

The Effect of Pressure on the Excited-State Intramolecular Proton Transfer of Polyhydroxyflavone[‡]

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The effect of pressure on excited-state intramolecular proton transfer (ESIPT) is studied in three polyhydroxyflavones: 3,7-dihydroxyflavone, 3,5,7-trihydroxyflavone (galangin), and 3,3',4',7-tetrahydroxyflavone (fisetin), dissolved in poly(methyl methacrylate) (PMMA), poly(vinyl acetate) (PVAc), poly(acrylic acid) (PAA), and cellulose acetate (CAC). The unusual photophysical properties of polyhydroxyflavone in PAA are interpreted in terms of protonation of the carbonyl oxygen. We calculate the ratio of the equilibrium constant at elevated pressure to that at 1 atm, $K(p)/K(0)$, and the difference of partial molar volume between the normal state and the phototautomer, ΔV . The results indicate that pressure effectively suppresses intramolecular proton transfer in polyhydroxyflavone. $K(p)/K(0)$ and ΔV show a strong pressure dependence in the range of 10–30 kbar.

Introduction

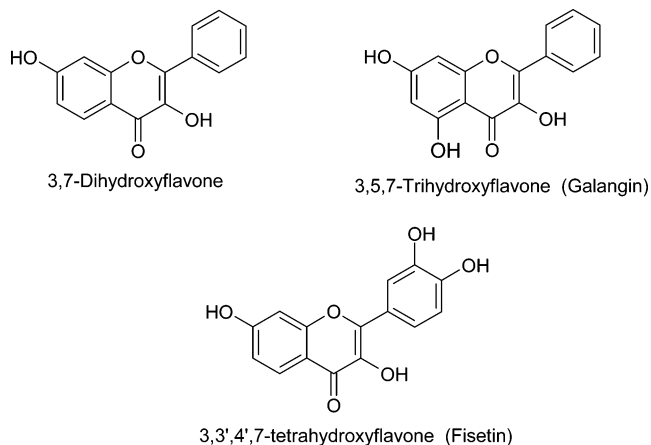
Polyhydroxyflavones, a group of natural organic compounds, have drawn extensive attention because of their anomalous photophysical properties.^{1–8} When excited by UV light, they undergo an ultrafast excited-state intramolecular proton transfer (ESIPT) accompanied by a large Stokes shift and dual emission bands. The emission bands are assigned to the normal excited state (N^*) and the phototautomer (T^*).

We have previously investigated the effect of high pressure on the proton transfer in 2-(2'-hydroxyphenyl) benzoxazole⁹ and in 3-hydroxyflavone.¹⁰ In the former case, pressure changes the distribution of isomers in the ground state; in the latter system, pressure suppresses formation of the phototautomer from the normal excited state. It is well established that intramolecular and intermolecular hydrogen bonds play an important role in ESIPT of 3-hydroxyflavone. Both intra- and intermolecular proton transfer were recently reported in 5-hydroxyflavone and 7-hydroxyflavone.¹⁶ What will happen, if we introduce possible competition from multiple hydroxyls in the chromone fragment and β -phenyl ring? To answer this question, we investigated the pressure effect on solid solutions of three polyhydroxyflavones (Scheme 1): 3,7-dihydroxyflavone, 3,5,7-trihydroxyflavone (galangin), and 3,3',4',7-tetrahydroxyflavone (fisetin). The molecules were dissolved in many different polymers. We present the results from PMMA, CAC, PVAc, and PAA, because in these media, the polyflavones all show dual emission bands, and because they are representative of the other polymers.

Experiment

3,7-dihydroxyflavone (99%), 3,5,7-trihydroxyflavone (galangin, 99%), and 3,3',4',7-tetrahydroxyflavone (fisetin, 99%) were

SCHEME 1: Structures of Three Polyhydroxyflavones



purchased from Aldrich and recrystallized in methanol before use. The polymers were also obtained from Aldrich: poly(methyl methacrylate) (PMMA, medium molecular weight), poly(vinyl acetate) (PVAc, medium molecular weight, $T_g \approx 35^\circ\text{C}$), poly(acrylic acid) (PAA, MW = 250 000, $T_g = 106^\circ\text{C}$), and cellulose acetate (CAC, acetyl content 39.8%). The polymers were used without further purification, because none emitted when irradiated at the excitation wavelength.

Sample preparation followed the procedure reported in previous papers.¹⁰ All solvents used in the experiment were photometric grade. The samples were dried and stored in a vacuum oven. The concentration of polyhydroxyflavone was 0.05 wt % in all cases. The operation of the diamond anvil cell (DAC) has been described in detail elsewhere.^{11–13} Emission is excited with the 325-nm line of a HeCd laser. The absorption apparatus utilizes light from a Xenon lamp, which is transmitted to and from the surface of the diamonds by optical fibers.

Fluorescence spectra are corrected by the efficiencies of PMT and grating. The DAC and fibers are transparent to 35000 cm^{-1} . Upon release of pressure, all spectra are highly reversible. In

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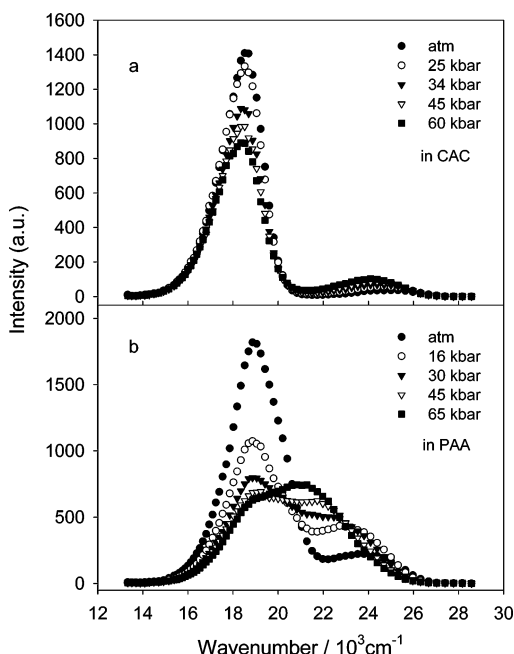
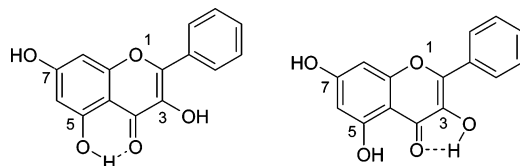


Figure 1. Fluorescence spectra of 3,7-dihydroxyflavone in CAC and PAA.

SCHEME 2: Two Possible Intramolecular Hydrogen Bonds



all cases, the results are the averages of at least two runs, with the scatter no more than $\pm 10\%$.

Results and Discussion

1. Effect of Pressure on Steady State of Polyhydroxyflavones. 3,5,7-Trihydroxyflavone (galangin) is nonfluorescent in all four polymers from 1 atm to 65 kbar, within our instrumental response limit. This result can be rationalized in terms of intramolecular hydrogen bonds. There are two possible competing intramolecular proton transfer reactions in 3,5,7-trihydroxyflavone, one is 3-H, and the other is 5-H. Intramolecular proton transfer usually occurs across a preexisting hydrogen bond.^{13,14} The two probable hydrogen bonds are shown in Scheme 2.

IR and NMR data have shown that the six-member-ring hydrogen bond is stronger than the five-member-ring one.^{15,16} So, the corresponding five-hydrogen transfer is predominating in the two competing proton-transfer reactions. In other words, the proton-transfer behavior of 3,5,7-trihydroxyflavone is more like 5-hydroxyflavone than 3-hydroxyflavone. 5-Hydroxyflavone was once thought to be nonluminescent, although later its fluorescence spectrum was obtained with laser excitation and a more sensitive detecting system.¹⁷ Its fluorescence quantum efficiency is only 4.8×10^{-6} , which is below our instrumental sensitivity. So, we will mainly discuss the results for 3,7-dihydroxyflavone and fisetin in what follows.

Typical fluorescence spectra of 3,7-dihydroxyflavone and fisetin in CAC and PAA show two peaks (Figures 1–2). The high-energy peaks increase with pressure, while the low-energy peaks decrease. It is reasonable to attribute the high-energy

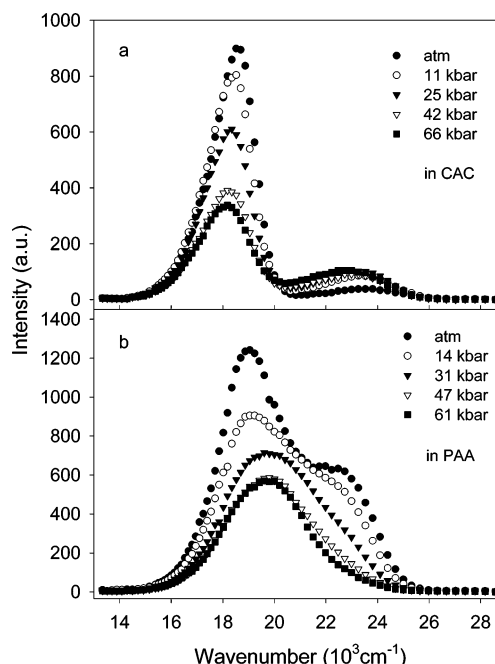


Figure 2. Fluorescence spectra of fisetin in CAC and PAA.

peaks to normal state fluorescence and the low-energy peaks to the excited-state intramolecular proton transfer products, or tautomers. For both 3,7-dihydroxyflavone and fisetin, in CAC, the position of the tautomer peaks remains unchanged, while the normal-state peaks gradually shift to the red with increasing pressure. In PMMA and PVAc, the shape of fluorescence is similar to that in CAC. In PMMA, below 10 kbar, only the tautomer peaks are observed for both compounds. In PAA, the intensities of normal-state fluorescence are higher than in other polymers at 1 atm and elevated pressure. One noticeable result is that with increasing pressure, the normal fluorescence red shifts toward the tautomer bands. As a result, above 30 kbar, the two bands are superimposed. Flavone derivatives exhibit weak fluorescence because of the efficient intersystem crossing in alkaline and neutral aqueous media. However, in acidic solution, their fluorescence intensities increase dramatically. The result has been interpreted in term of an acid–base interaction, which suppresses the intersystem crossing.¹⁸ As shown in Figure 3, absorption peaks red-shift much faster in PAA than in CAC. This implies that the protonation takes place in the ground state.

Taking account of the tautomer fluorescence positions, shapes, and the absorption spectra of fisetin in CAC and PAA, we can conclude that both the neutral and protonated molecules exist in the ground state in PAA, but the low-energy fluorescence (Figure 1b and Figure 2b) still originates from the tautomer, not from the excited state of the cation. The single broad emission bands in Figure 2b are the result of superposition of tautomer and normal fluorescence bands. Guharay et al. have observed the single broad emission band in highly polar solvents such as 2,2,2-trifluoroethanol.⁸ Two factors support our assumption: PAA is a weak polymeric acid and the normal fluorescence appears in Figure 1b at all pressures. As we suggested in a previous paper,¹⁰ neutral molecules may reach a tautomer state by one-proton transfer and by two-proton transfer as shown in Scheme 3.

2. Effect of Pressure on Equilibrium Constant *K* of 3,7-Dihydroxyflavone and Fisetin. The proton-transfer behavior of polyhydroxyflavone can be described by Birks scheme.¹⁹ The

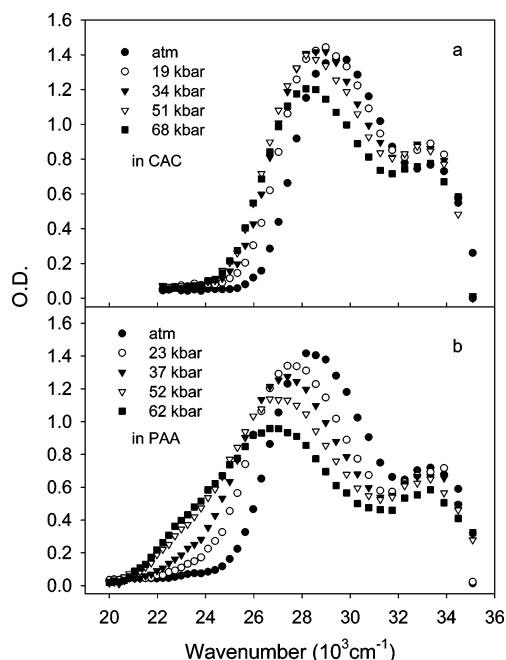


Figure 3. Absorption spectra of fisetin in CAC and PAA at different pressures.

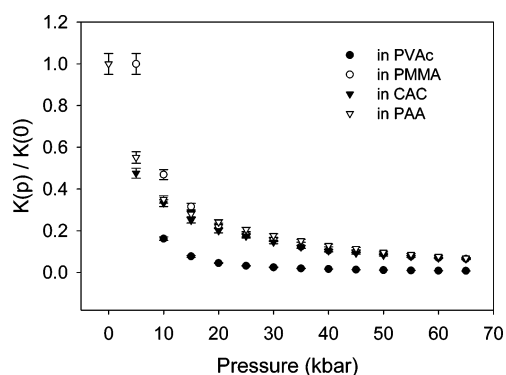
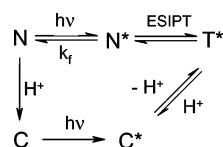


Figure 4. $K(p)/K(0)$ vs pressure for 3,7-dihydroxyflavone.

SCHEME 3: Proton-Transfer Reactions in PAA



ratio of equilibrium constant (K) is given by¹⁰

$$\frac{K(p)}{K(0)} = \frac{A_T(p)A_N(0)}{A_N(p)A_T(0)} \quad (1)$$

where p is the pressure, 0 refers to 1 atm, and A_N , A_T are the areas under the two fluorescence bands. The ratios of $A_T(p)/A_N(p)$ and $A_N(0)/A_T(0)$ are obtained by fitting the fluorescence spectra with two Gaussian peaks (using Peakfit 4). The ratio $K(p)/K(0)$ is calculated for 3,7-dihydroxyflavone (Figure 4) and fisetin (Figure 5). For fisetin in PAA, the two fluorescence bands are superimposed above 30 kbar, so this data is omitted in Figure 5.

As seen in Figure 4, $K(p)/K(0)$ drops by a factor of 5–10 between 1 atm and 10–20 kbar. This shows that pressure does not favor the intramolecular proton transfer.

3. Effect of Pressure on ΔV . ΔV is the difference in partial molar volume between the normal and tautomer states. From

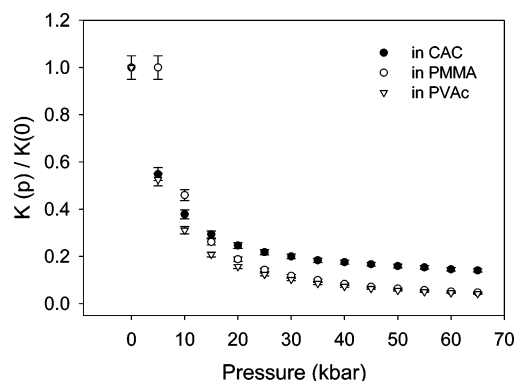


Figure 5. $K(p)/K(0)$ vs pressure for fisetin.

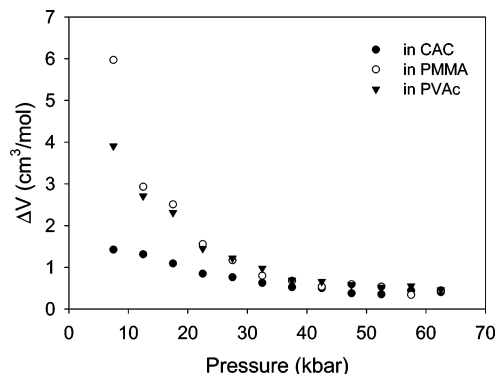


Figure 6. The effect of pressure on ΔV for 3,7-dihydroxyflavone in three polymers.

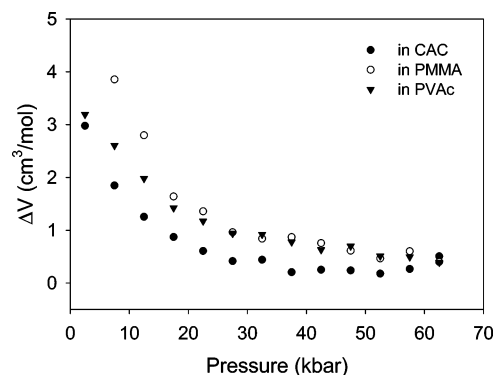


Figure 7. The effect of pressure on ΔV for fisetin in three polymers.

elementary thermodynamics, ΔV can be expressed by¹⁰

$$\Delta V = -RT \frac{\partial \ln K}{\partial p} = -RT \frac{\partial (A_T/A_N)}{\partial p} \quad (2)$$

Where R is the gas constant, $R = 8.314 \times 10^{-2} \text{ cm}^3 \text{ kbar mol}^{-1} \text{ K}^{-1}$, and T is the temperature.

The calculated values of ΔV for 3,7-dihydroxyflavone and fisetin in different polymers are shown in Figure 6 and Figure 7. ΔV decreases by over 1 order of magnitude in the range of 10–30 kbar.

Summary and Conclusions

We have investigated the effects of pressure on the excited-state intramolecular proton transfer of three polyhydroxyflavones in PMMA, PVAc, CAC, and PAA. The fluorescence of 3,5,7-trihydroxyflavone (galangin) is too weak to be observed in all matrices from 1 atm to 65 kbar. Its proton-transfer behavior is

more like 5-hydroxyflavone than 3-hydroxyflavone, which is interpreted in terms of intramolecular hydrogen bonds.

For 3,7-dihydroxyflavone and fisetin, the fluorescence intensity of the phototautomer decreases with pressure, while the fluorescence of the normal state increases. The results indicate that pressure greatly suppresses formation of the phototautomer. The abnormal photophysical behavior of 3,7-dihydroxyflavone and fisetin in PAA is the result of an acid–base interaction. The normalized equilibrium constant, $K(p)/K(0)$, and the difference in partial molar volume between normal and tautomer states, ΔV , decrease rapidly in the lower pressure region (1 atm–30 kbar). In summary, the proton-transfer behavior of 3,7-dihydroxyflavone and 3,3',4',7-tetrahydroxyflavone under pressure is similar to that of 3-hydroxyflavone, that is, the addition of the 7-OH and 3',4'-OH on the β -phenyl ring has little effect on proton transfer.

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