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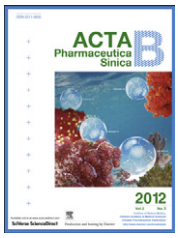

ORIGINAL ARTICLE

Fluorimetric analysis of paeonol in Chinese herbal medicine Cynanchi Paniculati Radix by aluminum ion-sensitized fluorescence

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KEY WORDS

Fluorescence analysis;
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Cynanchi Paniculati Radix (Xuchangqing);
3D fluorescence spectrum

Abstract A novel fluorimetric method for determination of paeonol, an active component of Chinese herbal medicine, is proposed. The method is based on the reaction of paeonol with aluminum ion in pH 4.4 HAc-NaAc buffer to form a fluorescent Al(III)-paeonol complex. The maximum excitation wavelength and emission wavelength of the complex were 296 nm and 455 nm, respectively. The fluorescence quantum yield of the complex was determined to be 0.053 at an excitation wavelength of 296 nm. A linear calibration curve covered the concentration range 0.017–1.2 μg/mL. The method has been applied to the analysis of paeonol in medicinal crop Cynanchi Paniculati Radix (Xuchangqing), and the results demonstrated that this method can be used for quality evaluation of crude drug Xuchangqing.

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1. Introduction

Paeonol (2-hydroxyl-4-methoxyacetophenone) is a major active component of Chinese herbal medicines such as Cynanchi Paniculati Radix (CPR, Xuchangqing in Chinese)[1,2] and Moutan Cortex (Mudanpi in Chinese)[3,4]. The Pharmacopoeia of the People's Republic of China recommends the content of paeonol as the quality index of CPR be no less than 1.3%[5]. Pharmacological evaluation revealed that paeonol possesses cardioprotective[6] and anti-diabetic[7] effects, inhibits the anaphylactic reaction[8], and is beneficial in the treatment of cardiovascular disorders[9] and colitis[10].

Some chromatographic methods, such as liquid chromatography[11,12], capillary electrophoresis[13–15] and gas/liquid chromatography-mass spectrometry[16–18], have been used for the determination of paeonol in medicinal plants and in blood plasma. Recently, quantum dots based fluorescence quenching method for the determination of paeonol in paeonol ointment was proposed[19]. The determination of paeonol by sensitized fluorescence has not been reported.

Chromatographic methods have the advantages of simultaneous separation and determination of various components with high accuracy and good precision[20]. They are the main method applied for analysis of complex medicinal and biological samples. However, in some cases such as routine analysis of Chinese herbal medicine, a sensitive, selective and simple method is preferable. Fluorimetric analysis has the advantages of high sensitivity and good selectivity compared to photometric methods and is simple, rapid and inexpensive compared to chromatographic methods. It can be suitable for analysis of Chinese herbal medicines containing a fluorescent component[21,22].

We observed in previous experiments that paeonol had no fluorescence essentially, but produced strong fluorescence when aluminum ion was added under acidic conditions. Based on this sensitized fluorescence, a fluorimetric method is described in this study to determine peaonol in Chinese herbal medicine CPR. It is a simple and environmentally friendly method in which water is used as the solvent to extract paeonol from CPR preparations and no separation procedure is needed.

2. Experimental

2.1. Apparatus

Fluorescence measurements were performed on a Hitachi (Tokyo, Japan) F-4500 fluorescence spectrophotometer equipped with a xenon lamp and 1 cm quartz cell. The excitation and emission slits (band pass) 5 nm/5 nm were used throughout the work. Absorption spectra were recorded using a Shimadzu (Kyoto, Japan) UV-2501PC recording spectrophotometer with 1 cm quartz cell. An Orion (Beverly, USA) 868 pH/ISE meter was used for pH measurement.

2.2. Chemicals and materials

Paeonol (serial No.: 0708–9704, a reagent for quantitative analysis, molecular weight: 166.7) and CPR (serial No.: 121514–200501, a comparison drug for qualitative identification) were purchased from the National Institute for the Control of Pharmaceuticals and Biological Products of China (Beijing, China). *L*-tryptophane ($C_{11}H_{12}N_2O_2$, biochemical reagent, chromatographic grade, molecular weight: 204.33) was purchased from the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). Aluminum chloride ($AlCl_3 \cdot 6\,H_2O$, molecular weight: 241.43), acetic acid (HAc, 36%), sodium acetic acid (NaAc) and all the other reagents were of analytical grade. The water used throughout the study was doubly-deionized and verified to be free from fluorescence.

The standard solution of 0.0330 mg/mL paeonol was prepared by dissolving 0.00330 g reagent in 100 mL water, and diluted to appropriate concentration with water as needed. The solution of 0.20 mg/mL *L*-tryptophane was prepared by dissolving 0.0050 g reagent in 25 mL water. The solution of 1.00 M $AlCl_3$ was prepared by dissolving 12.07 g $AlCl_3 \cdot 6\,H_2O$ reagent in 50 mL water. HAc-NaAc pH buffers were prepared by mixing different amounts of 2.0 M HAc and 2.0 M NaAc solutions.

2.3. Sample preparation

The CPR water extraction was undertaken by soaking 0.0250 g CPR powder in about 10 mL water for 12 h at room temperature. The mixture was filtered through a paper filter and washed several times with water. The filtrate was collected in a 25 mL volumetric flask, diluted to the mark with water and mixed well. The concentration of the water extract was expressed as 1.00 mg/mL (1.00 mg CPR per mL solvent).

2.4. General procedure for spectral measurement

A series of 10 mL volumetric flasks was prepared containing 1 mL 1 M NaCl (ionic strength of the solutions set to 0.1 M), appropriate amounts of HAc-NaAc buffer (pH 4.4), paeonol solution (or CPR water extract) and $AlCl_3$ solution. The mixtures were diluted to the mark with water and mixed well. After setting aside for 20 min, fluorescence or absorption spectra were measured at room temperature.

2.5. Measurement of fluorescence quantum yield

L-tryptophan (quantum yield 0.14 at excitation wavelength of 280 nm) was used as a reference in measuring quantum yield of Al(III)-paeonol complex. For the measurement, a 1.8 μg/mL *L*-tryptophan solution and a 4.33×10^{-4} μg/mL paeonol solution (containing 0.05 M $AlCl_3$, 0.1 M NaCl, and pH 4.41 HAc-NaAc buffer) were prepared. Absorption and fluorescence spectra were measured. Quantum yield of the Al(III)-paeonol complex was calculated by the following equation[23]:

$$Y_u = Y_r \frac{F_u}{F_r} \frac{A_r}{A_u} \quad (1)$$

where Y_u and Y_r were the fluorescence quantum yield of unknown and the resference, F_u and F_r were the integral fluorescence intensity of unknown and reference solutions, A_u and A_r were the absorbance of unknown and reference solutions at their excitation wavelengths, respectively.

Before measuring the quantum yield, excitation and emission spectra were corrected according to the operation manual of the F-4500 fluorescence spectrophotometer.