Solvent effects on the absorption and fluorescence spectra of rhaponticin: Experimental and theoretical studies

Yang Sun\textsuperscript{a,}\textsuperscript{1}, Xuhua Liang\textsuperscript{b,1}, Yingyong Zhao\textsuperscript{c}, Jun Fan\textsuperscript{b,*}

\textsuperscript{a} Department of Chemistry & Chemical Engineering, Xi'an University of Arts and Science, No. 168 Taibai South Road, Xi'an, Shaanxi 710065, China
\textsuperscript{b} School of Chemical Engineering, Northwest University, No. 229 Taibai North Road, Xi'an, Shaanxi 710069, China
\textsuperscript{c} College of Life Sciences, Northwest University, No. 229 Taibai North Road, Xi'an, Shaanxi 710069, China

HIGHLIGHTS
- The effect of various solvents upon the spectral properties of rhaponticin was discussed.
- The luminescent mechanism of rhaponticin was studied.
- Dipole moments of rhaponticin were compared by solvatochromic methods.
- Theoretical calculation using density functional theory.

ABSTRACT
Rhaponticin (RH) possesses a variety of pharmacological activities including potent antitumor, antitumor-promoting, antithrombotic, antioxidant and vasorelaxant effects. The fundamental photophysics of RH is not well understood. In this work, solvent effect on the photoluminescence behavior of RH was studied by fluorescence and absorption spectra. The bathochromic shift was observed in absorption and fluorescence spectra with the increase of solvents polarity, which implied that transition involved was $\pi \rightarrow \pi^{\ast}$. A quantitative estimation of the contribution from different solvatochromic parameters, like normalized transition energy value ($E^T_N$), was made using the linear stokes shift ($\Delta m$) relationship based on the Lippert–Suppan equation. The ground state and excited state dipole moments were calculated by quantum–mechanical second-order perturbation method as a function of the dielectric constant ($\varepsilon$) and refractive index ($n$). The result was found to be 2.23 and 3.67 D in ground state and excited state respectively. The density functional theory (DFT) was used to obtain the most stable structure, electronic excitation energy, dipole moments and charge distribution. The analysis revealed that the RH exhibited strong photoinduced intramolecular charge transfer (ICT), and the intermolecular hydrogen bonding ability of the solvent was the most important parameter to characterize the photophysics behavior of RH. The hydrogen bonding effect occurred at the localized electron-acceptor oxygen at the glycoside bond. The experimental and theoretical results would help us better understand the photophysical properties of RH.

Introduction
Rhaponticin (RH), a major representative of the stilbene glucoside compounds, exists widely in medicinal plant of Rheum L., such as Rheum officinale, Rheum undulatum, Rheum hotoense and Rheum palmatum [1]. RH possesses various biological effects including potent antitumor, antithrombotic, antioxidant and vasorelaxant activities [2]. Our previous researches have illustrated the structure, protein binding and metabolic distribution of RH [3–6]. The multiple pharmacological activities make it be worth carrying out a further study on the photophysical property of RH. According to the previous research result that the atoms of the stilbene moiety are nearly coplanar in the molecule of RH, and the dihedral angle between ring (C2–C7) and ring (C10–C15) is 9.0°. The hydrogen
bonds exist among the hydroxyl group (–OH), methoxy group (–OCH3) and O atom, and link the molecules into a complicated 3D framework [3]. Moreover, the hydroxyl groups at C-11 and methoxy group at C-12 of act electron donor and the glycoside fragment functions as a receptor unit, and there exists electronic transition as π → π*, belong to polyphenol derivatives. Therefore, RH would show optical property which is highly sensitive to its surroundings, it is reasonable to use it as a polarity probe and it also can be applied in medicine and chemistry.

Solvation effect is closely related to the nature and extent of solute–solvent interactions developed locally in the immediate vicinity of solute. One key approach to understand solvation effects is the solvent-induced changes in the electronic transition of solutes generally referred to as solvatochromism. The application of computational methods to studies in photophysics chemistry is instrumental in understanding phosphorescent mechanisms [7]. In recent years, approaches using density functional theory (DFT) have received large acceptance for describing the ground state properties of photoluminescence molecules, such as geometrical parameters, energy gap, dipole moments and charge transfer [8].

Until now, there is no report available in the literature on the luminescent mechanism of RH. Therefore, we focus on the photophysical properties of RH, and we present a comparative study of the experimental absorption and fluorescence spectra with DFT calculations in this work. These results would have a great significance in pharmacology and clinical medicine as well as methodology.

**Experimental**

RH (Fig. 1A) was isolated by authors from *R. hotoense* [3], and its purity was evaluated to be above 99% (HPLC and spectral analysis). Ultra high purity (UHP) water was prepared by a Millipore-Q SAS 67120 MOLSHEIM (France). The stock solution of RH (1.5 × 10⁻⁵ M) was prepared by dissolving appropriate amount of RH in 10 mL different solvents, and the solvents applied were analytical grade and used without further purification. All the absorption spectra were recorded by UV-2501PC spectrophotometer (Shimadzu, Japan) and the fluorescence spectra were carried out on an F-7000 fluorescence spectrometer (Hitachi, Japan) equipped with a 150 W Xenon lamp, and the slit width was 5 nm. All of the measurements were performed at 298 K. The solvatochromic method was used to calculate the dipole moment in ground and excited state. All calculations in the present study were performed by using the Amsterdam Density Functional package (ADF) 2009.01 program [9]. Geometry optimization in the ground state was carried out using B3LYP density functional calculations [10], with the DZP basis sets (all-electron double zeta plus polarization function) [11].

**Results and discussions**

**Solvent effects on the absorption and fluorescence spectra of RH**

Theoretical density functional theory (DFT) calculations were performed to gain more insight into the electronic properties of these systems that appear as promising molecules for light-harvesting applications [12]. Fig. 1B displays the minimum-energy molecular geometry computed for RH at the B3LYP–DZP level. As expected, RH exhibits the typical boat conformation, and the atoms of the stilbene moiety are coplanar, suggesting that an efficient π-conjugation can operate between the donor and acceptor units in RH molecule. The absorption and fluorescence spectra of RH in different solvents were presented in Fig. 2. The values of the absorption and fluorescence spectral maxima were listed in Table 1. As we know, the shift can be described as hypsochromic or bathochromic depending on whether the absorption maximum occurs at a shorter or longer wavelength respectively. As seen from Fig. 2A, the absorption maximum was obtained around 311 nm in dioxane and it was about 327 nm in water. In the present study, the absorption spectra shifted to longer wavelength with increasing solvent polarity, indicating RH exhibited a positive solvatochromism and π → π* transition. According to Jayabharathi, the bathochromic shift happens when the dipole moment of the compound increases during the electronic transition i.e., the dipole moment of excited state is higher compared to that in the ground state (μe > μg) and the excited state is formed in solvent cage of already partly oriented solvent molecules [13]. In such cases, the relaxed excited state S1 will be energetically stabilized relative to the ground state S0 and a significant red shift of the absorption will be observed.

At the same time, the fluorescence spectral studies of RH with excitation wavelength of 355 nm were carried out in different solvents (Fig. 2B), the peak maximum shifted from 370 to 403 nm (bathochromic shift) as the solvent polarity increased (dioxane to water). Meantime, the Stokes shift of RH in dioxane was 56 nm, whereas the Stokes shifts in water increased to 76 nm, respectively. The increased Stokes shift in polar protic solvents suggested that the fluorescent state of RH may be of the intramolecular charge transfer (ICT) feature.

The fluorescence intensity of RH decreased with the increasing solvent polarity (Table 1). On the one hand, the excitation state of RH is stabilized by protic solvents. On the other hand, ICT state is more likely to relax by non-radiative processes than by radiative processes, which results in lower intensities of RH in protic solvents [14,15]. In addition weak intermolecular hydrogen bonding effects of oxygen atom of the glycoside with protic solvents may induce electron distributions. When the acceptor (oxygen atom at glycoside band) is hydrogen-bonded, a more charge-separated chromophore is more stabilized (Fig. S1, Supporting materials). This might be the reason in protic solvents with hydrogen bonding
we chose the aprotic solvents in Table 1, from 2,4-dioxane to acetone, to avoid the hydrogen bonding between RH and protic solvents, to the band position of a solute molecule was obtained. In order to explain the absorption (16), quantum mechanical second-order perturbation theory of the electronic distribution on excitation. According to Lippert et al. and exited state of RH for it offers information about the change in the electron distribution, which will be affected by various solvents of varying permittivity (Table 1, the effect of solvents on the emission spectra of RH was more remarkable than that on absorption spectra (absorption and emission wavelength shifted 16 and 33 nm and from dioxane to water, respectively), which might imply that the less polar nature of RH in the ground state than in excited state and the ground state energy distribution was not affected by the solvents.

**Determination of dipole moments of RH**

The dipole moment of the ground and excited state is related to the electron distribution, which will be affected by various solvents. Thus, it is important to study the dipole moments of ground and exited state of RH for it offers information about the change in the electronic distribution on excitation. According to Lippert et al. [16], quantum mechanical second-order perturbation theory of absorption (νa) and fluorescence (νf) band shifts in different solvents of varying permittivity (ε) and refractive index (n) relative to the band position of a solute molecule was obtained. In order to avoid the hydrogen bonding between RH and protic solvents, we chose the aprotic solvents in Table 1, from 2,4-dioxane to acetone, and the related equation as followed:

\[
v_a - ν_f = m_1 f(ε, n) + \text{const}
\]

(1)

\[
v_a + ν_f = -m_2 [f(ε, n) + 2g(n)] + \text{const}
\]

(2)

where

\[
f(ε, n) = \frac{2n^2 + 1}{n^2 + 2} + \frac{1}{n^2} - \frac{n^2 - 1}{n^2 + 2}
\]

(3)

with

\[
g(n) = \frac{3}{2} \left(\frac{n^4 - 1}{n^2 + 2}\right)^2
\]

(4)

where ε is the dielectric constant and n is the refractive index.

\[
m_1 = \frac{2(μ_e - μ_g)^2}{hca^2}
\]

(5)

and

\[
m_2 = \frac{2(μ_e^2 - μ_g^2)}{hca^2}
\]

(6)

where h is the Planck’s constant and c is the velocity of light in vacuum, and m1 and m2 were calculated from the Eqs. (1) and (2) denoting the absorption and fluorescence band shifts. The plots of “Lippert” were listed in Fig. 3, and the values of ground state and excited state dipole moment, μg and μe, were obtained from Eqs. (5) and (6) [17,18]:

\[
μ_g = \frac{m_2 - m_1}{2} \left(\frac{hca^2}{2m_1}\right)^\frac{1}{2}
\]

(7)

\[
μ_e = \frac{m_2 + m_1}{2} \left(\frac{hca^2}{2m_1}\right)^\frac{1}{2}
\]

(8)

and
\[ \mu_s = \frac{m_1 + m_2}{m_2 - m_1} \mu_B (m_2 > m_1) \]  

(9)

The value of the solute cavity radius was calculated from the molecular volume by Suppan’s equation [19]:

\[ a = \left( \frac{3M}{4\pi\delta N} \right)^{\frac{1}{3}} \]  

(10)

Here \( N \) is the Avogadro’s number, \( M \) is the solute’s weight and \( \delta \) is the solid-state density of solute molecule. Then the dipole moment can be calculated by above parameters. Besides, according to Ravi et al. [20] the excited state dipole moment also can be obtained by following equation:

\[ \nu_a - \nu_y = 11307.6 \left[ \left( \frac{\Delta\mu}{\Delta\mu_B} \right)^2 \frac{\eta_B}{\eta_a} \right] E^N_{\text{TMS}} + \text{const} \]  

(11)

Since the values of \( \Delta\mu_B \) and \( \delta_B \) are known [9 D and 6.2 Å] [21], \( E^N \) is defined using water and tetramethylsilane (TMS) as extreme reference solvents with an equation [22]:

\[ E^N_T = \frac{E_T(\text{solvent}) - E_T(\text{TMS})}{E_T(\text{water}) - E_T(\text{TMS})} = \frac{E_T(\text{solvent}) - 30.7}{32.4} \]  

(12)

So the change in dipole moment is determined by

\[ \Delta\mu = \left( \frac{81m}{6.2/\alpha} \right)^{\frac{1}{3}} \]  

(13)

Here \( m \) is the slope of the linear plot of \( E^N_T \) versus Stokes shift (Fig. 3C). The linear \( E^N_T \) dependence of Stokes’s shift indicates the existence of general type of solute–solvent interaction in which the Stokes’s shift depends on dielectric constant and refractive index of the solvents (Fig. 3A and B), and such interactions could arise due to the H-bonding between RH and protic solvents. One can see that \( \mu_B = 2.23 \text{ D} \) and \( \mu_a = 3.67 \text{ D} \). The dipole moment of the RH in excited state is higher than that in ground state owing to the change in electronic distribution. This result means a more polar and stable structure in excited state than that of the ground state, which is consistent with the result of absorption spectra.

**Determination of quantum yield of RH**

The relative quantum yields of RH were determined by using Quinine bisulfate in 0.05 mol L\(^{-1}\) \( \text{H}_2\text{SO}_4 \) solution (\( \Phi = 0.546 \)) as the standard and were calculated through the following equation [23]:

\[ \Phi_R = \Phi_S \frac{A_R \chi \eta_S}{A_S \chi \eta_R} \]  

where \( \Phi \) is quantum yield of RH, \( F \) is integrated area under the corrected emission spectra, \( A \) is absorbance at the excitation wavelength, \( \lambda \) is the excitation wavelength, \( \eta \) is the refractive index of the solution, and the subscripts \( R \) and \( S \) refer to RH and the standard, respectively. The \( \Phi_R \) of RH in dioxane and water were found to be \( 7.1 \times 10^{-4} \) and \( 1.2 \times 10^{-4} \) respectively. The value is higher in dioxane as compared to in water. This suggests that the molecule stayed more in the excited state in dioxane which is an aprotic solvent. In water (protic solvent), the ground state of RH was more stabilized through intermolecular hydrogen bonding interactions and the spectra shifted to longer wavelength.

**Theoretical calculation using density functional theory**

Fig. S2 shows the orbital energy levels of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) and their energy gap for RH in dioxane, acetone, ethanol and water. And the calculated energy gaps, wavelengths of the lowest electronic transitions, oscillator strengths, and main orbital transition contribution and corresponding CI coefficients of the low-lying electronically excited states of RH in four solvents were listed in Table 2. The observed positive solvatochromism was reproduced by DFT calculations, and the maximum oscillator strengths were corresponding to HOMO → LUMO + 2 orbital transitions. The occupied orbital was mainly localized on the stilbene unit whereas the LUMO + 2 was centered on the glycoside part, suggesting a strong propensity for intramolecular charge transfer from the electron-donor hydroxyl groups at C-11 and methoxy group at C-12 to the glycoside acceptor, and therefore correspond to \( \pi \rightarrow \pi^* \) transitions. As seen from Fig. S2 that the energy level of the HOMO increased from \(-5.23 \text{ eV} \) in dioxane to \(-5.18 \text{ eV} \) in water, suggesting the intermolecular hydrogen bonding raised the energy level of the HOMO. Meantime, the energy level of the LUMO decreased slightly from \(-2.57 \text{ to } -2.58 \text{ eV} \) from dioxane to water, indicating that intermolecular hydrogen bonding...
showed less effect to the electronically excited state of RH than ground state [24]. The energy gap ($E_g$) between the HOMO and LUMO reduced from 2.66 to 2.60 eV from dioxygen to water. The reducing energy gap between the HOMOs and LUMOs was consistent with the absorption and fluorescence spectral red-shift of RH in polar solvents. On the other hand, from dioxygen to water, there was a quasi parallel energy stabilization of the HOMO–2 and HOMO and a concomitant small destabilization of the LUMO. These evolutions resulted in the decrease of the HOMO–LUMO and HOMO–(LUMO + 2) gap and thus in a bathochromic shift of the corresponding spectral behavior [25].

Moreover, the value of the dipole moments (Dm) in the excited states was 3.87 D (Table 3), and the difference between the dipole moments of the excited and ground states was 1.31 D, indicating that the value of dipole moment in the excited state of RH was high, which explained well the experimental observations that RH showed a bathochromic shift of spectra and the large Stokes shift with increasing solvent polarity. The properties of the frontier orbital and the energy gap have previously been shown to be in good agreement with experimental data.

Furthermore, the MOs for RH with hydrogen-bonded oxygen atom at glycoside bond were estimated. It could be seen from Fig. S3 that there was also a remarkable migration of the ICT character form the hydroxyl groups at C-11 and methoxy group at C-12 to the hydrogen-bonded oxygen atom part, which was mainly assigned to the transition from the HOMO to the LUMO. Compared to the absorption spectra in vacuum of RH, the calculated absorption wavelength of hydrogen-bonded RH was shifted to longer wavelengths (ca. 5 nm, Fig. S4). The difference between the dipole moments was increased, and the $E_g$ between HOMO and LUMO of hydrogen-bonded RH was 2.0 eV, which was lower than the one RH. Therefore, as shown in Fig. 2, the intermolecular hydrogen bonding can induce a red-shift of the fluorescence and absorption spectra. The DFT results were in good agreement with the spectral data in experiment.

### Conclusion

In this work, the excited-state photophysical behavior of RH has been studied by absorption and fluorescence spectra and DFT calculations. The observed solvatochromic shift of spectra was used to calculate the ground and excited state pole moment of 2.23 and 3.67 D by Lippert–Suppan equation, respectively. Moreover, the increased Stokes shift in polar solvents suggested that RH was intramolecular charge transfer (ICT) feature, and correspond to π → π* transitions. The photoluminescence behavior of RH was strongly modulated by the formation of intermolecular hydrogen bonds between RH and protic solvents. The geometric and electronic structures for the ground and excited states of RH and its solvation effect have been theoretically studied using the DFT method. Quantitative estimation of the relative contribution of several solvatochromic parameters indicates that the hydrogen-bond donor ability of the solvent was one of the factors governing the excited-state properties of RH, which were verified by DFT calculations. The intermolecular hydrogen bonding occurred at the localized electron-acceptor oxygen at the glycoside bond. These experimental and theoretical results would offer a rationale that contributes to understand the photophysical properties of RH, which would be available for clinical and analytical application of RH as active drug.

### Acknowledgements

This work was supported by the Doctoral Initiate Foundation of Xi’an University of Arts and Science (90506), National Scientific Foundation of China (81001622) and As a Major New Drug to Create a Major National Science and Technology Special from Ministry of Science and Technology of the People’s Republic of China (No. 2011ZX09401-308-34).

### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.saa.2012.10.013](http://dx.doi.org/10.1016/j.saa.2012.10.013).

### References


## Table 2

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Contrib.</th>
<th>CI component</th>
<th>Theory (nm)</th>
<th>Experiment (nm)</th>
<th>$E_g$ (eV)</th>
<th>$f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioxane</td>
<td>HUMO → LUMO + 2</td>
<td>0.951</td>
<td>315</td>
<td>311</td>
<td>2.66</td>
<td>0.243</td>
</tr>
<tr>
<td>Toluene</td>
<td>HUMO → LUMO + 2</td>
<td>0.904</td>
<td>320</td>
<td>314</td>
<td>2.63</td>
<td>0.246</td>
</tr>
<tr>
<td>Ethanol</td>
<td>HUMO → LUMO + 2</td>
<td>0.939</td>
<td>331</td>
<td>323</td>
<td>2.61</td>
<td>0.248</td>
</tr>
<tr>
<td>Water</td>
<td>HUMO → LUMO + 2</td>
<td>0.943</td>
<td>339</td>
<td>327</td>
<td>2.60</td>
<td>0.252</td>
</tr>
</tbody>
</table>

* The transition was shown by an arrow from one orbital to another, followed by its percentage CI (configuration interaction) component.

## Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental</th>
<th>Theoretical</th>
<th>Calculation</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_e$ (D)</td>
<td>$\mu_e$ (D)</td>
<td>$\Delta\mu$ (D)</td>
<td>$\lambda_{ex}$ (nm)</td>
</tr>
<tr>
<td>RH</td>
<td>2.23</td>
<td>3.67</td>
<td>1.44</td>
<td>2.56</td>
</tr>
<tr>
<td>RH–H</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6.82</td>
</tr>
</tbody>
</table>

* Oscillator strength.


