# Coupling of Large-Amplitude Side Chain Motions to the Excited-State H-Atom Transfer of Perylene Quinones: Application of Theory and Experiment to Calphostin C

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The excited-state intramolecular H-atom transfer reactions of hypocrellins B and A are compared with those of calphostin C. On the basis of the results of transient absorption measurements and *ab initio* quantum mechanical calculations, it is concluded that large-amplitude conformational changes are coupled to the H-atom transfer in calphostin C, just as they are in hypocrellins A and B. The calculations on this very large molecule with a very complex ground electronic state potential energy surface were made possible by the use of highly scalable electronic structure theory codes on large parallel computers.

## Introduction

The nearly symmetrical perylene quinones, hypericin, hypocrellins A and B, and calphostin C (Figure 1), are of interest because of their light-induced biological properties.[1−5] We have argued that they also present a fascinating system with which to study excited-state intramolecular H-atom transfer.[6−20]

Hypericin executes an intramolecular excited-state H-atom transfer in ∼10 ps.[20] This reaction is independent of solvent. In this respect, hypericin distinguishes itself from the other perylene quinones such as hypocrellins A and B, which both undergo excited-state H-atom transfer that is strongly viscosity dependent. Their H-atom transfer times in ethanol and octanol range from 50 to 100 ps (Table 1). The origin of this dependence lies in the presence of the seven-membered ring in the hypocrellin "bay region."

Three significantly populated species are observed in the ground state for hypocrellin A:[19] two "normal," that is untautomerized, species differing in the orientation of the seven-membered ring (i.e., a *gauche* or *anti* conformation about the C14−C13 bond or the C14−C16 bond) and a double tautomer in the *gauche* conformation (Figure 2). Conformational changes are coupled to both ground- and excited-state tautomerization in hypocrellin A.[19] Owing to the double bond in its seven-membered ring, hypocrellin B has higher structural rigidity in the ground state than does hypocrellin A. Consequently, only one tautomer/conformer of hypocrellin B is observed by NMR to be significantly populated in the ground state.[19] On the other hand, insofar as the double bond of hypocrellin B's seven-membered ring is conjugated to the $\pi$ system of the aromatic skeleton, one might expect that in the excited state the seven-membered ring is able to undergo a conformational change about the double bond. Thus, we suggest that excited-state conformational changes are coupled to the H-atom transfer in hypocrellin B just as *gauche*/*anti* changes are coupled to the H-atom transfer in hypocrellin A.[19,20]

Calphostin C is a perylene quinone isolated from *Cladosporium cladosporides*. It is a potent and specific inhibitor of protein kinase C.[21] To our knowledge, ours are the first time-resolved measurements obtained for calphostin C. Aside from the biological importance of investigating calphostin C, we were motivated by fundamental photophysical considerations. We originally hypothesized that if, as we suggest above, the viscosity-dependent time constants in hypocrellins A and B are a result of conformational changes of their seven-membered rings that are coupled to the H-atom transfer, then breaking this seven-membered ring ought to afford hypericin-like, viscosity independent, time constants of ∼10-ps.

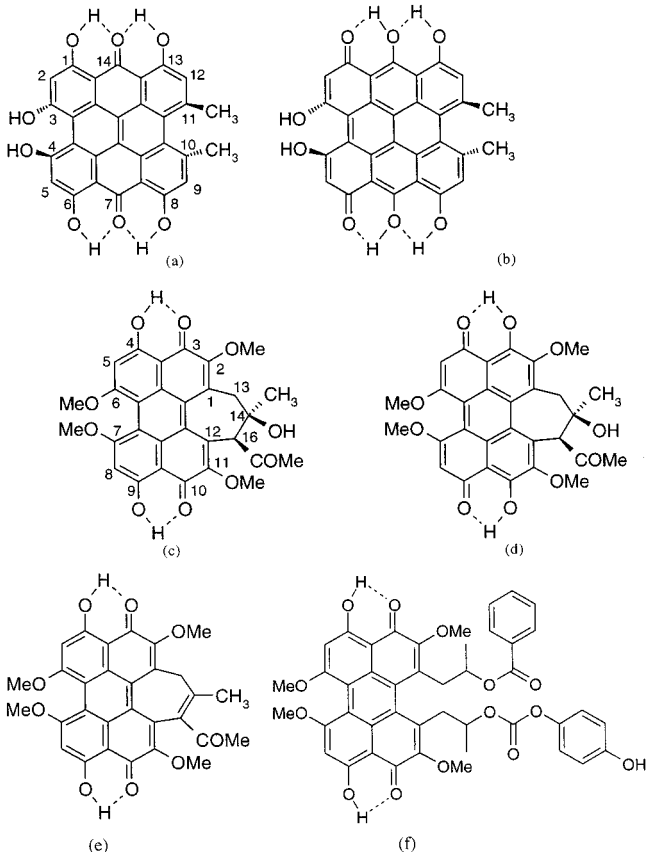


**Figure 1.** Structures of hypericin "normal" (a) and double tautomer (b) forms; hypocrellin A "normal" (c) and double tautomer (d) forms; hypocrellin B (e) and calphostin C (f).

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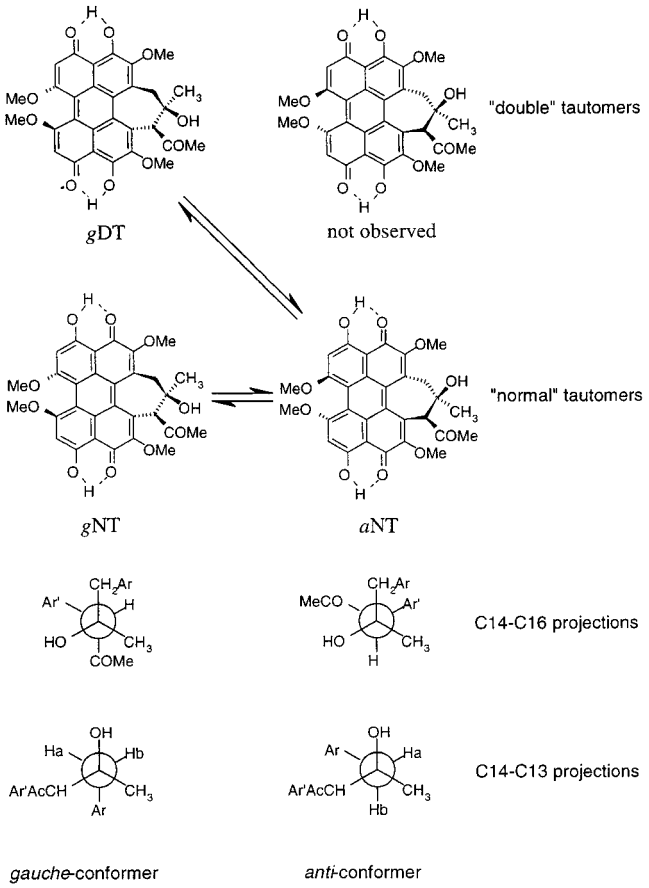


**Figure 2.** Conformational forms of the hypocrellin A normal and double tautomers. The *anti/gauche* nomenclature is based on the relative position of the methyl and acetyl moieties as depicted by the Newman projection: *gauche* double tautomer, *g*D, 60% of the ground-state population; *anti* normal tautomer, *a*N, 30%; *gauche* normal tautomer, *g*N, 10%.

**TABLE 1: Kinetic Parameters for Hypocrellin A, Hypocrellin B, and Calphostin C at 595 nm in Ethanol and Octanol**[a]

| probe molecule | solvent | $a_1$ | $\tau_1$ (ps) | $a_2$ | $\tau_2$ (ps) | $a_3$ | $\tau_3$ (ps) |
|---|---|---|---|---|---|---|---|
| hypocrellin A | EtOH | 0.23 | 55 | −0.46 | 1010 | 0.09 | ∞ |
| | OcOH | 0.02 | 103 | −0.72 | 1060 | 0.47 | ∞ |
| hypocrellin B | EtOH | 0.28 | 84 | 0.51 | ∞ | | |
| | OcOH | 0.26 | 109 | 0.14 | ∞ | | |
| calphostin C | EtOH | 0.37 | 104 | 0.01 | ∞ | | |
| | OcOH | 0.16 | 147 | 0.05 | ∞ | | |

[a] The data are fit to the following function: $\Delta A(t) = a_1[1 - \exp(-t/\tau_1)] + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\infty)$, where $a_1$ is the amplitude of the rising component; and the infinite time constant describes a process that does not decay on the experimental time scales (up to 500 ps full scale) used in this study.

The results presented here indicate that, contrary to our expectation, calphostin C, in which there is no seven-membered ring, behaves qualitatively very similarly to hypocrellins A and B. To rationalize this behavior, it is necessary to propose that there is some interaction of the bulky side chains in the bay region that retard the H-atom transfer reaction. Justification of this proposal is demonstrated by *ab initio* quantum mechanical calculations.

## Materials and Methods

Hypocrellin A and B were obtained from Molecular Probes and were used as received. Calphostin C was obtained from Sigma and used without further purification. Anhydrous HPLC grade solvents (Aldrich) were used as received. Steady-state absorbance spectra were obtained on a Perkin-Elmer Lambda 18 double-beam UV−vis spectrophotometer with 1-nm resolution. Steady-state fluorescence spectra were obtained on a Spex Fluoromax with a 4-nm band-pass and corrected for lamp spectral intensity and detector response. Transient absorption measurements were obtained with the apparatus described in detail elsewhere.[12]

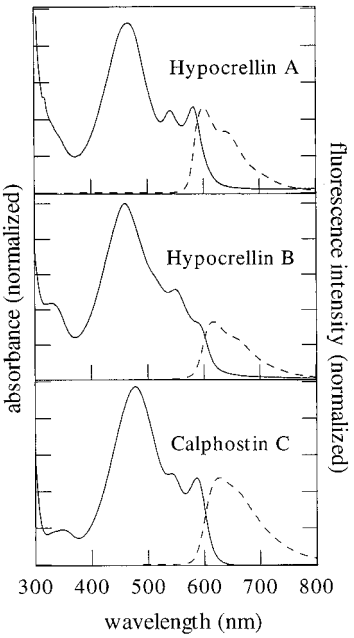


**Figure 3.** Absorption (−) and emission (- - -) spectra of hypocrellin A, hypocrellin B and calphostin C in ethanol. The spectra are normalized to the reddest absorption maximum. The excitation wavelength for the emision spectra is 477 nm.

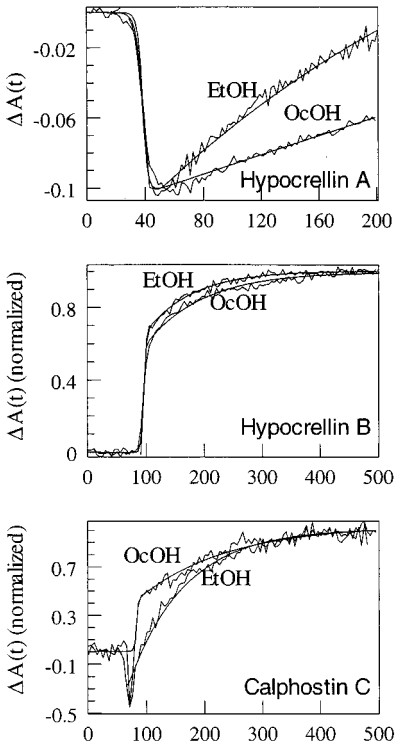


**Figure 4.** Transient absorbance traces of hypocrellin A, hypocrellin B, and calphostin C in ethanol and octanol at probe wavelength of 595 nm. The global fits to the data are presented in Table 1.

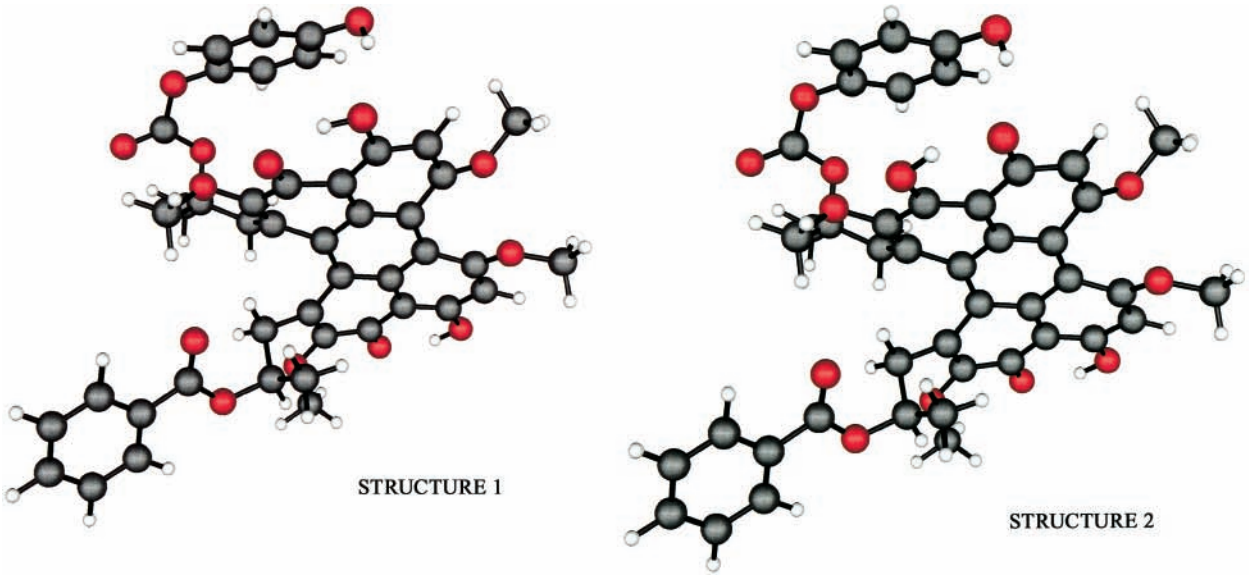


**Figure 5.** Optimized structures for calphostin C. White, black, and red atoms represent hydrogen, carbon, and oxygen, respectively.

For hypocrellin A, the data are fit to the following function:

$$\Delta A(t) = a_1[1 - \exp(-t/\tau_1)] + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\infty)$$

For hypocrellin B and calphostin C, however, two exponential components are sufficient to describe the data and the following form is used:

$$\Delta A(t) = a_1[1 - \exp(-t/\tau_1)] + a_2 \exp(-t/\infty)$$

In both cases, $a_1$ is the amplitude of the rising component and the infinite time constant describes a process that does not decay on the experimental time scales (up to 500 ps full scale) used in this study.

Geometry optimizations for various possible isomers of calphostin in its electronic ground state were performed using restricted Hartree−Fock (RHF) wave functions with the 3-21G* basis set. Various starting points for the geometry optimization were tried, including "flat" structures in which the side group and the H-atom transfer region are well separated, but all searches led to one of the two structures shown in Figure 1. Given the complexity of the potential energy surface (more than 50 heavy atoms and many low frequency torsional modes), it is certainly possible that other local minima exist. Nonetheless, it is likely that the structures given here are among the lowest energy isomers.

Once the optimized geometries were obtained, single point energy calculations were performed with the 6-31G(d) basis set, at both stationary points and at selected points along the linear least motion (LLM) path connecting the two. All calculations were carried out using the electronic structure code GAMESS.[22]

## Results and Discussion

Steady-state absorption and emission spectra of hypocrellins A and B and of calphostin C in ethanol are presented in Figure 3. Their excited-state H-atom transfer in octanol and ethanol, as monitored by transient absorbance, is illustrated in Figure 4 and summarized in Table 1. As indicated above, calphostin C lacks the seven-membered ring, which we have attributed to origin of the viscosity dependence of the H-atom transfer in hypocrellins A and B. The crucial feature revealed by the data is that its H-atom transfer reaction, nevertheless, exhibits a similar viscosity dependence, which we initially expected to be absent.

To rationalize this behavior, we hypothesized that there is some interaction of the bulky side chains that retard the H-atom transfer reaction. By means of *ab initio* calculations, we have discovered structures (Figure 5) in which the hydroxyl group of the phenolic side chain of calphostin C hydrogen bonds to the carbonyl and hydroxyl groups of the perylene quinone moiety. We propose that the large amplitude motion required for the displacement of this side chain from the carbonyl group is responsible for the slow H-atom transfer kinetics of calphostin C. We anticipate that analogues of the fundamental hypocrellin unit, 4,9-dihydroxyperylene-3,10-quinone, lacking bulky side chains, will exhibit hypericin-like 10-ps, viscosity independent time constants for H-atom transfer.

The two optimized structures both exhibit significant hydrogen bonding. The OH---OH distance in structure **1** is 1.995 Å, while the OH---O═ distance in structure **2** is 1.991 Å. The O---O distances are 2.84 and 2.79 Å, respectively. At the RHF/6-31G(d) level of theory, **1** is predicted to be about 4 kcal/mol lower in energy than **2**, but the two isomers should be considered to be approximately equal in energy, given the level of theory used here. The upper limit for the H-atom transfer barrier, obtained from the LLM path is about 23 kcal/mol. Both correlation corrections and a proper transition state search would be expected to lower this estimate.

In conclusion, the similarity of the viscosity dependence of the excited-state H-atom transfer reaction of calphostin C with that of hypocrellins A and B can be explained by the interaction of its side chain and carbonyl group. This interaction, in turn, is predicted by the results of *ab initio* quantum mechanical calculations. We note that these are vacuum calculations, and consequently they do not take into account any additional complications introduced by possible hydrogen bonding interactions of the phenolic side chain with the hydrogen bonding solvents used in this investigation. We note, for example, that the gas-phase dipole moments for structures 1 and 2 in Figure 5 are predicted to be 9.8 and 10.6 D, respectively. On the basis of a simple eletrostatic model (probably reasonable given the large dipoles), polar solvents are therefore expected to

stabilize both isomers and to favor structure 2 relative to structure 1.

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