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# Influence of cetophenolic and diphenolic intramolecular hydrogen bonding on the chromatographic and spectroscopic properties of hydroxyanthraquinones

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**Abstract**

Anthraquinones have been widely employed for dyeing textiles in combination with various mordants. Many papers have dealt with the modification of optical properties induced by the formation of dye complexes under various pH conditions. This paper deals with the characterization of diphenolic intramolecular hydrogen bonding via molecular modeling and cetophenolic interactions by FT-IR spectroscopy to determine their effect on the spectroscopic and chromatographic properties of hydroxyanthraquinones (anthraflavic acid, alizarin, quinizarin and purpurin). The formation of cetophenolic hydrogen bonding induces a substantial bathochromic shift to the visible absorption band. Moreover, it implies that the constitution of hypercyclised aromatic systems is potentially responsible for the fluorescence of quinizarin and purpurin. This study also demonstrates the modification of the chromatographic retention of dihydroxyanthraquinones on apolar stationary phase consequent to the monopolization of polar groups involved in the formation of such interactions.


*Keywords:* Anthraquinone; Intramolecular hydrogen bonding; Molecular modeling; FT-IR spectroscopy; Fluorescence; HPLC/PDA

## 1. Introduction

Anthraquinones are biosynthesised in the roots of numerous plants such as aloe, frangula, rhubarb and madder to protect themselves against fungi in the soil [1]. They have been widely used since ancient times due to their therapeutic and pharmacological properties such as anti-inflammatory, wound healing, analgesic, antipyretic, antimicrobial and antitumor activities [2–4]. Nevertheless, several anthraquinonic derivatives possessing a methyl or hydroxymethyl group on carbon-2 show mutagenicity. Indeed, under physiological conditions, they can be metabolised to an exomethylenic intermediate that forms covalent adducts with DNA and other macromolecules [5–8].

Moreover, these natural substances have been widely employed for dyeing textiles (cotton, wool or silk) in many parts of the world since ancient times [9–12]. Nevertheless, anthraquinonic systems cannot be directly fixed to fibres but their structure presents several complexation sites that induce their combination with various metal salts (aluminium and silver in particular). These metal ions, also called mordants, are essential for the dyeing process of textiles. Models generally proposed in specialised literatures [13–15] conferred to the metal element a role of intermediate between fibres and dyes but recent works [16,17] describe the formation of a dyed complex in which the true role of the metal element is to organize the space arrangement of the structure for its fixation.

It has been shown that organometallic charge transfer salts may exhibit optical switching [18]. More recently, the indirect role of hydrogen bonding in the modification of the optical

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properties of coordination complexes between metal ions and dihydroxyanthraquinones (DHAQ) was described [19–23]. These modifications can be attributed to the formation of intermolecular bonds via metallic ions. In this paper, we have investigated the intrinsic influence of intramolecular hydrogen bonding on chromatographic and spectroscopic properties of non-complexed hydroxyanthraquinones (HAQ). This work describes the characterization of intramolecular interactions by the combination of FT-IR spectroscopy and molecular modeling. Physicochemical properties of these dyed compounds were observed by HPLC/PDA and spectrofluorimetry.

## 2. Experimental

### 2.1. Chemicals

Solvents and reagents were all of analytical grade from Merck (Darmstadt, Germany). This study was realised on three dihydroxyanthraquinones (DHAQ) as anthraflavic acid (2,6-DHAQ), alizarin (1,2-DHAQ) and quinizarin (1,4-DHAQ) purchased from Extrasynthèse (Genay, France). It also concerns one trihydroxyanthraquinone (THAQ) like purpurin (1,2,4-THAQ) purchased from Acros Organics (Morris Plains, New Jersey, USA).

### 2.2. Materials and methods

#### 2.2.1. FT-IR spectroscopy

FT-IR spectra were recorded on a Nicolet (Madison, Wisconsin, USA) Avatar 360 ESP spectrometer equipped with a DTGS KBr detector and controlled by EZ OMNIC 6.0 software. Numerical data were obtained after 64 scans with 4 $cm^{-1}$ sensitivity. Samples were homogeneously crushed with anhydrous potassium bromide in a proportion of 1/20. The powder is then compressed under 10 $t/cm^2$ to form translucent pellets.

#### 2.2.2. Spectrofluorimetry

Experiments were carried out on a Jobin-Yvon (San Diego, California, USA) Fluoromax-2 spectrofluorimeter equipped with a 150 W xenon lamp and controlled by DATAMAX software. All measurements were realised at 25 °C in a quartz curve of 10 nm optical way considering a 5 nm slit opening in order to optimise the quality of resulting spectra.

Samples were solubilised in analytical grade methanol. Solutions were then exposed at 25 °C during 15 min in ultrasonic bath and filtered on 0.2 μm DynaGuard™ cartridge. Concentrations were optimised in order to obtain 0.07 intensity for the visible absorption band. The research of excitation and emission wavelength couple ($\lambda_{ex}/\lambda_{em}$) was manually realised using visible absorption wavelength, previously determined on UV–vis spectra as initial excitation wavelength. After recording fluorescence spectrum, emission wavelength was obtained. Then, the opposite process was realised in order to optimise excitation wavelength and so on until the final optimum wavelength couple was obtained.

#### 2.2.3. Molecular modeling

This theoretical approach was carried out in order to consider all essential molecular characteristics. The crucial step to realise such computerised investigations is the choice of adequate chemical programs. Thus, structures were created *in silico* in Cartesian coordinates and geometrically optimised by a molecular dynamic program (GHEMICAL 1.01). Taking into account the presence of electronic dyed systems extremely delocalised, this geometrical optimisation was completed by a semi-empirical minimization with a quantum mechanics program (MOPAC 6.0) using AM1 mathematical model including a minimum RMS gradient of 0.05 for charges/charges, charges/dipoles and dipoles/dipoles interactions. Lastly, minimization was performed with an *ab-initio* quantum mechanics program (GAMESS 6.0) using a mathematical model including a 6-311G orbital basis set with a minimum RMS gradient of 0.001.

#### 2.2.4. High-performance liquid chromatography with photodiode array detection (HPLC/PDA)

HPLC analysis was carried out with a chromatographic system consisting of a Spectra-Physics (San Jose, California, USA) SP-8800 ternary gradient pump, a Rheodyne 7125 injector equipped with a 20 μL loop, connected to a Merck $C_{18}$ reverse-phase column (LiChroCART Superspher, 5 μm 100 RP-18, 250 mm × 4 mm i.d.), coupled to a Waters (Milford, Massachusetts, USA) model 996 photodiode array (PDA) detector and controlled by MILLENNIUM$^{32}$ 3.05.01 software. The separation was performed at 35 °C with a binary elution mixture composed of acetonitrile (A) and bidistilled water (B) containing 0.01% trifluoroacetic acid (TFA). Chromatography was carried out for 50 min at a continuous flow-rate of 0.7 mL/min with the following solvent sequence: (i) isocratic period of 5 min with 25% of A, (ii) linear increase in 15 min from 25% to 70% of A, (iii) second isocratic period of 25 min with 70% of A, and (iv) linear increase in 5 min from 70% to 100% of A before returning to initial conditions in 10 min after analysis. Chromatograms were scanned at 250 nm.

Before injection, each sample (1 mg) was solubilised in 2 mL of analytical grade methanol. The solution is then exposed at 25 °C for 15 min in ultrasonic bath. After centrifugation, the supernatant is filtered on 0.2 μm DynaGuard™ cartridge.

## 3. Results and discussion

### 3.1. Determination of intramolecular hydrogen bonding

FT-IR spectroscopy is a suitable technique for determining characteristic functional groups and their chemical environment. It also contributes to the investigation of particular interactions like intramolecular hydrogen bonding [24,25]. In this case, the observation of C=O stretching wavenumber vibration could specify the hydroxyl substitution type of each anthraquinonic derivative.

The observation of all FT-IR spectra (Fig. 1) reveal a C=O stretching region located between 1667 $cm^{-1}$ and 1627 $cm^{-1}$.

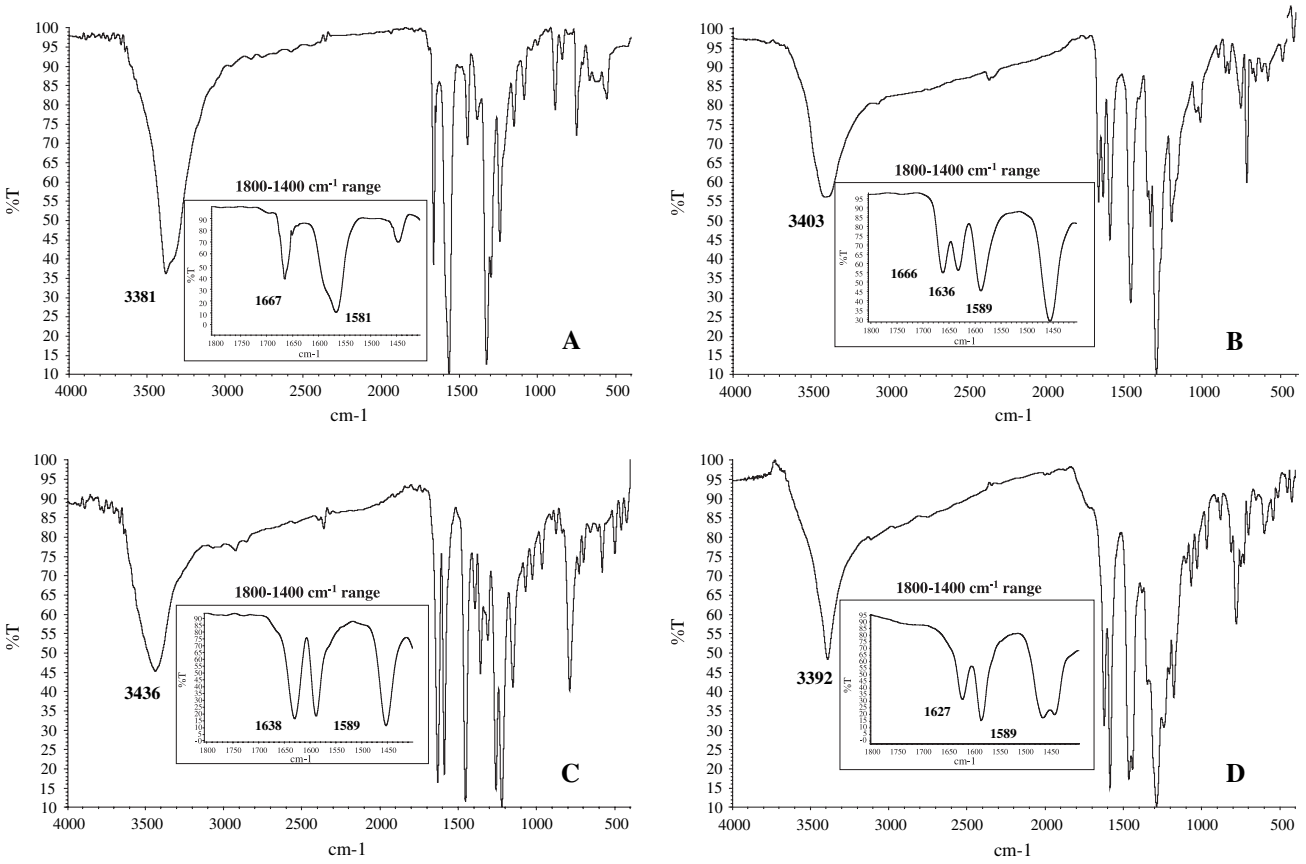


Fig. 1. FT-IR spectra of anthraflavic acid (A), alizarin (B), quinizarin (C) and purpurin (D).

Only one absorption band is determined at 1667 $cm^{-1}$, 1638 $cm^{-1}$ and 1627 $cm^{-1}$, respectively, for anthraflavic acid, quinizarin and purpurin while alizarin has two characteristic absorption bands at 1666 $cm^{-1}$ and 1636 $cm^{-1}$. The interpretation of C═O stretching wavenumber value is discussed in specialised literature. This phenomenon would come from the formation of intramolecular hydrogen bonding between oxygen of C═O and hydrogen coming from OH groups at C-1 and C-4 positions [21]. In this case, the presence of two absorption bands for alizarin and only one for the other structures is perfectly justified (Fig. 2). Nevertheless, this phenomenon does not explain the 11 $cm^{-1}$ gap between C═O stretching vibration of quinizarin (1638 $cm^{-1}$) and purpurin (1627 $cm^{-1}$). This variation is consequent to the modification of electronic density distribution induced by the hydroxyl substitution of aromatic system. Thus, the C═O stretching vibration is shifted to lower frequencies by the modification of the bond force constant induced by the electronic delocalisation. Indeed, previous works [26] show a diminution of free C═O stretching vibration wavenumber from 1675 $cm^{-1}$ for anthraquinone to 1625 $cm^{-1}$ for its amino substituted derivates. This phenomenon is consequent to the presence of amino group that supports electronic delocalisation. Nevertheless, as for other H-bonded molecular compounds, the IR spectrum of such anthraquinonic derivatives exhibits a broad O–H vibration band that excludes the observation of small O–H wavenumber shifts in the determination of hydrogen bonding. So, the characterization of such interactions is limited to the observation of C═O stretching wavenumber vibration, which requires a complementary theoretical study by molecular modeling for completing previous experimental observations.

Computerised investigations confirm the existence of cetophenolic hydrogen bonds between hydrogen of OH group at C-1 or C-4 and oxygen of C═O group at C-9 or C-10 and thus make possible to characterize the formation of diphenolic interactions between hydrogen of OH group at C-2 and oxygen

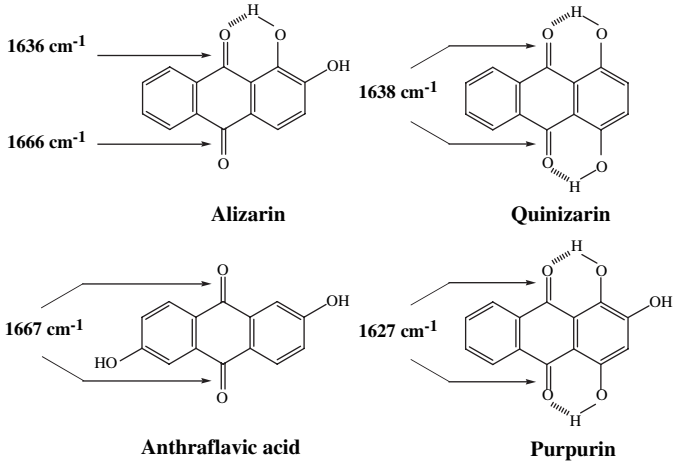


Fig. 2. Attribution of C═O stretching wavenumber vibrations.

of OH group at C-1 (Fig. 3). The 0.012 Å increasing of H-linked C═O bond length is a suitable complement of the determination of cetophenolic interactions, previously realised by FT-IR spectroscopy considering a 30 $cm^{-1}$ diminution of H-linked C═O stretching wavenumber vibration [27]. These cetophenolic interactions are involved in the formation of 6-element cycles whereas diphenolic bonds imply the formation of 5-element cycles. In the first case bond lengths are between 1.925 and 1.938 Å while they reach 2.137 Å when they correspond to the formation of more tended 5-element systems (only for both alizarin and purpurin). Moreover, all these intramolecular cyclizations are generated in the molecular medium plan. Indeed, cetophenolic interactions induce dihedral angles of about 10–12° measure whereas those corresponding to diphenolic hydrogen bonding are characterized by 1° dihedral angles. So, the formation of such cetophenolic and diphenolic hydrogen bonding contribute to increase in rigidity, planarity and electronic delocalisation of these dyed systems, which induce interesting consequences on their physicochemical properties.

### 3.2. Chromatographic and spectroscopic properties

High-performance liquid chromatography is an analytical technique in which organic compounds are separated through the stationary phase according to their affinity between solute, mobile and stationary phases. Considering experimental chromatographic conditions previously described and generally employed in the study of these substances [28,29], the use of a $C_{18}$ apolar stationary phase implies that organic dyes are eluted by decreasing polarity. However, anthraflavic acid, alizarin and quinizarin, which only differ by the positioning of their OH groups, have extremely distinct retention times ($t_R$) contrary to what it is expected to observe with such isomeric compounds (Fig. 4). In this case, it is not ''absolute'' polarity that governs the elution but ''free'' polarity after formation of intramolecular hydrogen bonding. Indeed, these interactions monopolize polar groups involved in their formation and determine chromatographic elution on apolar stationary phase employed herein. So, anthraflavic acid (with four free polar groups) is the first eluted compound at $t_R = 10.9$ min, alizarin (with only one free polar group) is the second at $t_R = 23.8$ min and quinizarin (with no free polar group) is the last separated DHAQ at $t_R = 34.1$ min.

Besides, the influence of such hydrogen bonding is not only restricted to the modification of chromatographic elution, but also concerns spectroscopic properties. The observation of UV–vis spectra of DHAQ (Fig. 5) reveals a spectroscopic behavior closely related to the number of intramolecular hydrogen bonding formed. Indeed, anthraflavic acid, which does not

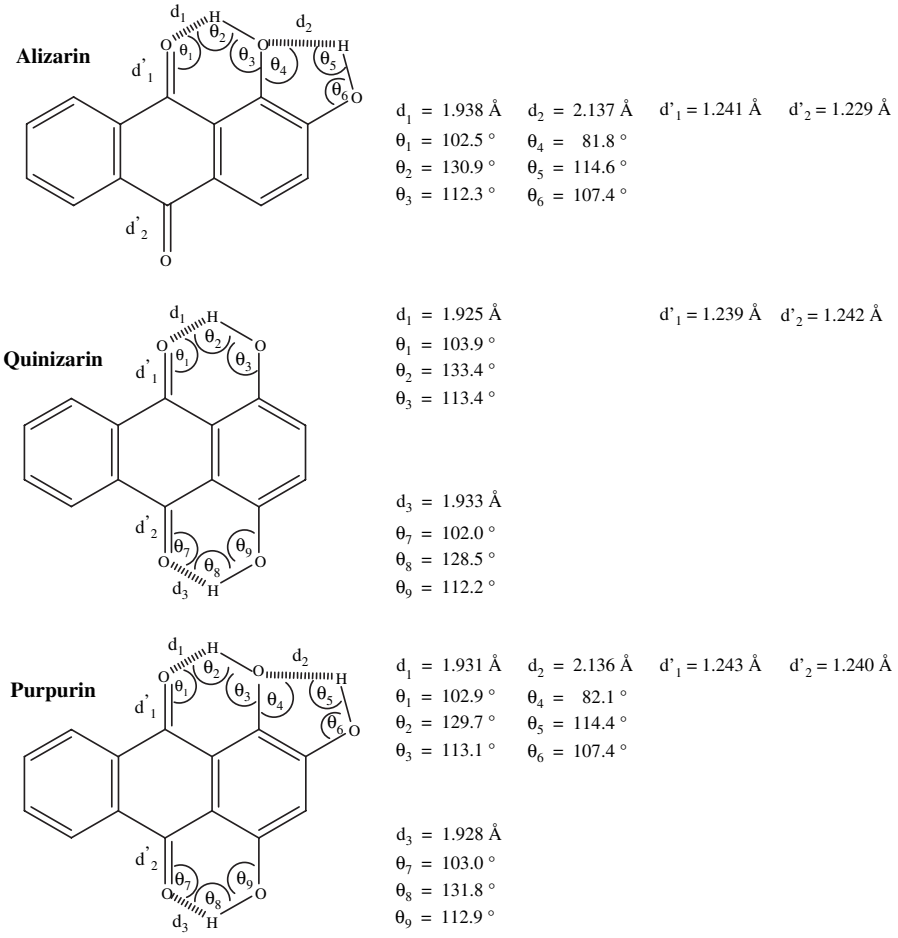


Fig. 3. Intramolecular hydrogen bonding properties resulting from molecular modeling.

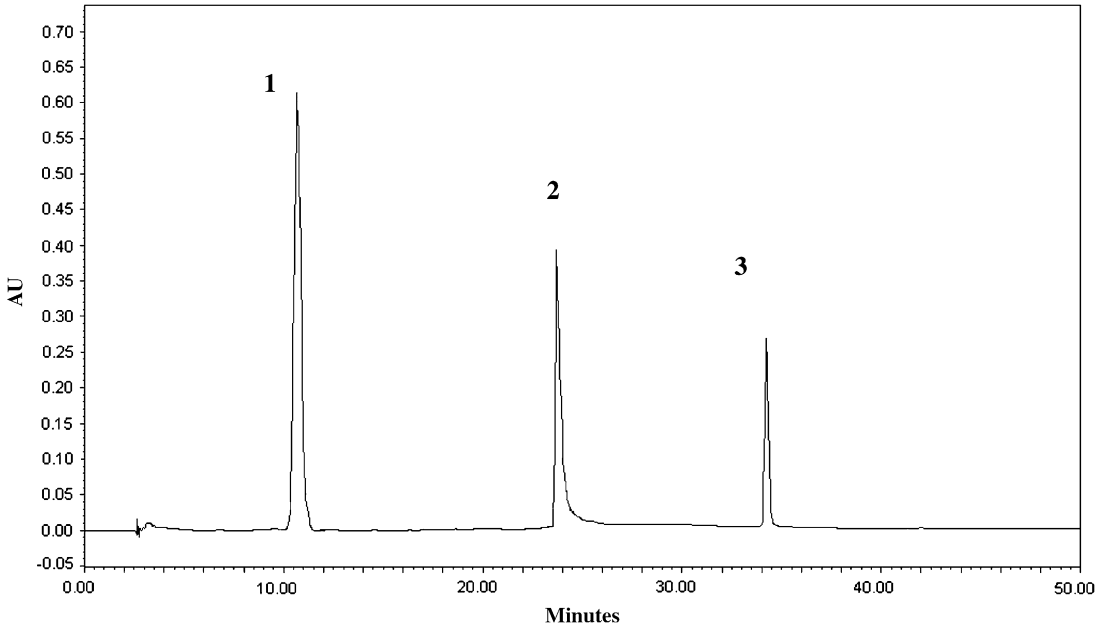


Fig. 4. Chromatogram of DHAQ at 250 nm. Anthraflavic acid (1), alizarin (2) and quinizarin (3).

form any hydrogen bonding, have no visible absorption band and its last absorption is located at about 328 nm. Alizarin and quinizarin, which form two hydrogen bonding each other, have distinct visible absorption band at 426 and 476 nm, respectively. Nevertheless, quinizarin (with two cetophenolic hydrogen bonding) absorbs at a visible wavelength higher than alizarin having one cetophenolic and one diphenolic hydrogen bonding. This bathochromic shift effect can be essentially assigned to cetophenolic intramolecular charge transfer transitions associated with charge migration from the OH group to the C═O group. Furthermore, all these anthraquinonic structures are found to be potential fluorimetric compounds by observing several characteristic elements such as rigidity, planarity and electronic delocalisation. Nevertheless, among all HAQ studied herein, only two of them, purpurin [30] and especially quinizarin, express a fluorescence in following experimental conditions $\lambda_{ex} = 467$ nm/ $\lambda_{em} = 561$ nm (Fig. 6). The particularity of these anthraquinonic dyes resides in the formation of two cetophenolic hydrogen bonding. It implies that the constitution of hypercyclised aromatic systems is mainly constituted by five 6-element cycles, which contributes to the increase in rigidity, planarity and electronic delocalisation, which in turn would explain, in this case, their fluorescence. In consequence, this paper

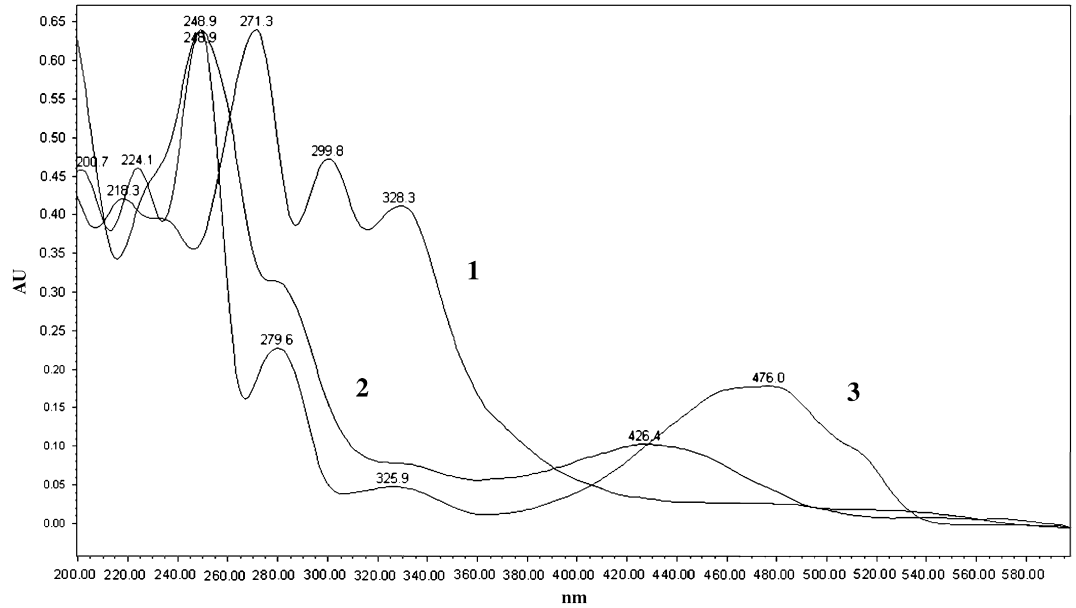


Fig. 5. UV–vis absorption spectra of DHAQ. Anthraflavic acid (1), alizarin (2) and quinizarin (3).

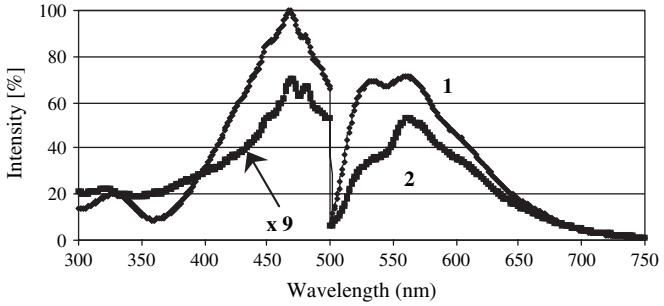


Fig. 6. Excitation and emission fluorescence spectra of quinizarin (1) and purpurin (2).

proposes an interesting correlation between structure and fluorescence, sensitive and selective spectroscopic phenomenon generally being not very predictable. It also constitutes the starting point of the foreseeable fluorescence comprehension of other natural potential fluorimetric markers such as pseudopurpurin (3-carboxypurpurin) in some madder roots and carminic acid in cochineal that also form, in particular, two cetophenolic hydrogen bonding. This predictable approach is very powerful in the preliminary choice of fluorimetric detection as sensitive analytical technique in HPLC studies of small amount samples.

## 4. Conclusions

These theoretical and analytical studies contribute to a better comprehension of the relations between structure and spectroscopic or chromatographic properties of several hydroxyanthraquinones. The combination of molecular modeling and FT-IR spectroscopy is very powerful to characterize all intramolecular hydrogen bonding existing in such aromatic systems. Computerised investigations give precious structural information and complete IR spectroscopic observations. Thus, they easily contribute to the determination of diphenolic hydrogen bonding not identified with the previous analytical technique.

Cetophenolic intramolecular electronic interactions induce substantial bathochromic shift on visible absorption band. Moreover, the formation of five 6-element cycles subsequent to the formation of two cetophenolic hydrogen bonding could explain the fluorescence of purpurin and quinizarin and allow a better comprehension of this complex spectroscopic phenomenon. Moreover, both diphenolic and cetophenolic cyclizations determine the chromatographic retention of dihydroxyanthraquinones on apolar stationary phase by monopolizing polar groups involved in their formation.

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