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ORIGINAL PAPER

Distinct Photoacidity of Honokiol from Magnolol

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Abstract Honokiol, 5,5′-diallyl-2,4′-dihydroxy- biphenyl, by comparison with its isomer magnolol, 5,5′-diallyl- 2,2′-dihydroxy- biphenyl, has been characterized by steady-state and time-resolved spectroscopy as well as ^{1}H NMR. Honokiol shows more complex pH dependence of absorption and fluorescence characteristics compared with magnolol. Honokiol possesses much weaker acidity than magnolol both in the ground and excited states. Its weak photoacidity is similar to that of 4-hydroxy- biphenyl or 4, 4′-dihydroxy- biphenyl rather than 2-hydroxy- biphenyl or 2, 2′-hydroxy- biphenyl. The electron effect and geometry configuration of substitution has been discussed.

Keywords Honokiol · Photoacidity · Substitution effect · Geometry configuration · Magnolol

Introduction

Honokiol and its structural isomer magnolol are biphenolic compounds present in the cones, barks, and leaves of *Magnolia officinalis* that has been used in the traditional Chinese medicine “houpu”. The compounds have activities of anxiolytic [1, 2], anti-bacterial [3, 4], and inhibiting contraction of smooth muscle [5]. In the late 1990s, honokiol has been found as a potent, highly tolerable anti-tumorigenic [6–8], and neurotrophic [9] compound. The distinguishable pharmacological effect of honokiol different from that of magnonlol evokes increasing interests of chemists and pharmacologists.

As shown in Fig. 1, the difference in structure between the two compounds exists: (1) They both have the same substituted groups such as 2-OH, 4- and 4′-allyl except that honokiol possesses 4′-OH while magnolol possesses 2′-OH; (2) Honokiol is asymmetric whereas magnolol is symmetric; (3) The dihedral angle of biphenol skeleton of honokiol is 57.14º [10] which is larger than 44.9º of magnolol [11], owing to the intramolecular H-bond between 2- and 2′- OH groups in the molecule of magnonol.

The acid-base properties of drug compounds are important to certain biochemical processes such as biological uptake, activity, transport and distribution [12–15]. Proton transfer, both in ground and excited states, plays a key role in many biological processes. Hydroxyl aromatic compounds are photoacids of which the acidity greatly increases upon spectroscopic excitation from the ground state. The proton-transfer reactions in ground and excited states of compounds are dependent on their electronic structures. In this work, we were interested in the photoacidity of honokiol which is quite distinct from that of magnolol.

Material and Methods

Honokiol and magnolol with 99% purity were purchased from Jiangsu Institute of Drug Control China. The water was redistilled without deoxygenation. Methanol was of analytical grade. The buffer solutions with pH from 4 to 12 were prepared to 0.05 mol·L^{-1} phosphate solutions that

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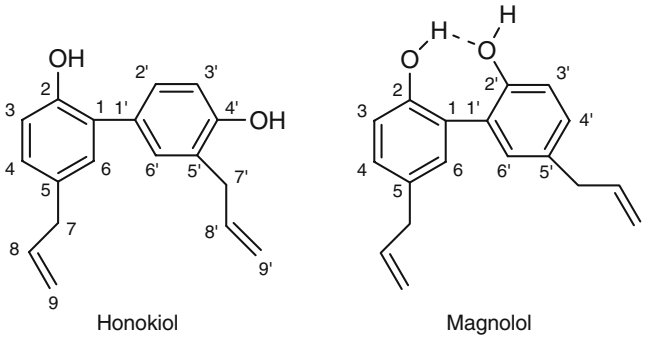

Fig. 1 Structures of honokiol and magnolol

were adjusted by sodium phosphate and hydrochloric acid, those with pH<4 and pH>12 were prepared by hydrochloric solution and sodium hydroxide solution, respectively. The pH was measured with a PHS-2C pH-meter (Shanghai Analytical Instrumental Factory, China). The ionic strength of the buffers was adjusted to 0.1 by a solution of potassium chloride.

Honokiol or magnolol was dissolved in methanol to prepare a 10^{-4} mol·L^{-1} stock solution. The stock solution was diluted with the buffer solutions to the concentration of 10^{-5} mol·L^{-1} for absorption determination, and 10^{-6} mol·L^{-1} for fluorescence determination, respectively. All experiments were performed at room temperature (22ºC). The absorption and steady-state fluorescence spectra were recorded on a Shimadzu UV-2100PC spectrometer and a Shimadzu FR-5301 spectrofluorometer respectively. ^{1}H-NMR spectra were recorded in the mixture of MeOD-D_2O v/v 5/5 on a Varian Gemimi series 300 MHz spectrometer. Transient fluorescence was detected using the time-correlated single-photon counting (TCSPC) method on an Edinburgh instrument.

Results and Discussion

Honokiol is a diprotic acid. It ionizes to form the monoanion and dianion species based on pH in both ground state and excited states. In this work, the relationship between deprotonation and spectral characteristics has been revealed for honokiol as shown in Fig. 2. Honokiol has been investigated by comparison with magnolol. To be convenient, H_2A, HA^- and A^{2-} denote the neutral, monoanion and dianion honokiol, H_2B, HB^- and B^{2-} denote the neutral, monoanion and dianion magnolol, respectively.

Absorption Spectra

The absorption spectra of honokiol in the wide pH range in aqueous solution shown in Fig. 3 (A1–4), demonstrate transformation between H_2A, HA^- and A^{2-}. At pH below 8.6, the absorption spectra with the lowest energy absorption peak at 290 nm, which are attributed to the neutral species H_2A, are changeless as shown in Fig. 3-A1. With pH increasing from 8.6, the spectra begin to make a change, which could be divided into such stages as: (1) From 8.6 to about 9.8, the peak at 253 nm decreases concomitant with the peak at 290 nm increasing; (2) From 9.8 to 10.5, the lowest energy peak increases concomitant with a significant red-shift to 297 nm, as shown in Fig. 3-A2; (3) From 10.5 to about 12.0, the continual red-shift occurs over a wide range from 297 nm to 314 nm, as shown in Fig. 3-A3; (4) Further basification up to pH above 12, the single absorption peak remains stable at 314 nm, as shown in Fig. 3-A4.

In comparison with honokiol, magnolol is much simpler in the pattern of absorption spectral change with pH. As shown in Fig. 4A, the absorption spectra of magnolol with the lowest energy absorption peak at 284 nm are invariable below pH 6; the peak at 284 nm disappears and a new peak at 316 nm arises with pH increasing from 6 to about 10. The spectral transformation can be attributed to the first deprotonation, the peaks at 284 nm and 316 nm are assigned to H_2B and HB^-, respectively. In the pH range from 10 to 13, the spectra remain invariable to that of HB^-. The absorption titration curves are presented as the inset in Fig. 4A. At pH above 13, however, the lowest energy absorption undergoes a blue-shift upon further basification, which can be attributed to the second deprotonation.

NMR Spectra of Honokiol

The variation of absorption spectra of honokiol with pH is confusing. To demonstrate the response of honokiol to pH changing, we have measured the H^1-NMR spectra in the range of pH from 7 to 14. The experiments were performed in 5/5 v/v CD_3OD-D_2O mixture because of the poor solubility of honokiol in pure water. The H^1-NMR spectra are presented in Fig. 5. No signal of new hydrogen atom is observed, which suggests that only proton dissociation reaction occurs.

Because the dissociation rate is higher than the NMR time scale, only a single peak of an adjacent nucleus can be observed at intermediate position [16, 17]. The observable chemical shift δ_{obs} is the average of the individual chemical shifts of the species H_2A (δ_{H_2A}), HA^- (δ_{HA^-}) and A^{2-} (δ_A^{2-}), the weighting coefficients being the pH dependent molar fractions (x_{H_2A}, x_{HA^-} and x_A^{2-}): $\delta_{obs} = x_{H_2A}\delta_{H_2A} + x_{HA}\delta_{HA} + x_{A-}\delta_{A-}$. So the deprotonation constant can be estimated by NMR titration [17, 18]. The pH dependence of the chemical shifts has been shown in Fig. 6. However, in all the titration curves only one inflection point is observed. The aromatic hydrogens H2′, H3′ and H6′ are sensitive to deprotonation of 4′-OH,

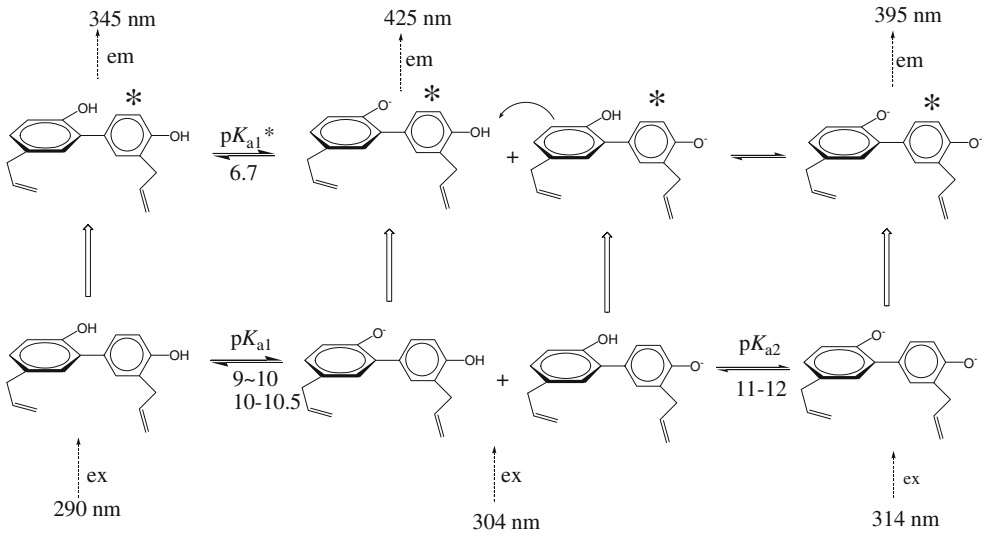

Fig. 2 Spectral characteristics and deprotonation of honokiol in the ground and excited state

whereas H3, H4 and H6 to 2-OH. Among all of the hydrogen atoms, H3′ and H3 are the most sensitive. In their titration curves the inflection points are at around pH 11.5 and 11.8, respectively. This demonstrates that pK_a of the two OH groups are very close and the acidity of 2-OH is slightly higher than that of 4′-OH, which is confirmed by the signals of the other aromatic hydrogen atoms. The inflection points of the titration curves are at pH 10.9, 11.6 for H2′, H6′ *vs.* those at 11.8, 12.0 for H4, H6 respectively.

Ground-state Deprotonation Constants of Honokiol

Honokiol has two monoanion species, of which the absorption spectra should be alike and different to each other. Obviously, the observed absorption spectrum is the addition of the individual spectra according to their molar fractions. ^{1}H-NMR titration has demonstrated that the ground-state acidity of 2-OH and 4′-OH is similar but not exact the same. So the small difference in acidity between 2-OH and 4′-OH results in appearance of absorption spectra changing complexly with pH for honokiol. In the range of pH from 8.6 to 9.8 the change is mainly due to the deprotonation of 2-OH, with pH increasing about from 9.8 the change mainly due to deprotonation of 4′-OH. At pH 10.5, honokiol exits mainly in the monoanion HA^-. It is estimated approximately that the value of pK_a of 2-OH is 9~10 and the pK_a of 4′-OH is 10~10.5. The spectral transformation of honokiol in the range of pH from 10.5 to 12 is attributed to the second proton dissociation. The value of the p$K_{a,2}$ is estimated approximately in the range of 11~12. With respect to magnonol, the two 2-OH groups are chemical equivalent because of the symmetrical structure, so there is one monoanion species which simplifies the pH dependence of the spectrum.

Steady-state Fluorescence Spectra

The pH dependence of fluorescence spectra of honokiol is complex in aqueous solution. The spectral transformation undergoes the following stages as shown in Fig. 3 (F1-4): (1) In acidic solutions, the fluorescence displays an emission at 345 nm with the excitation band at 290 nm. With pH increasing from 5 to 8.6, the emission at 345 nm slowly decreases while the emission at 425 nm slowly increases. (2) With pH increasing from 8.6 to around 10.5, the spectral transformation is accelerated, and the new fluorescent species with $\lambda_{ex}/\lambda_{em}$ at 304/425 nm becomes dominating. (3) With pH further increasing to 12, the fluorescence species with $\lambda_{ex}/\lambda_{em}$ at 290/345 nm is disappearing and that with $\lambda_{ex}/\lambda_{em}$ at 304/425 nm is decreasing in concomitant with another new fluorescence with $\lambda_{ex}/\lambda_{em}$ at 314/395 nm emerging. (4) With pH increasing from 12 to 14, the fluorescence with $\lambda_{ex}/\lambda_{em}$ at 314/395 nm is dominating. To sum up, honokiol changes its fluorescence from $\lambda_{ex}/\lambda_{em}$ at 290/345 nm into $\lambda_{ex}/\lambda_{em}$ at 304/425 nm, then into $\lambda_{ex}/\lambda_{em}$ at 314/395 nm with pH increasing, which could be assigned to H_2A, HA^- and A^{2-}, respectively. As shown in Fig. 7, two inflection points exist at pH 6.7 and 9.6 in the curve of the fluorescence intensity of $\lambda_{ex}/\lambda_{em}$ at 290/345 nm *vs*. pH. Considering the excited species HA^-* emits rather lower at 345 nm, the values of pK_{a1} and pK_{a1}* of honokiol are estimated approximately to be 6.7 and 9.6 according to the inflection points in the titration curve.

In aqueous solutions over a broad pH range, magnolol has an invariable emission at 400 nm, which is attributed to the excited species HB^-* as shown in Fig. 4F. The pH dependence of the fluorescence intensity can be described as the following: (1) At pH below 4, the fluorescence intensity increases with pH increasing. (2) In the pH range

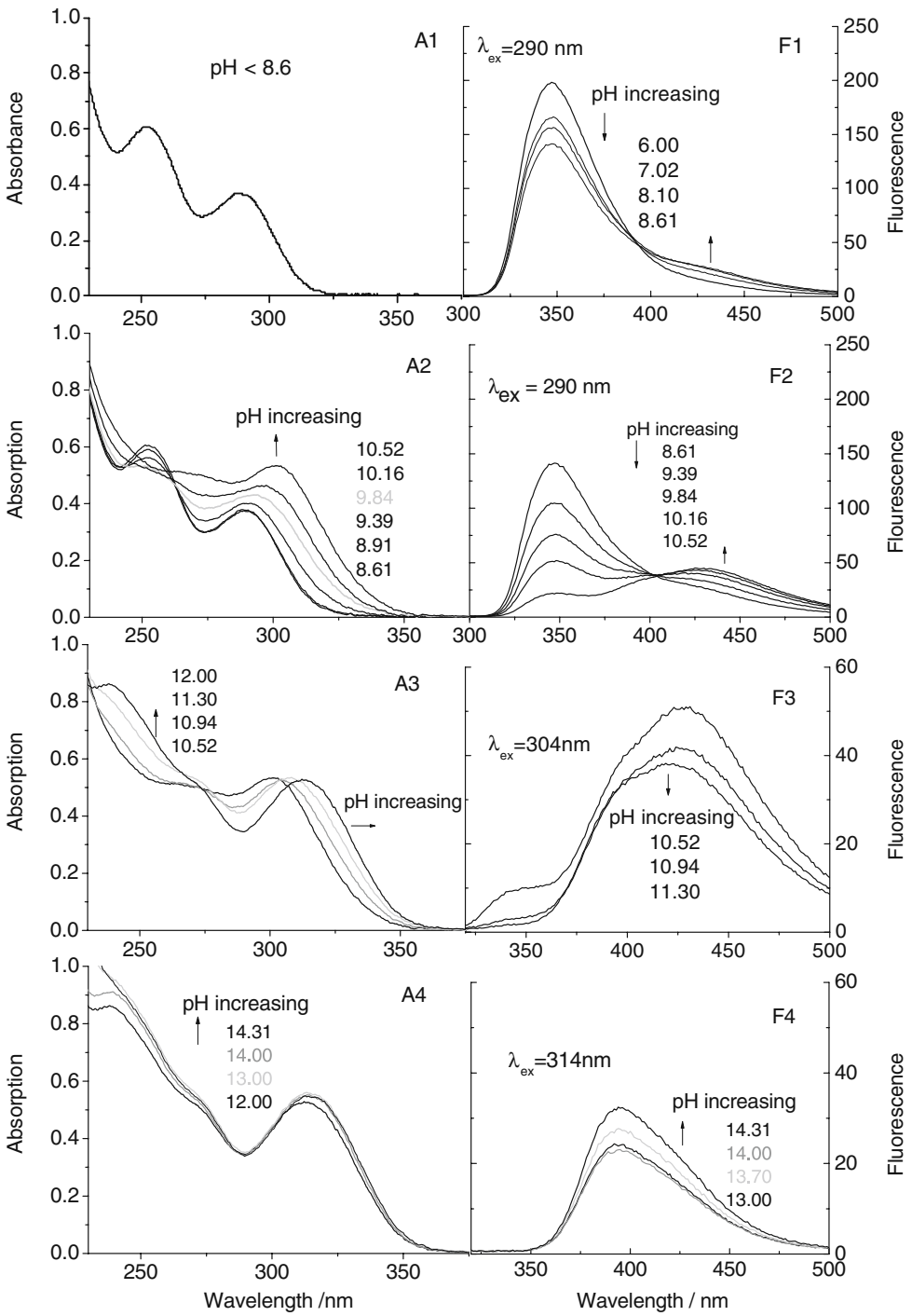

Fig. 3 Absorption (A1, A2, A3 and A4) and fluorescence (F1, F2, F3 and F4) spectra of honokiol under different pH conditions

from 4 to 6, the fluorescence is invariable. (3) When pH increasing from 6 to 10, the excitation band red-shifts from 284 nm to 316 nm, and the fluorescence intensity excited at 316 nm increases. The excitation spectral transformation matches the absorption well. (4) With pH increasing from 10 to 13, the spectra remain invariable. (5) When pH increasing above 13, the fluorescence intensity rapidly decreases.

To investigate the behaviors of honokiol in different protophilic solvents, we have also measured its fluorescence spectra in methanol and in water-ethanol mixtures, as shown in Fig. 8A 1-2. Honokiol displays the fluorescence of H_2A with $\lambda_{ex}/\lambda_{em}$ at 290/345 nm in pure and acidic methanol and the fluorescence of HA^- with $\lambda_{ex}/\lambda_{em}$ at 304/425 nm in basic methanol. The spectral pattern is the same in water as in pure methanol. But the fluorescence intensity

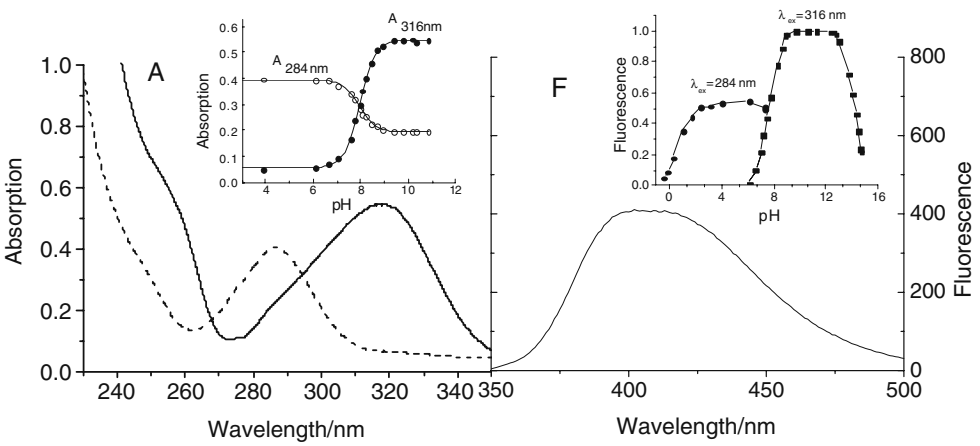

Fig. 4 Absorption (A) and fluorescence (F) spectra of magnonol (The insets are the titration curves of absorption and fluorescence)

decreases with water content increasing in the methanol-water mixtures. In contrast to honokiol, magnolol displays the emission of the neutral species H_2B at 355 nm only in methanol, as shown in Fig. 8B 1-2. It undergoes a transformation from the emission at 355 nm to that at 400 nm with water content increasing in the methanol-water mixtures.

Time-resolved Fluorescence Measurement of Honokiol

The time-resolved fluorescence of honokiol in phosphate buffer solutions in pH range from 6.0 to 12.0 has been detected using TCSPC technique. The monitoring wavelengths $\lambda_{ex}/\lambda_{em}$ were selected at 290/345 and 304/425 nm for H_2A^* and $HA^{-}*$, respectively, considering a minimal overlap between the emission bands of H_2A^* and $HA^{-}*$ (see Fig. 8A2). The results of de-convolution fit, such as lifetimes τ, pre-exponential factors α and goodness of fit χ^2, are collected in Table 1. The emission of H_2A^* at 345 nm decays single-exponentially at pH 6.0, and two-exponentially at pH above 6.0. The emission of $HA^{-}*$ at 425 nm decays single-exponentially at pH 9.19, two-exponentially when pH above 9.19, and three-exponentially at pH 12.0.

The single-exponential delay of H_2A^* suggests the absence of excited state proton transfer at pH 6. The delay time determined to be 1.7 ns is the lifetime of H_2A^*. At pH above 6, the fluorescence delay with $\lambda_{ex}/\lambda_{em}$ at 290/345 nm is two-exponential due to the deprotonation of H_2A^*. As shown in Table 1, the pH dependence of the

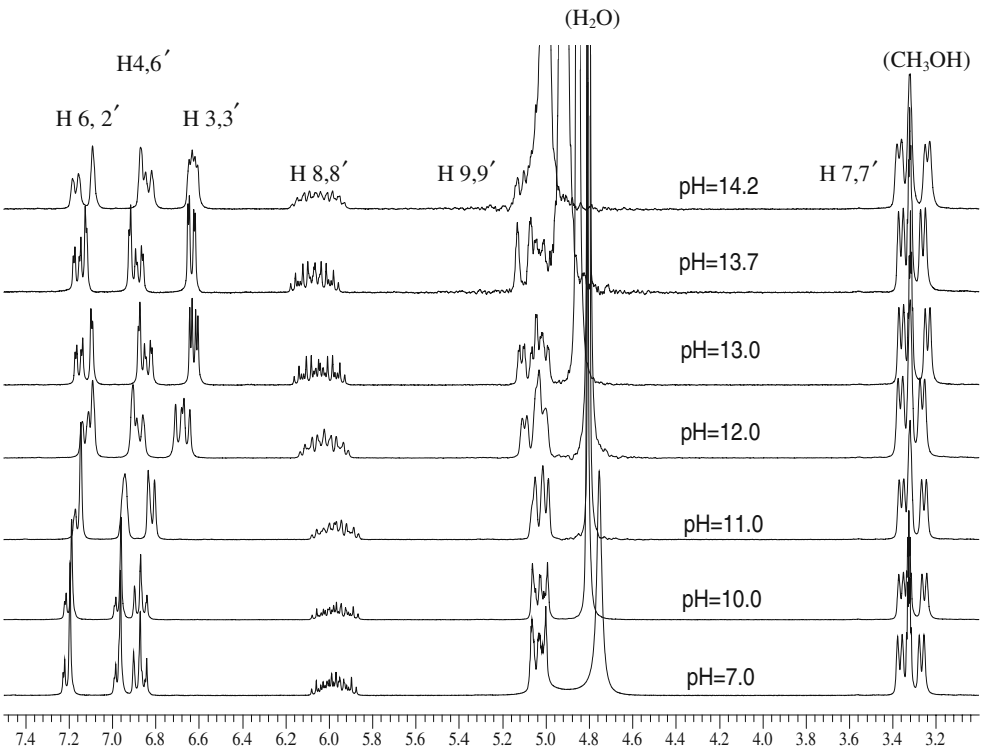

Fig. 5 H^1-NMR spectra of honokiol in mixture of CD3OD-D2O v/v 5/5 under different pH conditions

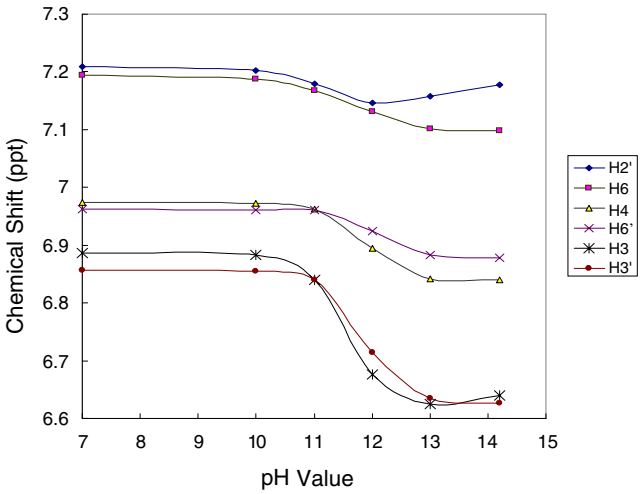

Fig. 6 H^1-NMR titration curves of honokiol in the mixture of CD3OD-D2O v/v 5/5

lifetimes τ_1 and τ_2 suddenly changes at pH 9.2, this may be caused by that the ground state deprontonation of H_2A occurs. Because of feeble fluorescence of H_2A* at 425 nm, the emission of H_2A* at 425 nm can be ignored, so the time-resolved signal of HA^-* is single-exponential at pH 9.2. Its delay time is 1.7 nm. However, with pH increasing from 9.8, the emission displays two-exponential, the delay time and the pre-exponential factor α depend on pH. In this case, HA^- exists in two anions, which are excited simultaneously at 304 nm resulting in two excited species of HA^-*. Thus, the decay curve is due to the delay of the two excited species of HA^-*. The values of τ and α are essentially dependent on not only the molar extinction coefficients at 304 nm and the concentrations of the two ground-state species, but also the kinetic parameters of conversion between the two excited species. When pH increasing to 12.0, the three-exponential delay of the fluorescence with $\lambda_{ex}/\lambda_{em}$ at 304/425 nm demonstrates the presence of the new excited species A^{2-}*, this agrees with the steady-state spectra of honokiol at pH 12.0.

Excited-state Deprotonation of Honokiol

Acidity of aromatic hydroxyl greatly increases upon spectroscopic excitation from the ground state [19, 20]. Magnolol is a "super" photoacid with the excited-state constant $pK_{a,1}$* determined to be 0.57, 7 units lower than the ground-state constant $pK_{a,1}$ of 7.54 [21]. Such photoacidity of magnolol promotes the neutral magnolol molecule to transfer the proton to the water molecule upon excitation, resulting in the emission of the excited monoanion HB^-* at 400 nm even at very low pH. For the same reason, the emission of H_2B* at 355 nm decreases and that of HB^-* at 400 nm increases with water content increasing in the methanol-water mixtures.

In contrast with magnolol, honokiol is a rather weaker photoacid. The excited konokiol H_2A* is a too weak acid to transfer its proton to water. As discussed above, in the ground state honokiol exists overwhelmingly in the form of H_2A when pH below 8.6, so the fluorescence spectra display the emission of H_2A* in methanol, methanol-water mixtures, and even in pure water. With pH increasing from 5, however, the emission of H_2A* begins to decrease and the emission of HA^-* increases, whereas with pH increasing from 8.6 the absorption of H_2A begins to change. The asynchronous changes of emission and absorption with pH demonstrate that honokiol is somewhat photoacidic. With monitoring the fluorescence of $\lambda_{ex}/\lambda_{em}$ at 290/345 nm upon pH in the range from 5 to 8.6, the value of pK_{a1}* of honokiol is estimated by fluorescence titration to be 6.7, which is about 3 units lower than that of pK_{a1}. The photoacidity of honokiol is also confirmed by the time-resolved measurement. The time-resolved signal of H_2A* is single-exponential in the absence of excited state proton transfer at pH 6. Above pH 6, H_2A* delays two-exponentially, indicating that excited state proton transfer occurs. This agrees to that the steady-state fluorescence of $\lambda_{ex}/\lambda_{em}$ at 290/345 nm decreases faster above pH 6, as shown in Fig. 7.

The spectral shifts that occur upon dissociation in the excited state can be used to calculate the change in pK_a that occurs upon excitation. This is known as the Förster cycle [19].The difference in pK_a values between the ground and excited states of AH and A^- can be estimated by

$$pK_a - pK_a^* = \frac{Nh(\nu_{HA} - \nu_{A^-})}{2.303RT}$$

where ν_{HA} and ν_{A^-} are the frequencies of the lowest absorption bands of the neutral form and the deprotonic form; R is the gas constant; T is the temperature; h is the Planck constant and N is Avogadro's number. Supposing the value of pK_{a1}* of honokiol is 6.7, its pK_{a1} is estimated to be 10.0 by the Förster cycle. This value of pK_{a1} is very close to the value determined by absorption titration.

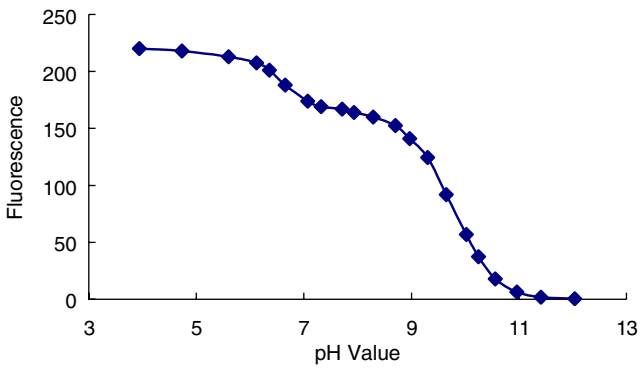

Fig. 7 Curve of the fluorescence intensity *vs*. pH with $\lambda_{ex}/\lambda_{em}$ at 290 /345 nm for Honokiol

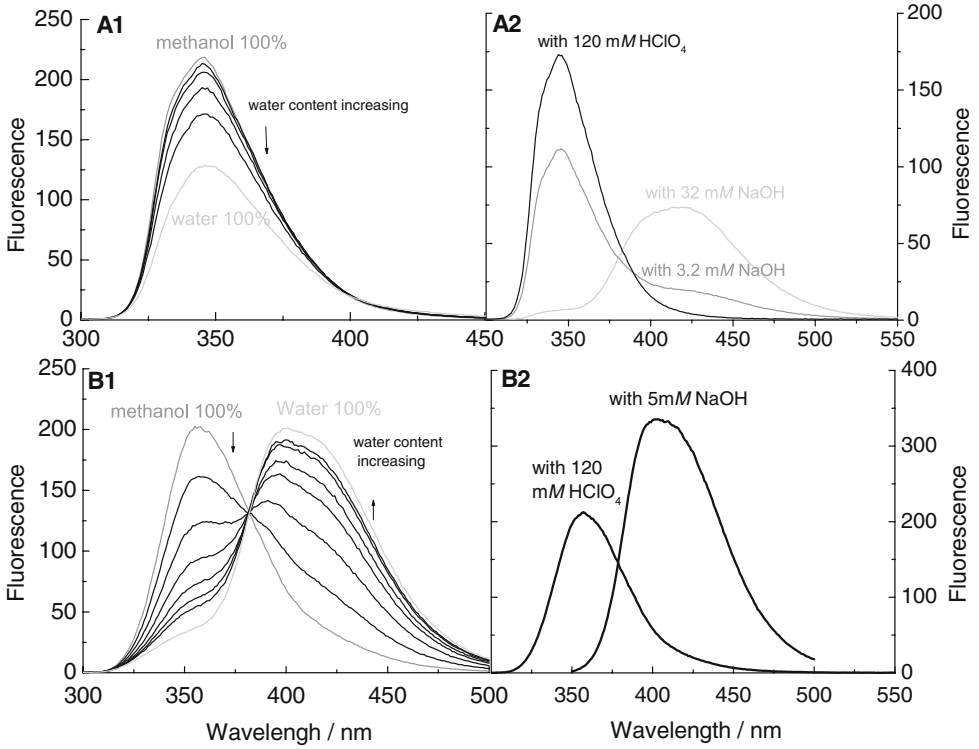

Fig. 8 Emission spectra of honokiol (A1, A2) and magnolol (B1, B2) in methanol-water mixtures (A1, B1) and in acidic or basic methanol (A2, B2)

Differences in Acidity between Honokiol and Magnonol

The substitution effect on deprotonation has been investigated extensively for phenol [22–24]. The extent of deprotonation depends on the extent to which electrons are withdrawn from the oxygen atom to the aromatic ring. An electron substituent will donate (or withdraw) electrons to (or from) the *ortho* and *para* positions in the ground state, but to the *ortho* and *meta* positions in the excited state. This causes differences in behavior between substituted aromatic hydroxyls in the ground and excited states [24–26].

For 4-hydroxy-biphenyls, the electron withdrawing group of phenyl is at the *para* position of 4-OH and affects 4-OH mainly in the ground state; for 2-hydroxy-biphenyls, the phenyl group is at *ortho* position of 2-OH and affects 2-OH both in the excited and ground states. This agrees with the fact that pK_a* of 4-hydroxy-biphenyl is 8-9 units higher than that of 2-hydroxy-biphenyl whereas the values of their pK_a are close to each other [27, 28]. For the sake of being clear in comparison, the pK_a of those hydroxybiphenyl compounds is collected in Table 2. As shown in Table 2, 2-hydroxyl- and 2, 2′-dihydroxy-biphenyl possesses strong photoacidity. They decrease the values of pK_{a1} upon excitation by about 9 or 7 units of pK. With respect to 4-Hydroxy- and 4, 4′-dihydroxy-biphenyl, they barely show excited-state deprotonation, 4, 4′-dihydroxy-biphenyl shows absorption and fluorescence characteristics similar

Table 1 Results of deconvolution fit for the fluorescence decays of honokiol under different pH conditions

pH	$\lambda_{ex}/\lambda_{em}$=290/345nm					$\lambda_{ex}/\lambda_{em}$=304/425nm						
	τ_1 /ns	α_1	τ_2 /ns	α_2	χ^2	τ_1 /ns	α_1	τ_2 /ns	α_2	τ_3 /ns	α_3	χ^2
6.02	1.69	1.00			1.028		–	–	–	–	–	–
6.62	1.35	0.98	12.0	0.02	1.056		–	–	–	–	–	–
7.73	1.32	0.97	6.73	0.03	1.039		–	–	–	–	–	–
8.67	1.28	0.97	14.4	0.03	1.059		–	–	–	–	–	–
9.19	1.49	0.97	6.31	0.03	1.031	1.70	1.00	–	–	–	–	1.056
9.76	1.47	0.97	5.41	0.03	1.005	1.47	0.93	4.88	0.07	–	–	1.045
10.71	1.50	0.97	0.32	0.03	1.016	1.00	0.63	2.44	0.37	–	–	1.194
12.02	–	–	–	–	–	0.51	0.22	1.60	0.75	8.34	0.03	1.110

Table 2 Collection of pK_a of hydroxybiphenyl compounds ('a' from ref. 27, 'b' from ref. 28, 'c' from ref. 29)

	pK_{a1}	pK_{a1}*	pK_{a2}
[a,b] 2-hydroxy-	10.0	1–2	
[c] 2,2′-dihydroxy-	7.2	0.6	14.2
[a,b] 4-hydroxy-	9.55	9–10	
[a] 4,4′-dihydroxy-	9–10	8.5–9.5	11–12

to that of 4-hydroxy-biphenyl except that above pH 12 [28, 29]. The dihydroxybiphenyl compounds have similar acidity to their corresponding monohydroxybiphenyl compounds except that 2, 2′-dihydroxy-biphenyl has ground-state acidity much stronger than 2-hydroxy-biphenyl. This could be explained by the H-bond between 2-OH groups in the molecule of 2, 2′-dihydroxy- biphenyl, which stabilizes the monoanion of 2, 2′-dihydroxy- biphenyl.

Although the exact values of pK_a of honokiol are not obtained because of the spectral overlapping, it is estimated approximately to be 9~10 and 10~10.5 for pK_{a1} and 6.7 for pK_{a1}*. The comparison in acidity of honokiol and magnolol is shown in Table 3.

Magnonol, 5, 5′-diallyl-2,2′-dihydroxy-biphenyl, has its pK_{a1}* and pK_{a1} very close to that of 2, 2′-dihydroxybiphenyl [21, 29]. So the electron effect of 5, 5′-diallyl can be ignored. With respect to honokiol, 5, 5′-diallyl- 2, 4′-dihydroxy- biphenyl, it shows an absorption characteristics like the mixture of 2-hydroxy- biphenyl and 4-hydroxy-biphenyl, whereas its fluorescence characteristics similar to 4-hydroxy- or 4, 4′-dihydroxy- biphenyl rather than 2-hydroxy- or 2, 2′-dihydroxy- biphenyl. Naturally, one may think of the geometric configurations of magnolol and honokiol. Owing to the intramolecular H-bond between 2-OH groups, magnolol has a smaller dihedral angle than honokiol. The small dihedral stabilizes the conjugation system between the two benzene rings both in the ground and excited state, and strengthens the electron-withdrawing effect of phenyl. For honokiol, the larger dihedral angle reduces the conjugation effect so that honokiol behaves its ground-state acidity like the monohydroxy-biphenyls. So the values of pK_{a1} and pK_{a1}* of magnolol are much lower than that of honokiol even though the two isomers both possess the 2-OH group in their molecules. Since the Hückel coefficient of electron-withdrawing group is greater in the excited state than in the ground state [24, 26, 30], 2-OH in honokiol molecule shows its excited-state acidity stronger than 4,4′-dihydroxy- biphenyl, despite much weaker than 2,2′-dihydroxy- biphenyl.

Considering honokiol like 2-hydroxy-biphenyl without H-bond between benzene rings, however, the ground- and excited-state acidities of its 2-OH would be similar to that of 2-OH of 2-hydroxy- biphenyl, but not similar to that of 4-OH of 4-hydroxy- biphenyl. It is interesting that honokiol shows a weak photoacidity like 4-hydroxy- or 4, 4′-hydroxy-biphenyl rather than 2-hydroxy- or 2, 2′-hydroxy-biphenyl.

In the sequence of excited state deprotonation, the first event that occurs upon excitation is redistribution of the π electron cloud, producing the electron-density characteristic of the excited state. Supposing that photoacidity depends on a larger intramolecular charge transfer from the oxygen atom to the aromatic ring upon excitation for the anion than for the acid, the variation in dipole moment upon excitation should be a good monitor for the photoacidity of hydroxybiphenyls [19, 28]. Owing to the bond of C1-C1′ freely whirling and lower symmetry, honokiol has a smaller dipole moment in the ground state than magnolol. It is presumably that the dipole moment of anionic HA^- would not change significantly upon excitation. So, the decrease in the electronic charge density on the oxygen atom of 2-OH upon excitation for HA^- would be not significantly larger than that for H_2A.

Conclusion

Honokiol shows more complex absorption and fluorescence characteristics than magnolol. If only considering the electron effect of substitution, 2-OH of honokiol would show the ground- and excited- state acidities similar to 2-OH of magnolol. But our work has revealed that magnonol possesses much stronger acidities than honokiol both in the ground and excited states. One could explain this phenomenon by the difference in geometry configuration between the isomers. Like honokiol, 2-hydroxy-biphenyl also has no H-bond between the two benzene rings. However, 2-hydroxy-biphenyl is a "super" photoacid. It is strange that

Table 3 Comparison of pK_a between magnolol (adopted from ref.17) and honokiol with different determination methods

Method	Honokiol			Magnonol		
	pK_{a1}	pK_{a2}	pK_{a1}*	pK_{a1}	pK_{a2}	pK_{a1}*
Absorption titration	2-OH:9~10 4′-OH:10~10.5	11~12		7.37		
Fluorescence titration	2-OH: 9.6		6.7	7.54	14.38	0.57
Föster cycle	10.0					3.60

honokiol shows its weak photoacidity like 4-hydroxy-biphenyl rather than 2-hydroxy-biphenyl. This phenomenon may be caused by the reason that honokiol has a small dipole moment in its molecule. The degree of dipole moment changes of HA^- would be not remarkable upon excitation in comparison with H_2A. This induction needs to be verified by a further investigation.

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