# Structure and Energetics of Ground-State Hypericin: Comparison of Experiment and Theory

**J. W. Petrich,* M. S. Gordon,* and M. Cagle**

*Department of Chemistry, Iowa State University, Ames, Iowa 50011*

*Received: October 24, 1997; In Final Form: December 10, 1997*

Calculations of the energies of the ground-state un-ionized tautomers of hypericin have been performed at the RMP2/6-31G(d) level of theory, using geometries obtained with the 3-21G basis set and Hartree−Fock wave functions. It is found that only the so-called normal form is likely to be populated at room temperature and that only two of the three possible double tautomers correspond to minima on the potential energy surface. The effect of continuum aqueous solvation on the tautomer energies is negligible. The O---O distances between which the proton is transferred are reported and are consistent with that required for an adiabatic proton transfer, i.e., ∼2.5 Å. All 156 vibrational frequencies are tabulated and may be viewed at www.msg.ameslab-.gov. For example, the vibrations in the range 320−660 $cm^{-1}$ are coupled with O---O vibrations. The vibrations that are most clearly O---O vibrations occur in the range 400−500 $cm^{-1}$. Twisting of the backbone occurs in a wide range of frequencies, from 230 to 1150 $cm^{-1}$, while the motion corresponding to an inversion of the (nonplanar) backbone occur at very low frequencies, 80−150 $cm^{-1}$. The results of these calculations are discussed in terms of ground-state heterogeneity of hypericin that has been invoked to explain its spectra and excited-state kinetics.

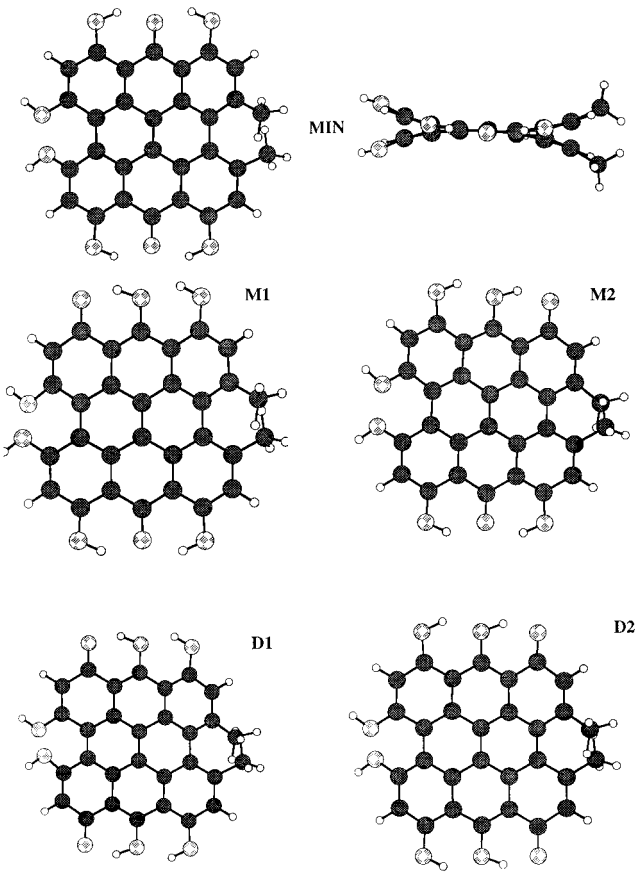
## Introduction

The naturally occurring polycyclic quinone hypericin (Scheme 1) is of current interest because of its light-induced[1] antiviral (especially anti-HIV) and antitumor activity.[2−11] In addition, hypericin has attracted attention for its antidepressant activity.[12−15] The interaction of light with hypericin and hypericin-like chromophores is clearly of fundamental biological importance. To understand and eventually to exploit these properties of hypericin, it is essential to elucidate its nonradiative excited-state processes. We have undertaken this task using the tools of ultrafast absorption and emission spectroscopy (see refs 16−20 and citations therein). We have also proposed a means of exploiting the biological activity of hypericin.[21,22] Hypericin and its analogues have been the subject of several reviews.[22,25]

Our original argument[16−20] for the presence of intramolecular excited-state proton transfer in hypericin is as follows. The hypericin analogue lacking labile protons, mesonaphthobianthrone, is significantly fluorescent and has optical spectra that resemble those of hypericin only when its carbonyl groups are protonated. In hypericin, the fluorescent state grows in on a time scale of several picoseconds, as measured by the rise time of stimulated emission. Therefore, the combined observations of the requirement of protonated carbonyls for strong hypericin-like fluorescence and the rise time of fluorescence in hypericin were taken as evidence for intramolecular excited-state proton transfer in hypericin. (We use the term "proton transfer" very loosely. Our current knowledge of the excited-state transfer process is not detailed enough to specify whether the process is a proton or an atom transfer.) This argument has consistently been borne out by the study of tetra- and hexamethoxy analogues of hypericin[16,17,19] and by the observation of a deuterium isotope effect in the hypericin analogue, hypocrellin.[18]

Of special relevance to the role of labile protons for the light-induced biological activity of hypericin is the observation that hypericin acidifies its surroundings upon light absorption.[26−30] The role of photogenerated protons takes on additional signifi-

**SCHEME 1: Optimized Structures for Potential Energy Minima; Two Views Are Shown for the "Normal Isomer" MIN**




* To whom correspondence should be addressed.

S1089-5639(97)03442-7 CCC: $15.00 
Published on Web 02/06/1998

cance in the context of the growing body of literature implicating pH decreases with pharmacologically important functions, such as virucidal activity (optimum pH values are important in the life cycles of many enveloped viruses),[32] antitumor activity,[33,34] apoptosis (a form of cell death associated with DNA fragmentation and chromatin condensation),[35−37] and the subcellular distribution of hexokinase.[38]

One possible argument against intramolecular excited-state proton transfer in hypericin is the observation of near-mirror-image symmetry between its absorption and emission spectra. Such symmetry is typically taken as a signature of negligible structural changes between the absorbing and the emitting species. Intramolecular excited-state proton transfer usually generates a broad structureless emission spectrum that bears little resemblance to the absorbance spectrum. 3-Hydroxyflavone provides a good example.[39]

There are at least two ways to respond to this objection. It is possible that the structural changes induced by intramolecular proton transfer do not significantly effect the electronic structure of the tautomeric species (Scheme 1) in such a way as to destroy the mirror-image symmetry. It is also possible, as we have argued elsewhere,[16−20] that the ground state of hypericin is already partially tautomerized or exists in an equilibrium between various isomeric species and that this ground-state heterogeneity yields the observed mirror-image symmetry between absorption and emission spectra.

High-level quantum chemical calculations will have much to offer in understanding these problems. The subject of this article is the ab initio computation of the energies of the ground-state tautomeric forms of hypericin with the goal of determining to what extent they contribute to the ground-state heterogeneity discussed above. Several molecular mechanics studies of hypericin and its derivatives[40−43] and an ab initio calculation of the normal form of unsolvated hypericin ionized in the bay region have been performed.[44] Density functional calculations of hypericin fragments such as emodin have also been performed.[45,46] Ours is the first work, to our knowledge, that presents a detailed comparison of the energetics of all of the ground-state hypericin tautomers connected by hydrogen transfers at a reliable ab initio level of theory.

## Computational Methods

Initial structures for all minima and transition states were obtained with the semiempirical AM1[47] molecular orbital method. Using these structures as starting points, the geometries were then optimized at the restricted Hartree−Fock (RHF) level of theory, using the 3-21G basis set.[48] At each RHF/3-21G stationary point, the Hessian (matrix of energy second derivatives) was calculated using finite double differencing of the analytic gradients. The Hessian is used to determine the nature of each stationary point (positive definite for a local minimum, one negative eigenvalue for a saddle point) and to provide an estimate of the vibrational frequencies and the vibrational zero-point energy (ZPE) to be discussed below.

To provide an improved estimate of the energetics, the relative energies of the tautomers and transition states were predicted by performing single-point energies at the 3-21G geometries. These single-point energies were carried out at the RHF level of theory using the 6-31G(d) basis set,[49] denoted RHF/6-31G(d)//RHF/3-21G, and with second-order perturbation theory (MP2[50]) using the 3-21G basis set, MP2/3-21G//RHF/3-21G. Assuming the effects of basis set improvement and introduction of electron correlation (MP2) are additive, one can estimate the relative MP2/6-31G(d) energies.

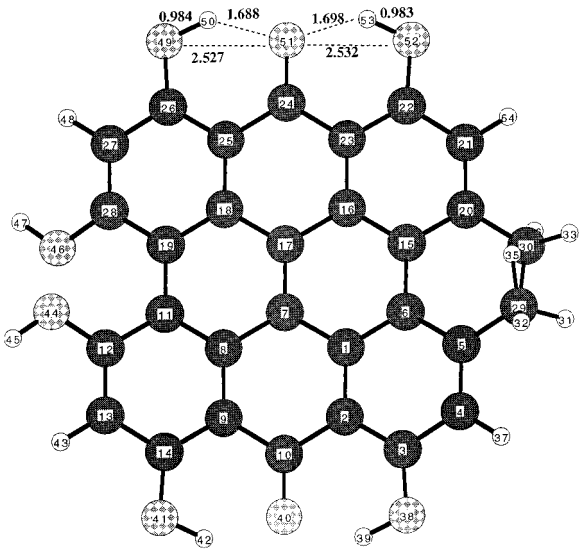



**Figure 1.** RHF/3-21G hypericin global minimum. Bond lengths in Å.

The effects of aqueous solvation on the relative energetics were estimated by using a simple self-consistent reaction field (SCRF)[51] model. Since this model relies on the interaction between the dipole moment of the solute and the dielectric of the solvent (in this case, water), and since the dipole moments of the tautomers are all rather small, the predicted SCRF solvent effects on the relative energies are negligible and are not discussed further.

All calculations discussed here were performed with the electronic structure code GAMESS.[52]

## Results and Discussion

**Structures and Vibrational Frequencies.** A schematic of the minima found on the hypericin potential energy surface (PES) is given in Scheme 1. The only structures investigated in this work are those that are related to the global minimum by a transfer of a hydrogen atom; isomers that correspond to $-$OH or $-CH_3$ rotations were not explored. In Scheme 1 the global minimum is shown in two perspectives, to emphasize the nonplanar overall structure. This distortion from planarity is caused by interactions between the pendent $-$OH and $-CH_3$ groups. RHF/3-21G geometry optimization of the structure with these four groups removed leads to a planar minimum. On the other hand, optimization with the two OH groups restored but in $C_{2v}$ symmetry to maintain planarity of the aromatic backbone is not a minimum on the PES, nor is the full $C_s$ hypericin structure with the planar backbone.

The global minimum (traditionally referred to as the "normal" species) on the hypericin PES has $C_2$ symmetry. There are two tautomers (M1 and M2 for *mono*) that arise from the transfer of one hydrogen and two additional tautomers (D1 and D2 for *duo* or double) that arise from a double H transfer. A third double transfer structure is possible, in which at the "top" of the molecule an H is transferred from a hydroxyl group nearest to the bay hydroxyls and at the "bottom", from a hydroxyl group nearest to the bay methyl groups. But this tautomer, D3, does not correspond to a stationary point on the PES at the RHF/3-21G level of theory.

The essential features of the geometries of the minima and identified transition states are shown in Figures 1−4. The two hydrogen bonds in the global minimum are similar, with O---H distances just under 1.7 Å. These clearly correspond to the interaction of hydroxy hydrogens with one of the carbonyl lone

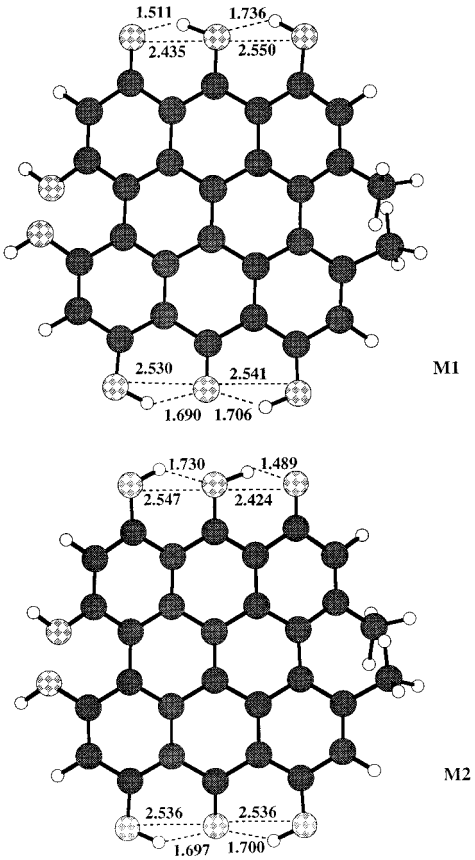



**Figure 2.** RHF/3-21G structures for mono-H transfer tautomers, M1 and M2. Bond lengths in Å.

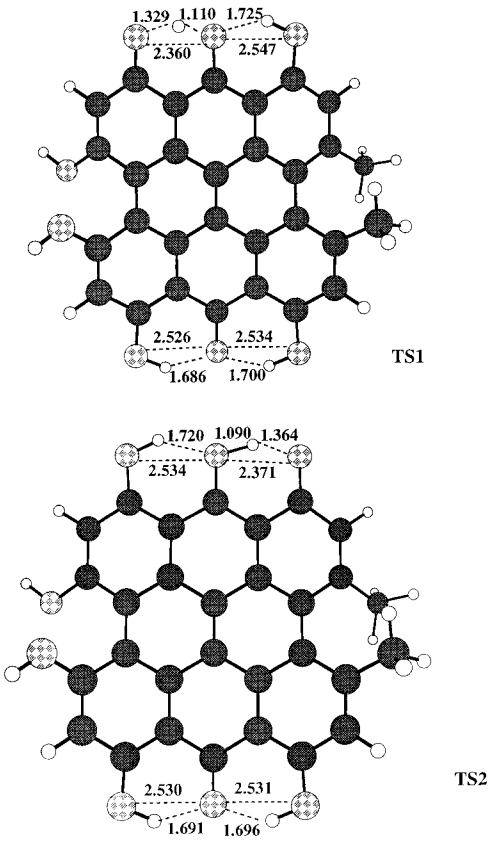



**Figure 3.** RHF/3-21G structures for transition states TS1 and TS2. Bond lengths in Å.

pairs. When one of the hydrogens is transferred to the central carbonyl oxygen to form M1 or M2 (Figure 2), the terminal ═O---H interaction increases considerably, as is evidenced by the large (nearly 0.2 Å) decrease in the hydrogen bond distance to about 1.5 Å. At the same time, the O---O distance decreases by nearly 0.1 Å. On the other hand, the −O---H interaction is weaker than the ═O---H hydrogen bond (note the increase in the hydrogen bond distance), since the hydroxy oxygen lone pairs are not lined up as well as those of the carbonyl oxygen. The O---O distances between which the proton is transferred are reported and are consistent with that required for an adiabatic proton transfer, i.e., ∼2.5 Å.[18,20,58]

The transition states (TS1 and TS2) that connect the global minimum with M1 and M2 are shown in Figure 3. Both transition states are characterized by a compressed structure on the side of the molecule in which the transfer is occurring. This phenomenon has been noted for other hydrogen transfer reactions[53,54] and serves to minimize the energy of the transition states. The geometries of the double H transfer tautomers, D1 and D2, are shown in Figure 4. Both of these structures have $C_2$ symmetry, and their overall characteristics are similar to those discussed for M1 and M2.

Hypericin has 54 atoms and therefore 156 vibrational frequencies. Rather than try to list these frequencies for all tautomers, they are summarized here in qualitative terms for the global minimum. The reader is referred to the web site www.msg.ameslab.gov to view animations of all frequencies. Recall that harmonic vibrational frequencies calculated at this level of theory are typically about 10% too high. The highest calculated frequencies, in the range 3500−3930 cm$^{-1}$, correspond to O−H stretches, while the C−H stretches are in the 3200−3400 cm$^{-1}$ range. A large number of frequencies in the range 1500−1750 cm$^{-1}$ may be characterized as backbone breathing + C═O stretching motions. There are several other frequencies in the same range that correspond to backbone breathing motions, some of which are coupled with distortions of the methyl groups or the pendent OH groups.

A large number of vibrations may be characterized as internal bending and stretching in the backbone of the molecule. These are in the range 320−1500 cm$^{-1}$. The vibrations in the low end of this range (320−660 cm$^{-1}$) are coupled with O---O vibrations. The vibrations that are most clearly O---O vibrations occur in the range 400−500 cm$^{-1}$. Twisting of the backbone occurs in a wide range of frequencies, from 230 to 1150 cm$^{-1}$, while the motion corresponding to an inversion of the (nonplanar) backbone occur at very low frequencies, 80−150 cm$^{-1}$. After reducing the predicted vibrational frequencies by 10%, these predicted vibrational frequencies are consistent with two recent experimental SERRS studies.[59,60] These studies find strong features in the 1250−1400 cm$^{-1}$ range corresponding to in-plane motions and in the 310−630 cm$^{-1}$ range corresponding to out-of-plane motions.[60]

**Energetics.** The relative energies for the minima and transition states are summarized in Table 1 and Scheme 2. Improving the basis set from 3-21G to 6-31G(d) increases the energies relative to the global minimum, but the addition of corrections for electron correlation via MP2 decreases the relative energies. The net effect is to decrease all of the relative energies by a few kcal/mol. M1 and M2 are predicted to be within 10 kcal/mol of the global minimum, but the reverse barriers are very small, on the order of 1 kcal/mol, so it is unlikely that these tautomers would be present except at low temperatures. The two double hydrogen transfer tautomers, D1 and D2, are considerably higher in energy than these transition

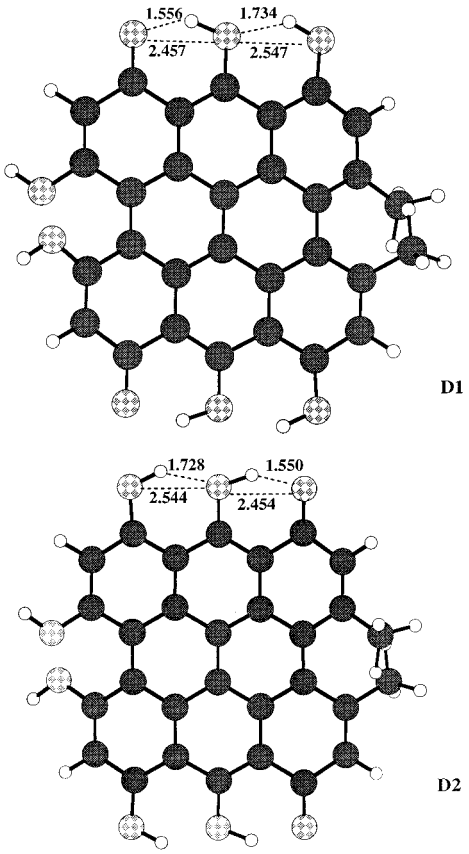


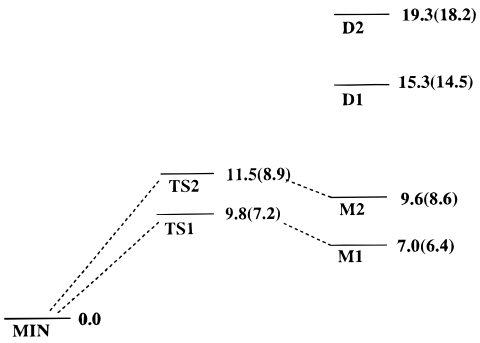

**Figure 4.** RHF/3-21G structures for double-H transfer tautomers, D1 and D2. Bond lengths in Å.

**TABLE 1: Relative Energies (kcal/mol) as a Function of Basis Set and Correlation at 3-21G Geometries**

| structure | 3-21G | 6-31G(d) | 6-31G(d)+ ZPE | MP2/ 3-21G | MP2/ 6-31G(d)[a] |
|---|---|---|---|---|---|
| MIN | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| T1 | 7.6 | 9.2 | 8.6 | 5.4 | 6.4 |
| T2 | 9.2 | 11.4 | 10.4 | 7.4 | 8.6 |
| TS1 | 9.7 | 13.2 | 10.6 | 6.3 | 7.2 |
| TS2 | 11.1 | 14.6 | 12.0 | 8.0 | 8.9 |
| D1 | 16.5 | 19.2 | 18.4 | 11.6 | 14.5 |
| D2 | 19.7 | 23.2 | 22.1 | 15.8 | 18.2 |

[a] Estimated value, including 3-21G vibrational zero-point energy; see text. MIN is traditionally referred to as the "normal species".

**SCHEME 2: Estimated MP2/6-31G(d)//RHF/3-21G Relative Energies (kcal/mol) of Minima and Transition States; Energies Including Vibrational Zero-Point Corrections Are Given in Parentheses**




states, so no attempt was made to identify transition states connecting them with the other structures.

As noted earlier, the $C_s$ structure, in which the backbone is forced to be planar, is not a minimum on the potential energy surface, due to the repulsive methyl−methyl and OH−OH interactions. At the RHF/3-21G level of theory, the relative energy of this species is rather high, 48 kcal/mol above the global minimum and therefore 28 kcal/mol above the highest energy tautomer, D2. While improved basis sets and correlation corrections will certainly modify these values, it is likely that the conclusion that the planar structure is rather unstable will not change. This is of some interest, since in solution it is possible that deprotonation might occur, potentially reducing the symmetry from $C_2$ in the neutral to $C_1$. This raises the possibility that two structural isomers might exist, separated by a very large inversion barrier with a planar backbone.

## Conclusions

The most important result of the calculations reported here is that at room temperature only the normal tautomeric form of hypericin is significantly populated in the ground state, although it is possible that other tautomers could be stabilized by particular solvents. This is consistent with earlier molecular mechanics calculations.[40] As noted in the Introduction, we have invoked a ground-state equilibrium between tautomeric or isomeric forms of hypericin, and its analogue hypocrellin, to rationalize the mirror-image symmetry between their absorption and emission spectra and the differences between their respective excited-state kinetics. The existence of such heterogeneity is supported by the observation of dependence of the excited-state kinetics of hypericin with respect to excitation or emission wavelength,[16,17,19,20] as well as that of its continuous absorption spectrum from the ultraviolet to the visible.

The absence of stable ground-state tautomeric forms in equilibrium with the normal form leads us to look elsewhere for the origin of the ground-state heterogeneity. In this light, we recall that the hypericin skeleton is twisted (Scheme 1).

As noted earlier, the $C_s$ structure, in which the backbone is forced to be planar, is not a minimum on the potential energy surface, due to the repulsive methyl−methyl and OH−OH interactions in the bay regions. It has been argued that the bay hydroxyl groups have a p$K_a$ either of 1−2[55,57] or of 7.[56] If a bay hydroxyl is deprotonated in the ground state and if the solvent prevents it from cyclizing with its un-ionized partner, then it is possible that two structural isomers might exist, separated by a very large inversion barrier. (Future calculations addressing the ionized species will need to include discrete solvent molecules in order to account for specific solute−solvent interactions.)

On the basis of the results presented here, for un-ionized tautomers at the RMP2/3-21G level of theory, we conclude that the notion of ground-state heterogeneity is still valid for rationalizing the spectra and the excited-state kinetics of hypericin but that this heterogeneity must find its origin in the deprotonation of the bay hydroxyl groups.

**Acknowledgment.** This work was supported by a grant from the National Science Foundation (CHE-9613962). The most demanding of the calculations reported here were performed on the IBM SP2 at the Cornell Theory Center, and the support of the CTC is gratefully acknowledged. Calculations were also performed on IBM workstations and high end Power Macintoshes, obtained from grants from the National Science Foundation and the Air Force Office of Scientific Research.

## References and Notes

(1) Carpenter, S.; Kraus, G. A. *Photochem. Photobiol.* **1991**, *53*, 169−174.

(2) Meruelo, D.; Lavie, G.; Lavie, D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5230−5234.
(3) Lavie, G.; Valentine, F.; Levin, B.; Mazur, Y.; Gallo, G.; Lavie, D.; Weiner, D.; Meruelo, D. *Proc. Natl. Acad. Sci. U.S.A.* **1989,** *86*, 5963−5967.
(4) (a) Hudson, J. B.; Lopez-Bazzocchi, I.; Towers, G. H. N. *Antiviral Res.* **1991,** *15*, 101−112. (b) Hudson, J. B.; Zhou, J.; Chen, J.; Harris, L.; Yip, L.; Towers, G. H. N. *Photochem. Photobiol*. **1994,** *60*, 253−255.
(5) Lopez-Bazzocchi, I.; Hudson, J. B.; Towers, G. H. N. *Photochem. Photobiol*. **1991,** *54*, 95−98.
(6) Degar, S.; Prince, A. M.; Pascaul, D.; Lavie, G.; Levin, B.; Mazur, Y.; Lavie, D.; Ehrlich, L. S.; Carter, C.; Meruelo, D. *AIDS Res. Hum. Retroviruses* **1992,** *8*, 1929−1936.
(7) Meruelo, D.; Degar, S.; Amari, N.; Mazur, Y.; Lavie, D.; Levin, B.; Lavie, G. In *Natural Products as Antiviral Agents;* Chu, C. K., Cutler, H. G., Eds.; Plenum Press: New York, 1992; pp 91−119.
(8) Treating AIDS with Worts. *Science* **1991**, *254*, 522.
(9) Thomas, C.; Pardini, R. S. *Photochem. Photobiol*. **1993**, *55*, 831−837.
(10) Thomas, C.; MacGill, R. S.; Miller, G. C.; Pardini, R. S. *Photochem. Photobiol*. **1992**, *55*, 47−53.
(11) Lenard, J.; Rabson, A.; Vanderoef, R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 158−162.
(12) Kreitmair, H. *Pharmazie* **1950**, *5*, 556−557.
(13) Linde, K.; Ramirez, G.; Mulrow, C. D.; Pauls, A.; Weidenhammer, W.; Melchart, D. *Br. Med. J.* **1996**, *313*, 253−257.
(14) Suzuki, O.; Katsumata, Y.; Oya, M.; Bladt, S.; Wagner, H. *Planta Medica* **1984**, *50*, 272−274.
(15) Anker, L.; Gopalakrishna, R.; Jones, K. D.; Law, R. E.; Couldwell, W. T. *Drugs Future* **1995**, *20*, 511−517.
(16) English, D. S.; Zhang, W.; Kraus, G. A.; Petrich, J. W. *J. Am. Chem. Soc*. **1997**, *119*, 2980−2986.
(17) English, D. S.; Das, K.; Zenner, J. M.; Zhang, W.; Kraus, G. A.; Larock, R. C.; Petrich, J. W. *J. Phys. Chem. A* **1997**, *101*, 3235−3240.
(18) Das, K.; English, D. S.; Petrich, J. W. *J. Phys. Chem. A* **1997**, *101*, 3241−3245.
(19) English, D. S.; Das, K.; Ashby, K. D.; Park, J.; Petrich, J. W.; Castner, Jr., E. W. *J. Am. Chem. Soc*. **1997**, *119*, 11585−11590.
(20) Das, K.; Smirnov, A. V.; Snyder, M. D.; Petrich, J. W. Picosecond linear dichroism and absorption anisotropy of hypocrellin: Toward a unified picture of the photophysics of hypericin and hypocrellin. *J. Phys. Chem. A*, submitted.
(21) Carpenter, S.; Fehr, M. J.; Kraus, G. A.; Petrich, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12273−12277.
(22) Kraus, G. A.; Zhang, W.; Fehr, M. J.; Petrich, J. W.; Wannemuehler, Y.; Carpenter, S. *Chem. Rev.* **1996**, *96*, 523−535.
(23) Duran, N.; Song, P.-S. *Photochem. Photobiol*. **1986**, *43*, 677−680.
(24) Diwu, Z. *Photochem. Photobiol*. **1995**, *61*, 529−539.
(25) Lown, J. W. *Can. J. Chem*. **1997**, *75*, 99−119.
(26) Fehr, M. J.; McCloskey, M. C.; Petrich, J. W. *J. Am. Chem. Soc*. **1995**, *117*, 1833−1836.
(27) Fehr, M. J.; Carpenter, S. L.; Wannemuehler, Y.; Petrich, J. W.; *Biochemistry* **1995**, *34*, 15845−15848.
(28) Sureau, F.; Miskovsky, P.; Chinsky, L.; Turpin, P. Y. *J. Am. Chem. Soc*. **1996**, *118*, 9484−9487.
(29) Chaloupka, R.; Sureau, F.; Kocisova, E.; Petrich, J. W. *Biophys. J.*, submitted.
(30) Fehr, M. J.; Carpenter, S. L.; Petrich, J. W. *Biochem. Biophys. Res. Commun.* **1994**, *4*, 1339.
(31) Racinet, H.; Jardon, P.; Gautron, R. *J. Chim. Phys.* **1988**, *85*, 971−977.
(32) Pinto, L. H.; Holsinger, L. J.; Lamb, R. A. *Cell* **1992**, *69*, 517−528.
(33) Newell, K. J.; Tannock, I. F. *Cancer Res*. **1989**, *49*, 4447−4482.
(34) Newell, K. J.; Wood, P.; Stratford, I.; Tannock, I. *Br. J. Cancer* **1992**, *66*, 311−317.
(35) Barry, M. A.; Reynold, J. E.; Eastman, A. *Cancer Res*. **1993**, *53*, 2349−2357.
(36) Li, J.; Eastman, A. *J. Biol. Chem*. **1995**, *270*, 3203−3211.
(37) Gottlieb, R. A.; Nordberg, J.; Skowronski, E.; Babior, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 654−658.
(38) Miccoli, L.; Oudard, S.; Sureau, F.; Poirson, F.; Dutrillaux, B.; Poupon, M. F. *Biochem. J.* **1996**, *313*, 957−962.
(39) (a) Brucker, G. A.; Swinney, T. C.; Kelley, D. F. *J. Phys. Chem.* **1991**, *95*, 3190−3195. (b) Strandjord, A. J. G.; Barbara, P. F. *J. Phys. Chem.* **1985**, *89*, 2355−2361. (c) McMorrow, D.; Kasha, M. *J. Phys. Chem*. **1984**, *88*, 2235−2243. (d) Brucker, G. A.; Kelley, D. F. *J. Phys. Chem.* **1987**, *91*, 2856−2861. (e) Schwartz, B. J.; Peteanu, L. A.; Harris, C. B. *J. Phys. Chem.* **1992**, *96*, 3591−3598.
(40) Etzlstorfer, C.; Falk, H.; Müller, N.; Schmitzberger, W.; Wagner, U. G. *Monatsh. Chem.* **1993**, *124*, 751−761.
(41) Etzlstorfer, C.; Falk, H.; Müller, N. *Monatsh. Chem.* **1993**, *124*, 431−439.
(42) Etzlstorfer, C.; Falk, H.; Müller, N. *Monatsh. Chem.* **1993**, *124*, 431−439.
(43) Etzlstorfer, C.; Falk, H. *Monatsh. Chem.* **1994**, *125*, 955−961.
(44) Etzlstorfer, C.; Falk, H.; Mayr, E.; Schwarzinger, S. *Monatsh. Chem.* **1996**, *127*, 1229−1237.
(45) Ulicny, J.; Ghomi, M.; Jobic, H.; Miskovsky, P.; Berthier, G. *Biol. Macromol. Dyn.*; Buttner, H., Ferrand, M., Eds.; Adenine Press: New York, 1997; pp 105m-109.
(46) Ulicny, J.; Ghomi, M.; Jobic, H.; Miskovsky, P.; Aamouche, A. *J. Mol. Struct.* **1997**, *410/411*, 497−501.
(47) Stewart, J. J. P. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1.
(48) Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc*. **1980**, *102*, 939.
(49) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys*. **1972**, *56*, 2257.
(50) Pople, J. A.; Binkley J. S. *Int. J. Quantum Chem. Symp*. **1976**, *10*, 1.
(51) Szafan M.; Karelson M. M.; Katritzky A. R.; Koput, J.; Zerner, M. C. *J. Comput. Chem.* **1993**, *14*, 371.
(52) Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A., Jr. *J. Comput. Chem*. **1993**, *14*, 1347.
(53) Nguyen, K. A.; Gordon, M. S.; Truhlar, D. G. *J. Am. Chem. Soc*. **1991**, *113*, 1596.
(54) Gordon, M. S. *J. Phys. Chem*. **1996**, *100*, 3974.
(55) Freeman, D.; Frolow, F.; Kapinus, E.; Lavie, D.; Lavie, G.; Meruelo, D.; Mazur, Y. *J. Chem. Soc., Chem. Commun.* **1994**, 891−892.
(56) Eloy, D.; Le Pellec, A.; Jardon, P. *J. Chim. Phys.* **1996**, *93*, 442−457.
(57) Altmann, R.; Falk, H. *Monatsh. Chem.* **1997**, *128*, 571−583.
(58) Borgis, D.; Hynes, J. T. In *The Enzyme Catalysis Process*; Cooper, A., Houben, J. L., Chien, L. C., Eds.; NATO ASI Series; Plenum Press: New York, 1989; Vol. 178, p 293.
(59) Wynn, J. L.; Cotton, T. M. *J. Phys. Chem.* **1995,** *99*, 4317.
(60) Sánchez-Cortés, S.; Miskovsky, P.; Jancura, D.; Bertoluzza, A. *J. Phys. Chem*. **1996,** *100*, 1938.