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Journal of Photochemistry and Photobiology A: Chemistry 186 (2007) 202–211
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Proton transfer of magnolol in ground and excited states

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Received 5 June 2006; received in revised form 17 July 2006; accepted 12 August 2006
Available online 23 August 2006

Abstract

Magnolol, 5′,5-di-2-propenyl-[1,1′-biphenyl]-2,2′-diol, has been characterized by steady-state and time-resolved spectroscopy as well as ^{1}H MR and ^{13}C NMR. And the proton transfer reactions both in ground and excited states have been investigated. The binary acid enhances its acidity upon excitation at the first deprotonation reaction and exhibits strong photoacidity. The relationship between the spectroscopic property and the geometric conformation of magnolol with unique biphenyl group has been discussed.

Keywords: Mgnolol; Photoacid; Geomitrc conformation

1. Introduction

Magnolol is the major phenolic element of the plant medicine "Houpo" (*Magnolia officinalis*), which is used in the treatment of chest tightness and asthma. It has various pharmacological functions such as effecting on smooth muscle cells [1,2], inhibiting muscle contraction [3], possessing antioxidant effects approximately thousandfold stronger than alpha-tocopherol [4], increasing cytoplasmic free Ca^{2+} through a phospholipase C-mediated pathway, involving in a new activation mechanism closely associated with intracellular Ca^{2+} mobilization [5], and producing neurotrophic effects in primary cultured rat cortical neurons [6], relieving age-related neuronal loss in the hippocampus [7]. However, less investigation in the spectroscopic property has been reported although the fluorescence and absorption spectra of magnolol has been used in assay of *M. officinalis* and its pharmaceutical preparations.

Proton transfer, both in ground and excited states, plays a key role in many biological processes. The acid-base properties of drug compounds are important to certain biochemical processes such as biological uptake, activity, transport and distribution. As is well known, hydroxyarenes are photoacids of which the acidity greatly increases upon spectroscopic excitation from the ground state. For instance, naphthol, phenol and their substituted derivatives have been extensively investigated both experimentally and theoretically [8–11]. The absorption of a photon by a photoacid triggers a succession of reactions contributing to the overall phenomenon of excited-state proton transfer (ESPT). It has been noticed that the overall action of light on such systems is completely different, though ESPT is an important step in all of them [12–17]. The proton-transfer reactions in ground and excited states of compounds are closely related to their electronic structures. The acidity of hydroxyarene shows a unique dependence on the structure of the photoacid.

We are interested in the photoacidity of magnolol because of its unique structure of biphenyl group (as shown in Fig. 1) and, in particular, because of the relative lack of study on photoacids in the biological literature. Magnolol possesses a symmetrical structure, in which hydroxyl groups are in the *ortho*-position of phenyl group and in the *para*-position of allyl group. The degree of the dihedral between the two benzene rings is affected by the intramolecular H-bonding between the two hydroxyl groups of which deprotonation determines the photoacidity of magnolol. We have noticed the following important features of magnolol related to the investigation of magnolol photochemistry at our experimental conditions:

1. The acidity of neutral magnolol molecule increases dramatically upon electronic excitation. Although magnolol is a binary acid with two hydroxyl groups, only the first proton

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doi:10.1016/j.jphotochem.2006.08.006

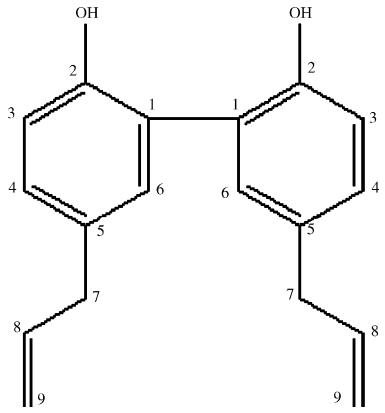

Fig. 1. Structure of magnolol.

dissociation of excited-state has been observed. In aqueous solutions the pK_{a1} value decreases by about 7 units upon excitation.

2. Neutral magnolol can transfer its proton to water but not to methanol in the excited state, demonstrating single-peak fluorescence (around 400 nm) in aqueous solution. The emission band of excited monoanic magnolol HA^{-*} appears in a wide pH range from 0 to 14 with the intensity dependent on pH. The emission band of excited neutral form H_2A^* at around 355 nm appears in acidic and neutral methanol and almost disappears in water even at sufficiently low pH because of the strong photoacidity and the occurrence of proton-quenching. An apparent pK^*_{a1} of excited-state is 0.57 from determination by fluorescence titration.
3. The first proton dissociation of ground-state results in red-shifted absorption. The pK_{a1} 7.54 and pK_{a2} 14.38 of ground-state have been obtained by fluorescence titration. Steady-state and time-resolved spectroscopy and ^{1}H NMR and ^{13}C NMR indicate no other reaction but the second deprotonation occurs in strongly basic aqueous solution. Along with the second deprontonation, an unusual blue-shifted absorption has been observed and attributed to the change in geometric conformation of biphenyl group which takes place in the transition from monoanion HA^- to dianion A^{2-} of ground-state.

2. Experiment

Magnolol was isolated from the cortex of *M. officinalis* Rhed. The 99% purity was determined by HPLC. Water was deionized and redistilled. Solvents were used without detectable fluorescent impurities. The buffer solutions with pH from 4 to 12 were prepared with 0.1 mol L^{-1} solution of sodium phosphate and 0.1 mol L^{-1} solution of hydrochloric acid, those with pH < 4 were prepared with hydrochloric solution and those with pH > 12 with 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 mol L^{-1} with sodium chloride.

All experiments were performed at room temperature (22 °C). The UV absorption spectra were recorded on a Shimadzu UV-2101PC spectrometer. Steady-state fluorescence spectra of nondeoxygenated solutions were recorded on a Shimadzu FR-5301 spectrofluorometer. Transient fluorescence was detected using the time-correlated single-photon counting (TCSPC) method on an Edinburgh instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemimi series 300 MHz spectrometer.

3. Result

3.1. Absorption spectra and absorption spectroscopic titration

During the study of the pH dependence of absorption and emission, aggregation of magnolol should be avoided. Magnolol shows good linearity in the Beer's law plot ($\varepsilon = 6650$ mol L^{-1} cm^{-1} at 284 nm) up to 0.1 mmol L^{-1} in neutral aqueous solution. Thus, the absorption spectroscopic titration was performed in water with the concentration of 3×10^{-5} mol L^{-1}.

We investigated the absorption and fluorescence spectra in aqueous solutions with pH from −0.3 to 14.7. In order to discuss the results distinctly, the spectral data were divided into three parts: (1) the strongly acidic region, pH from −0.3 to 3; (2) the weakly acidic–basic region, pH from 6 to 10; (3) the strongly basic region, pH from 13 to 14.7.

The absorption spectra at all pH values were shown in Fig. 2A, C and E, demonstrating transformation between neutral, monoanionic and dianionic species. At low pH, the low-energy absorption peak with a maximum at 284 nm appeared in aqueous solution. With pH increasing from 6 to 9, the low-energy absorption gradually shifted to a new peak with a maximum at 316 nm. Two clear isoabsorptive points at 267 and 294 nm were observed. The spectral transformation could be attributed to the first deprotonation reaction, the absorption band at 284 nm and that at 316 nm were assigned to the neutral magnolol H_2A and the monoanionic magnolol HA^-, respectively. With further basification up to pH 13, both the absorption maximum did not change. Upon increasing concentration of NaOH from 0.1 to 6.0 mol L^{-1}, the absorption peak with a maximum at 316 nm was gradually blue-shifted to 309 nm. The first deprotonation constant of ground-state pK_{a1} could be determined by monitoring the absorbance at 316 nm where H_2A has no absorbance. The UV-absorbance titration curve at 316 nm was presented in Fig. 3A with the inflection point at around pH 7.37.

3.2. Steady-state fluorescence spectra in methanol solution

The emission of magnolol in methanol was different from that in water. The emission bands in neutral methanol coincided with that in acidic methanol with a maximum at 355 nm. With addition of NaOH to methanol, the emission band at 355 nm disappeared and a new emission band at 400 nm emerged, see Fig. 4. In methanol with addition of $HClO_4$ up to 0.1–3.6 mol L^{-1}, a strong proton quenching was observed. The quenching curve was shown in Fig. 5. Water acted effectively to change the