study of hypocrellin B. The seven-membered ring of hypocrellin B contains a double bond and is consequently more rigid than that of hypocrellin A. Only one ground-state conformer/tautomer is observed by NMR for hypocrellin B.

1. Recent Theoretical and Computational Approaches

Previous quantum mechanical calculations also indicate that for hypericin the ground state is much less heterogeneous than we had believed. Based on $ab$ initio calculations, (RMP2/6-31G(d) level of theory, using geometries obtained with the 3-21G basis and Hartree-Fock wavefunctions) only one hypericin species, the “normal” form, is populated in the ground state for an unionized gas phase species [67] (Fig. 1.6).

Here we present more recent calculations using analytical instead of numerical Hessians, as was done in the earlier calculation. The initial structure used for hypericin was taken from previous work [67]. The structure was then reoptimized at the restricted Hartree-Fock (RHF) level theory, using the 6-31G(d) basis set. The Hessian (matrix of energy second derivatives with respect to Cartesian coordinates) of the optimized structure was calculated using a recently developed analytic Hessian method [68]. Diagonalization of the Hessian provides harmonic normal modes and corresponding vibrational frequencies [69]. Transition states and minima are indicated by 1 and no imaginary frequency mode, respectively. Hessian calculations provide vibrational frequencies and a diagnostic for the nature of a stationary point.

There are two types of Hessian calculations: seminumerical, using a finite difference of analytic gradients, and fully analytic. The analytic approach employed in our method is usually preferable due to the significantly increased accuracy of the calculated vibrational frequencies as well as its considerable time savings. The relative efficiency and accuracy of analytic Hessians increase with the size of the molecule. All calculations presented were performed with the quantum chemistry program GAMESS [70].
The structure of hypericin in its “normal” form is presented in Figures 1.7 and 1.8. It was established [67] that this C2 structure is the global minimum on the ground electronic state. The functional groups on carbon atoms 1, 13, and 14 (Figs. 1.1 and 1.7) are of primary interest. In particular, the distances between the oxygen atoms are crucial in understanding the H-atom-transfer kinetics and has been discussed by us in detail in our considerations of a unified picture of the hypericin and hypocrellin photophysics [34, 48, 71]. In the present calculation, the distances of the hydrogen bonds involving both O-H bonds are about 1.75 Å, slightly longer than in the previous work in which the 3-21G basis set was used [67].

2. Recent Experimental Approaches Our most recent attempt to investigate the problem of ground-state heterogeneity experimentally uses tunable pump laser pulses derived from a home-made optical parametric amplifier (Fig. 1.9). A white light continuum is used to seed a two-stage optical parametric amplifier pumped by the second harmonic of a regeneratively amplified Ti-sapphire laser
operating at 815 nm. Our system is based on the design by Greenfield and Wasielewski [72]. A 10% beam splitter is used to divert a small fraction of the compressor output for continuum generation. A combination of a λ/2 plate (HWP) and a thin-film polarizer (TFP) are used to control the exact intensity of the red before focusing it into a 1-mm-thick fused silica. The continuum generated is passed through a short-pass filter to eliminate any remaining fundamental

Figure 1.7. Structure of hypericin in the “normal” form (7,14-dioxo tautomer). See the text for details.

Figure 1.8. Side-on view of the hypericin “normal” form (7,14-dioxo tautomer). See the text for details.
and then through a HWP to obtain the right polarization for type II phase matching for the 3-mm-thick type II BBO crystal. The remaining IR is then reduced with a $2\times$ telescope and passed through a 2-mm-thick LBO-I. The blue light generated at 407 nm is split into two parts by a combination of HWP and TFP. We used roughly equal amounts of blue to pump the first and second stage of the OPA. In the first stage, we used a single 500-mm lens to control the spot size of the blue at the crystal. Care was taken to avoid continuum generation by the blue itself, by not focusing it directly at the crystal. We have found that using a single long-focal-length lens in the first stage instead of using a telescope significantly increases the stability of the output of the first stage. The white light and the blue were combined with a dichroic beam splitter and spatially and temporally overlapped in the BBO-II. In the second stage, the residual blue spot size was reduced with a $3\times$ telescope and passed through a HWP to have the matching polarization for type-I phase matching. The parametric output from the first stage and the residual blue were again combined with a dichroic beam splitter and spatially and temporally overlapped at the 2-mm-thick type-I BBO. Finally, a filter is used to select the tunable output in the visible region by rejecting the idler and the residual blue. Typically, the OPA
gives about 1 μJ of energy and is tunable from 475 to 700 nm. Tunability is achieved by angle tuning of the two BBO crystals simultaneously.

This tunable source was used to investigate the transient absorption kinetics of hypericin in DMSO as a function of pump wavelength (Fig. 1.10). The startling result is that using pump wavelengths from 495 to 600 nm, the data can be fit globally by a sum of two exponentials, which except for two traces exhibits the ~10-ps component characteristic of H-atom transfer. Fit results are compiled in Table 1.1.

Thus, both experimental and theoretical work continue to point toward the remarkable conclusion that hypericin in the ground state exists in only one tautomeric or conformational form, as indicated by both optical and NMR spectroscopies.

**Figure 1.10.** Transient absorption kinetics for hypericin as a function of pump wavelength. In each panel, the first value corresponds to the pump wavelength; the second, to the probe wavelength. The significant feature of these data is that they can be uniformly fit to two time constants, of which one corresponds to the characteristic H-atom-transfer time of ~10 ps. The results of a global fit to these data are compiled in Table 1.1.
Figure 1.10. (Continued)
B. Are There Multiple H-Atom Transfers in the Perylene Quinones?

Given the structure of the perylene quinones of interest here (Fig. 1.1), with either one (hypocrellin A) or two (hypericin), hydroxyl groups peri to a carbonyl on either end of the molecule, one is naturally inclined to inquire how many hydrogen atoms are transferred in the excited state. And, if more than one is transferred, one must necessarily inquire whether the process is stepwise or concerted. (It is useful to note the difference between a concerted and a synchronous reaction. A concerted reaction takes place in a single kinetic step, with no reaction intermediate, in which some of the changes in bonding take place to different extents in different parts of the reaction. A synchronous reaction is one in which all the bond-making and bond-breaking processes take place at the same time and proceed at the same extent during the reaction [73, 74]. It is a common error to assume that concertededness implies synchrony.) The availability of hypomycin B [1], where there is only one peri hydroxyl group and only one intramolecular hydrogen bond, provides an excellent means to investigate these questions.

Hypomycin B is unique in that it has only one intramolecular hydrogen bond as opposed to the two in hypocrellin A and the four in hypericin (Fig. 1.1). Picosecond transient absorption data for hypomycin B fail to reveal any stimulated

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**TABLE 1.1 Absorption Transients for Hypericin in DMSO as a Function of Pump Wavelength**

<table>
<thead>
<tr>
<th>λ Pump (nm)</th>
<th>λ Probe (nm)</th>
<th>a₁</th>
<th>a₂</th>
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<tr>
<td>495</td>
<td>560</td>
<td>-0.09</td>
<td>1</td>
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<td>495</td>
<td>600</td>
<td>0.23</td>
<td>-1</td>
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<tr>
<td>495</td>
<td>610</td>
<td>0.39</td>
<td>-1</td>
</tr>
<tr>
<td>495</td>
<td>660</td>
<td>0.20</td>
<td>-1</td>
</tr>
<tr>
<td>520</td>
<td>560</td>
<td>-0.15</td>
<td>1</td>
</tr>
<tr>
<td>520</td>
<td>600</td>
<td>-0.13</td>
<td>1</td>
</tr>
<tr>
<td>520</td>
<td>610</td>
<td>0.22</td>
<td>-1</td>
</tr>
<tr>
<td>520</td>
<td>660</td>
<td>0.20</td>
<td>-1</td>
</tr>
<tr>
<td>600</td>
<td>610</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>600</td>
<td>660</td>
<td>—</td>
<td>-1</td>
</tr>
</tbody>
</table>

*The absorption transients are fit globally to a sum of two exponentials: ΔΛ(t) = a₁ exp (-t/τ₁) + a₂ exp (-t/τ₂). The two time constants are kept fixed for all the kinetic traces at values of 10 and 5000 ps for τ₁ and τ₂, respectively. The value of 5000 is approximately the value of the fluorescence lifetime of hypericin, which is long on the time scale used to acquire these kinetic traces and may be considered to be infinite.*
emission, let alone rise time in stimulated emission (unlike hypocrellin and hypericin), which we have interpreted in terms of excited-state H-atom transfer (Fig. 1.11). Furthermore, a global analysis of the excited-state kinetics at four different probe wavelengths in MeOD and MeOH yields no significant difference in the excited-state kinetics: The time constants are 82 and 75 ps, respectively. Transient absorption data for hypomycin B in other solvents, such as DMSO and buffer-DMSO mixtures (data not shown), also failed to reveal any rise time in the stimulated emission. In the context of our previous arguments and criteria for identifying H-atom transfer in hypericin, hypocrellin, and their analogs, one might hastily conclude that hypomycin B does not undergo this process.

Figure 1.11. Comparison of the kinetic traces of hypomycin B in MeOD (solid lines) and in MeOH (dashed lines). The probe wavelengths are given in the top right hand corner of each panel. A global fit was carried out to fit the decays of the several wavelengths. In MeOH, the global time constant was 82 ps, whereas that in MeOD was 75 ps; $\lambda_{\text{ex}} = 407$ nm.
If subsequent experiments do indeed demonstrate that excited-state H-atom transfer does not occur in hypomycin B, then one may draw the conclusion that multiple transfers (either concerted or stepwise) must occur in these perylene quinones and that by frustrating the process in one half of the molecule, the process in the other half is impeded. At this point, such reasoning is speculative and contrary to the growing body of evidence provided by theory and experiment. As indicated above, quantum mechanical calculations indicate that the double-H-atom transfer in hypericin [67] and in the perylene quinone nucleus [75] of hypocrellin is energetically unfavorable compared to the single-transfer event. Experiments for hypericin in which one half of the molecule cannot participate in H-atom transfer owing to protonation of the carbonyl group (or even perhaps complexation with a metal ion) [76] also indicate that the transfer process can still occur.

Given the richness of the photophysics of these perylene quinones and their attendant complexity, it is premature to conclude that hypomycin B does not execute an excited-state H-atom transfer. Certainly, much more work is required to reach a proper judgment. This will include fluorescence upconversion experiments with picosecond resolution in order to interrogate the entire time scale of relevance as well as to monitor only emission, which can simplify the analysis [48]. For example, the failure to detect stimulated emission for hypomycin B may simply be the result of the presence of a strongly absorbing species in the same spectral region. Also, experiments must be performed to determine if hypomycin B, like hypericin and hypocrellin A, acts as an excited-state acid. It is clear, however, that hypomycin B is an important system with which to test and refine our current understanding of these naturally occurring perylene quinones. One of the most important questions that the study of hypomycin B might fruitfully address is the elucidation of the reaction coordinate in the H-atom-transfer process in the perylene quinones.

As we have discussed in depth elsewhere, despite the similarities in the structures of hypericin and hypocrellin, which are centered about the perylene quinone nucleus, their excited-state photophysics exhibit rich and varied behavior. The H-atom transfer is characterized by a wide range of time constants, which in certain cases exhibit deuterium isotope effects and solvent dependence. Of particular interest is that the shortest time constant we have observed for the H-atom transfer is $\sim 10$ ps. This is exceptionally long for such a process, 100 fs being expected when the solute H atom does not hydrogen bond to the solvent [62]. That the transfer time is so long in the perylene quinones has been attributed to the identification of the reaction coordinate with skeletal motions of the molecule [48, 50].

We have previously observed that when hypericin is bound to human serum albumin, it no longer undergoes an excited-state H-atom transfer. Assuming that the binding occurs through the interaction of one of the two carbonyl groups of hypericin and the N$_1$-H of the single tryptophan residue (W214), which would
necessarily impede H-atom transfer on this half of the hypericin molecule, we suggested that the absence of H-atom transfer in the complex indicated concerted, double-H-atom transfer in the excited state of hypericin [36]. We suggested that H-atom transfer is completely impeded when hypericin binds to HSA because skeletal motion is coupled to the H-atom transfer [48, 50]. Fluorescence anisotropy measurements of the HSA–hypericin complex indicate that the hypericin is rigidly bound and that there is no rapid restricted motion of hypericin relative to the protein. By analogy, one might argue that if H-atom transfer does not occur in hypomycin B, it is not because the process requires that two H atoms be in flight but because the required skeletal motion is restricted by the presence of the O-C-O bond. Although this response is plausible, it is not easy to reconcile it with the observation that hypericin undergoes H-atom transfer in a glass at low temperatures (the energy of activation is 0.05 kcal/mol) [37], where the amplitude of skeletal motion would seem to be less than that in the HSA matrix. These sorts of problems and questions continue to illustrate the need for further elucidation of the reaction coordinate for the H-atom transfer in hypericin and its analogs.

To conclude this section, we note that our assignment of excited-state H-atom transfer to the primary photoprocess in hypericin, hypocrellin, and their derivatives has occasioned some objections, to which we refer in the introduction and that we address in detail elsewhere [48, 71]. An additional concern, which has been brought to our attention and which is relevant in the light of the previous discussion, is the following. As we note above, we have measured the energy of activation for the H-atom transfer in hypericin to be 0.05 kcal/mol (or \( \sim 20 \text{ cm}^{-1} \)). The absence of an isotope effect for the hypericin reaction (and for the \( \sim 10\)-ps reaction in hypocrellin A) indicates that the reaction coordinate is not the hydrogen atom coordinate (which theoretical and experimental results suggest is \( \sim 1450 \text{ cm}^{-1} \) in the hypericin triplet [77]) and consequently must involve the skeletal motions noted above. It has been suggested by an anonymous colleague that if there is a slow H-atom transfer that is not limited by the H-atom coordinate, “then it must be that vibrational excitation of oxygen or ring modes is what limits the rate. This would correspond to a significant vibrational barrier and hence large activation energy.” We disagree with the last statement. Quantum mechanical calculations [67] (see also www.msg.ameslab.gov/Group/Supplementary_Material/Hypericin/) indicate that there are four calculated frequencies (unscaled) below 100 cm\(^{-1}\): 40 cm\(^{-1}\), out-of-plane motion of oxygens and carbons; 48 cm\(^{-1}\), oxygen and carbon displacements; 80 cm\(^{-1}\), mostly OH oxygen motion; and 84 cm\(^{-1}\): mostly OH oxygen motion. There is no dearth of low-frequency vibrations in large biological molecules, as the calculations and many experiments suggest [78–80], and we believe that such motions may indeed be coupled to the H-atom transfer in these perylene quinone systems.
Our previous results on hypericin indicate that excited-state H-atom transfer occurs even when one of the carbonyls is prohibited from accepting a hydrogen. The presence of such a transfer is apparent under very acidic conditions in AOT reverse micelles and cannot be excluded upon chelation of Tb$^{3+}$ \cite{76}. There is thus no evidence for a concerted H-atom-transfer mechanism in hypericin. In the present study, contrary to our initial expectations, we are not even able to demonstrate that hypomycin B executes an excited-state H-atom transfer; hence our investigation sheds no light on the general question of how many H atoms are transferred in the perylene quinones and whether the transfer is concerted or stepwise. On the other hand, if further investigation reveals that H-atom transfer does not occur in hypomycin B, the result would have considerable implications for an understanding of the reaction coordinate for the H-atom transfer.

C. Identifying the H-Atom Translocation

Above we addressed the absence of a deuterium isotope effect for the 10-ps component of hypericin and hypocrellin and the mirror image symmetry between the absorption and the emission spectra in hypericin and hypocrellin. We recognize, however, that no matter how satisfactory one finds our reasoning, a direct demonstration of an excited-state H-atom transfer is required, which entails measurements of the carbonyl or hydroxyl stretching frequency as a function of time subsequent to laser excitation. A first step in this process is the identification of vibrational modes that indicate translation of the H-atom between the enol and the keto oxygens. We have addressed this problem by employing time-resolved infrared spectroscopy to hypericin and to two of its analogs that cannot undergo excited-state H-atom transfer (Fig. 1.12) \cite{77}. The salient feature of these spectra is in the region between 1400 and 1500 cm$^{-1}$, where a strong band is present for hypericin and is absent in both O-hexamethoxy hypericin, which lacks hydrogen atoms that can coordinate to the carbonyl, and in the reduced analog, which lacks the necessary carbonyls. The ground-state infrared spectrum of hypericin is included at the top of Figure 1.12. Ab initio quantum mechanical calculations, at the Hartree-Fock 3-21G level \cite{77}, reveal strong normal modes in this spectral region for the triplet species of the normal form of hypericin and the 6,14- and 7,13-dioxo monotautomers (Fig. 1.1). Calculations were not performed for the triplet species of the double tautomers. Assignments of these modes have been given elsewhere \cite{77}. Our most recent calculations on the ground-state 7,14-dioxotautomer using the analytical Hessian method and the 6-31G basis set provide the results compiled in Table 1.2 and the corresponding theoretical infrared spectrum depicted in Figure 1.13.
**Figure 1.12.** Comparison of the transient infrared spectra on the microsecond time scale of hypericin, O-hexamethoxy hypericin, and a hypericin analog that lacks carbonyl groups (the hexaacetoxy analog). The salient feature of the data is that the latter two compounds, which cannot execute excited state H-atom transfer owing to the absence of either labile protons or appropriate carbonyl groups, lack the feature at $\sim 1450 \text{ cm}^{-1}$. Ab initio calculations at the Hartree-Fock 3-21G level for the normal and two monotautomeric forms of the hypericin *triplets* indicate normal modes with substantial O•••H•••O character in the region $\sim 1400$–$1460 \text{ cm}^{-1}$ [77]. While these preliminary results do not demonstrate a time-resolved H-atom transfer, they do clearly point to a region of the spectrum that must be investigated in further studies. Hypericin and hexamethoxy hypericin, *solid line*, 0–1 $\mu$s and *dashed line*, 14–18 $\mu$s; reduced analog, *solid line*, 0–0.5 $\mu$s, and *dashed line*, 7–9 $\mu$s.
The major finding of this study is that a vibrational mode corresponding to H-atom translocation can been identified in hypericin by the joint contributions of synthetic, computational, and spectroscopic methods. Identification of this mode is only a first step in providing a direct demonstration of excited-state intramolecular H-atom transfer in hypericin and its analogs. There is considerable work to be accomplished. As indicated elsewhere [77], ab initio calculations predict that the normal modes in this region of the spectrum are close for the normal and the monotautomeric forms. The direct observation of the formation of the tautomer will require both adequate temporal and spectral resolution.

It must be remembered, furthermore, that the identification of the H-atom translocation mode is not equivalent to the identification of the reaction coordinate. We have attributed the absence of a deuterium isotope effect on the excited-state H-atom transfer (for the \(~10\)-ps component in hypericin and hypercrellin A) to the zero-point energy in the proton coordinate lying above the barrier, with the H-atom being effectively delocalized between the two oxygen atoms. Consequently, the reaction coordinate for the excited-state H-atom transfer cannot be identified with the proton coordinate, and it must be concluded that other intramolecular motions are in fact responsible for the process. Temperature-dependent measurements indicate that these motions are extremely low amplitude, $E_a = 0.05$ kcal/mol for hypericin [37]. Because the nature of this motion is not yet identified, we refer to it as the skeleton coordinate [48, 71, 82]. We propose that it is the time scale for this latter conformational change.

### TABLE 1.2 Calculated Frequencies of the Normal Mode Vibrations of the Ground State 7,14-Dioxotautomer Hypericin Species

<table>
<thead>
<tr>
<th>Frequency (cm$^{-1}$)</th>
<th>Scaled Frequency$^a$</th>
<th>IR Intensity</th>
<th>Description$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1262</td>
<td>1123</td>
<td>16</td>
<td>C(3)-O-H, C(4)-O-H, C(2)-O, C(5)-O; motions and ring breathing</td>
</tr>
<tr>
<td>1341</td>
<td>1194</td>
<td>20</td>
<td>Ring breathing</td>
</tr>
<tr>
<td>1523</td>
<td>1356</td>
<td>13</td>
<td>C(1)-O-H, C(6)-O-H, C(8)-O-H, C(13)-O-H; motions and planar ring breathing</td>
</tr>
<tr>
<td>1741</td>
<td>1550</td>
<td>14</td>
<td>Peripheral carbons stretch in plane and ring breathing</td>
</tr>
<tr>
<td>1840</td>
<td>1638</td>
<td>53</td>
<td>Peripheral carbons stretch up and down in phase</td>
</tr>
<tr>
<td>3835</td>
<td>3413</td>
<td>12</td>
<td>O-H stretches on C(1), C(6), C(13)</td>
</tr>
<tr>
<td>4107</td>
<td>3655</td>
<td>6</td>
<td>O-H stretches on C(3), C(4)</td>
</tr>
</tbody>
</table>

$^a$The calculated frequencies are scaled by a factor of 0.89 [81].

$^b$See Figure 1.7 for atom labelling.
that determines the observed H-atom-transfer time. The exact nature of the conformational changes that are coupled to the H-atom-transfer reaction in hypericin and hypocrellin has yet to be identified [82].

III. CONCLUDING REMARKS

We presented an overview of what we consider to be the current outstanding problems in understanding the photophysics of hypericin and its analogs. Many questions remain unanswered, and a wealth of theoretical and experimental techniques will be required to address them. It is surprising that given the richness of the physical, chemical, biological, and physiological behaviors of hypericin and...
the class of molecules to which it belongs, their importance has not been widely appreciated.

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