Subsequent isotope dilution analysis demonstrated 95% conversion of **3** into **4** within 10 h. Under similar conditions, aminoSA gave only 0.9% conversion into **4**.

The results reported here leave little doubt that AHBA, the precursor of $mC_7N$ units, is produced by the proposed new variant of the shikimate pathway shown in Scheme I. Efforts are now underway to purify and characterize the enzymes catalyzing this new metabolic pathway and to establish their structural and evolutionary relationships to the normal shikimate pathway enzymes.

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## Direct Evidence of Excited-State Intramolecular Proton Transfer in 2′-Hydroxychalcone and Photooxygenation Forming 3-Hydroxyflavone

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2′-Hydroxychalcones are widely distributed in plants and are precursors in vivo for different classes of flavonoids and isoflavonoids.[1,2] There have been several attempts to determine if 2′-hydroxychalcones are true intermediates in the biosynthesis of flavonoids.[3] Numerous reports have focused on studies of the photocyclization of 2′-hydroxychalcones, forming 4-flavanones in polar solvents.[4-6] However, to our knowledge, the relaxation dynamics of the excited 2′-hydroxychalcone have not been reported. Both NMR studies and semiempirical calculations indicate that the intramolecularly hydrogen-bonded species of 2′-hydroxychalcone (2HC, structure a in Figure 1) should dominate in the ground state. The 12.5 ppm downfield shift of the hydroxyl proton peak in benzene is indicative of the intramolecular hydrogen bond. The configuration of a is similar to *o*-hydroxybenzaldehyde types of excited-state intramolecular-proton-transfer (ESIPT) molecules.[7] Therefore, exploring the possibility of intramolecular proton transfer is intriguing and may provide valuable mechanistic information about the photochemistry of 2HC. In this communication we report the first observation of ESIPT for 2HC and its photooxygenation in nonpolar solvents, forming a well-known ESIPT molecule, 3-hydroxyflavone.

Figure 2 shows the absorption and photolysis-time-dependent emission of 2HC in aerated *n*-hexane at room temperature. For each measurement a fresh sample solution was prepared in the dark, and the emission was collected from the first six shots under the minimum excitation energy in order to avoid photodecomposition.[8] The maxima of the first and second absorption bands are ~354 and 314 nm with absorption extinction coefficients of $\sim 1.0 \times 10^4$ and $2.5 \times 10^4$ $L^{-1}$ $cm^{-1}$, respectively (Figure 2A). When we utilized a red-sensitive diode array coupled with laser excitation (the third harmonic, 355 nm, of a Nd:YAG laser), a weak and broad emission with a maximum at ~635 nm was observed (Figure 2Ba). The fluorescence yield, $\Phi_f$, is estimated to be $\sim 10^{-5}$ in *n*-hexane. Therefore, attempts to measure this emission using a commercially available fluorometer were unsuccessful. This emission is not quenched by oxygen, and its lifetime is beyond the limit of the resolution of the intensified detector (≪5 ns). Since the emission intensity is linearly proportional to the excitation energy and the prepared 2HC concentration, the possibility of the emission originating from a dimer or excimer of 2HC has also been excluded. The possibility that the observed fluorescence results from an impurity has been ruled out by employing several different methods of purification for 2HC. The intensity of the 635-nm emission is identical for all solutions prepared. In addition, although obtaining the excitation profile for this emission is not possible, no 635-nm emission was observed when the excitation wavelength was tuned from 500 to 590 nm, the likely absorption region of an impurity. We therefore conclude that the 635-nm emission originates from the $S'_1 \rightarrow S'_0$ (prime indicates the tautomer state) transition, where $S'_1$ is populated through $S_0 \rightarrow S_1$ absorption of conformer a followed by rapid ESIPT (Figure 1).

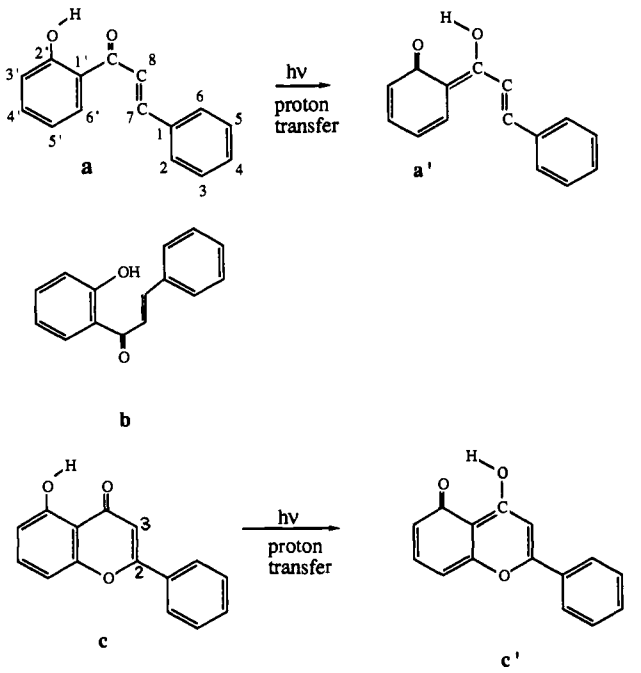


**Figure 1.** Structure of a, 2HC (normal, closed form); a′, 2HC (tautomer form); b, 2HC (normal, open form); c, 5-hydroxyflavone (normal form); and c′, 5-hydroxyflavone (tautomer form).

This observation is consistent with our earlier proposal that the tautomer emission wavelength can be qualitatively predicted using the simple Huckel approach by counting the number of nonaromatic conjugated double bonds of the tautomer.[9] For the case of 5-hydroxyflavone (Figure 1c) the tautomer species (c′) has five conjugated double bonds and exhibits an emission maximum at ~670 nm, which is ~3000 $cm^{-1}$ lower in energy than that of 5-hydroxyflavanone, in which the $C_2$–$C_3$ double bond is hydrogenated. Since the 2HC tautomer (a′) has a similar structure and the same number of nonaromatic conjugated double bonds as 5-hydroxyflavone, the assignment of the 635-nm band to tautomer emission can be rationalized.

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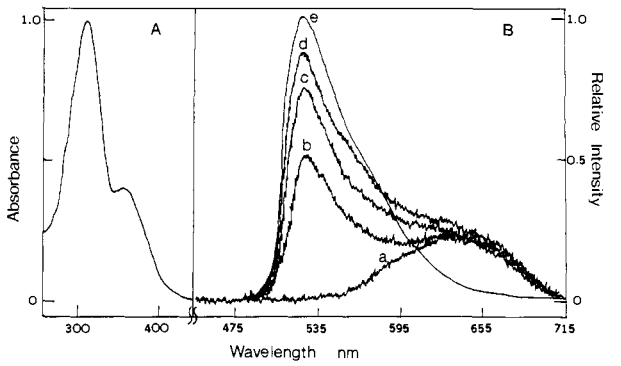
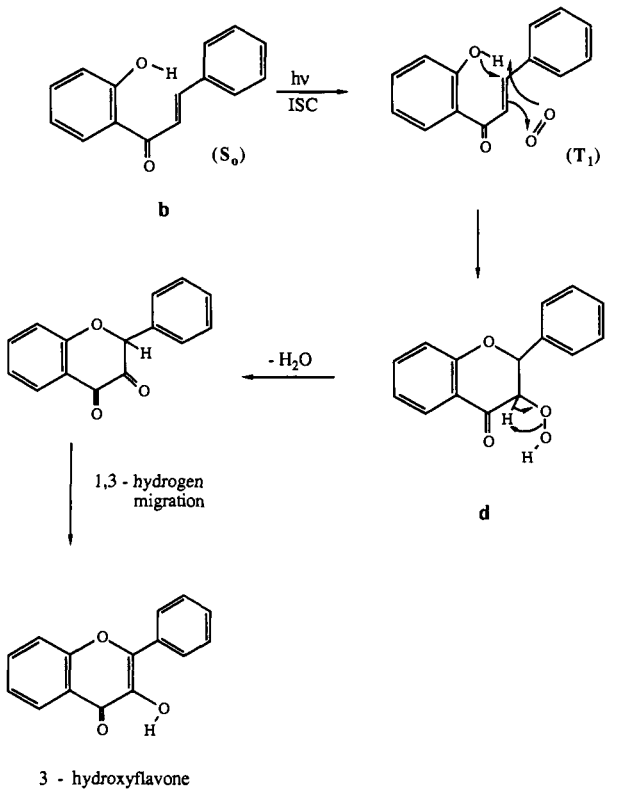



**Figure 2.** (A) The absorption spectrum of 2HC. (B) The time-dependent emission spectra of 2HC excited at 355 nm (5 mJ/cm$^2$, 2 Hz) with a time period of (a) 6 shots, (b) 1 min, (c) 3 min, (d) 5 min. The emission of 3-hydroxyflavone is shown in part e.

The failure to observe this emission previously may be partially due to its low yield and unexpected long wavelength. However, the major reason is believed to be the dominant interfering green emission of the photoproduct. The photolysis-time-dependent fluorescence spectra of 2HC in aerated *n*-hexane are also shown in Figure 2B. The growth of a time-dependent shorter-wavelength emission band is apparent. The maximum of this time-dependent green emission is ~525 nm. After 355-nm photolysis (5.0 mJ/cm$^2$, 2 Hz) for 30 min, where the ratio of the intensity at 525 nm to that at 670 nm is ~20, it is convenient to examine the excitation origin of the 525-nm band by conventional fluorometric techniques. The excitation spectrum monitored at 525 nm has a maximum at 338 nm with spectral features identical to those of 3-hydroxyflavone, in which the occurrence of excited-state intramolecular proton transfer, resulting in tautomer emission, has been extensively studied.[10] In addition, both photoproduct and 3-hydroxyflavone give the same fluorescence lifetime of 3.2 ± 0.5 ns in *n*-hexane. Thus, the assignment of the 525-nm emission of the photoproduct to 3-hydroxyflavone is definitive. It is noted that the absorption spectrum of 2HC changed negligibly during the photolysis. In addition, the intensity of the 635-nm tautomer emission holds nearly constant (see Figure 2B), indicating that the yield of 3-hydroxyflavone is small. However, a trace of 3-hydroxyflavone production can give observable tautomer emission due to its unusually high yield ($\Phi \sim 0.36$).[11] No 3-hydroxyflavone emission was observed when the solution was degassed by three freeze–pump–thaw cycles.

The reaction of 2′-hydroxychalcones with $^1\Delta_g$ $O_2$ has been studied using a dye sensitization technique to verify the potential role of $^1\Delta_g$ $O_2$ in the genesis of flavonoids.[12-14] Thus, the role of singlet molecular oxygen in 3-hydroxyflavone formation through direct photolysis of 2HC was investigated by solvent deuterium isotope dependent studies. The yield of 3-hydroxyflavone measured by the intensity of the tautomer emission is the same in cyclohexane ($C_6H_{12}$) and $C_6D_{12}$, while the ratio of the lifetime ($\tau$) of

**Scheme I**




$^1\Delta_g$ $O_2$, $\tau(C_6D_{12})/\tau(C_6H_{12})$, is ~15.[15] Similar results were observed in benzene and benzene-$d_6$ in which the $\tau(C_6D_6)/\tau(C_6H_6)$ of $^1\Delta_g$ $O_2$ is even higher (~20).[16] Thus the participation of $^1\Delta_g$ $O_2$ in the formation of 3-hydroxyflavone can be eliminated in the direct photolysis of 2HC. Molecular analysis indicates that structure b rather than a is favorable for the formation of 3-hydroxyflavone (see Scheme I). However, we note that the NMR study indicates that conformer a dominates in nonpolar solution due to the intramolecular hydrogen bond formation. Therefore, proposing conformer b as a precursor for photooxygenation is a fairly severe assumption. It implies the existence of a trace, but non-negligible, concentration of conformer b in equilibrium.[17] The stabilization of conformer b may be rationalized by the weak interaction between the 2′-hydroxyl oxygen and $C_7$, which carries a partially positive charge due to the conjugation of the $\alpha,\beta$-unsaturated ketone. This also suggests the alternative interpretation that the population of b is due to a hydrogen-bonding impurity in *n*-hexane. Although several different solvent-drying methods were employed and the tautomer emission of 3-hydroxyflavone was still observed during the photolysis, the role of solvent impurity cannot be ascertained at this stage.

In summary, a photooxygenation mechanism incorporating a spin-allowed triplet state of conformer b with $^3\Sigma_g$ $O_2$ is tentatively proposed in Scheme I. The proposed mechanism involves the attack of the 2′-hydroxyl group on $C_7$ to give a hydrogen peroxide intermediate d, which subsequently undergoes $H_2O$ elimination and 1,3-hydrogen migration, forming the product 3-hydroxyflavone. In the dye-sensitized $^1\Delta_g$ $O_2$ + 2HC reaction, despite unsuccessful attempts to isolate free hydroperoxide, the hydroperoxide intermediate could be inferred from the positive KI–AcOH test.[12-14] A similar intermediate is reasonably expected in the case of direct photolysis of 2HC.

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(17) It is believed that the concentration ratio [b]:[a] is $\ll 10^{-2}$. The normal Stokes shifted emission of b was not detectable in this study.