

Unusual Color Change of Vinylpyranoanthocyanin–Phenolic Pigments

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Anthocyanins are responsible for an interesting variety of colors in flowers, red fruits, and plant-derived foodstuffs. Experimental evidence is presented supporting an unusual color change from red to blue during storage. It has been investigated as a function of temperature at constant pH (pH <2). The color properties of the vinylpyranoanthocyanin pigments, usually designated portisins, have been studied in this work using both experimental and theoretical techniques. The visible spectra obtained from the two approaches were in good agreement. This behavior was explained by a reversible physical–chemical change due to electronic and vibrational properties.

KEYWORDS: Portisins; color change; experimental and theoretical approaches; UV/VIS spectrum; storage

INTRODUCTION

Anthocyanins constitute the largest group of natural water-soluble pigments and are responsible for a great variety of colors in flowers and red fruits. These compounds undertake also a very important role in the color presented by several plant-derived foodstuffs. The color of young red wines is due to the presence of anthocyanins that are extracted from red grape skins during fermentation. During wine aging, the color gradually shifts to red/orange as a result of several chemical reactions (mainly oxidation). These processes yield new anthocyanin-derived pigments. The transformations involved in the formation of anthocyanin derivatives were first described to arise from the dimerization between anthocyanins (1) and condensation of anthocyanins and flavanols in the presence or absence of acetaldehyde (2–9). The development of more accurate analytical methods allowed identification of new families of pigments, namely, pyranoanthocyanins, which were shown to result from the reaction of anthocyanins with small molecules such as acetaldehyde (10), acetoacetic acid (11), pyruvic acid (12), vinylphenol (13), vinylguaiacol (14), vinylcatechol (15), and vinylcatechin (16).

A striking new class of anthocyanin-derived pigments occurring in Port red wines was found to display a very unusual bluish color under acidic conditions (17). Their structural characterization by LC-DAD/MS, NMR, and studies performed in model solutions revealed that their structural features comprise a pyranoanthocyanin moiety linked to a flavanol molecule through a vinyl bridge. These pigments were found to occur in aged Port wines and belong to the group of portisins. Moreover, other compounds newly displaying a structure similar to that of the other reported portisins were detected (18). These latter have a

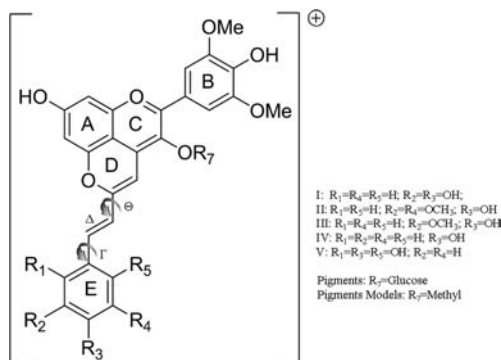


Figure 1. Structure of pigments and respective models I–V: I, vinylpyranomalvidin-3-glucoside–catechol; II, vinylpyranomalvidin-3-glucoside–syringol; III, vinylpyranomalvidin-3-glucoside–guaiacol; IV, vinylpyranomalvidin-3-glucoside–phenol; V, vinylpyranomalvidin-3-glucoside–phloroglucinol. Θ , Δ , and Γ represent the dihedral angles associated.

phenolic moiety replacing the flavanol group in their structure (Figure 1). These new pigments were shown to possibly arise from the reaction between carboxypyrananthocyanins and hydroxycinnamic acids (caffeic, coumaric, sinapic, and ferulic acids), both present in red wines (18).

These pigments revealed a maximum absorbed wavelength (λ_{\max}) (~540 nm) bathochromically shifted when compared with that of the respective carboxypyrananthocyanin precursor (λ_{\max} ~ 510 nm) (Figure 2). Nevertheless, the λ_{\max} of these pigments is not as bathochromically shifted as that of the initially reported portisins bearing a flavanol moiety (18). These latter display an unusual bluish color under acidic conditions (19) with a λ_{\max} at ~570 nm.

Intriguingly, during the experimental studies carried out in model solutions, the portisin pigments (I–V) revealed an unusual color change when frozen in water.

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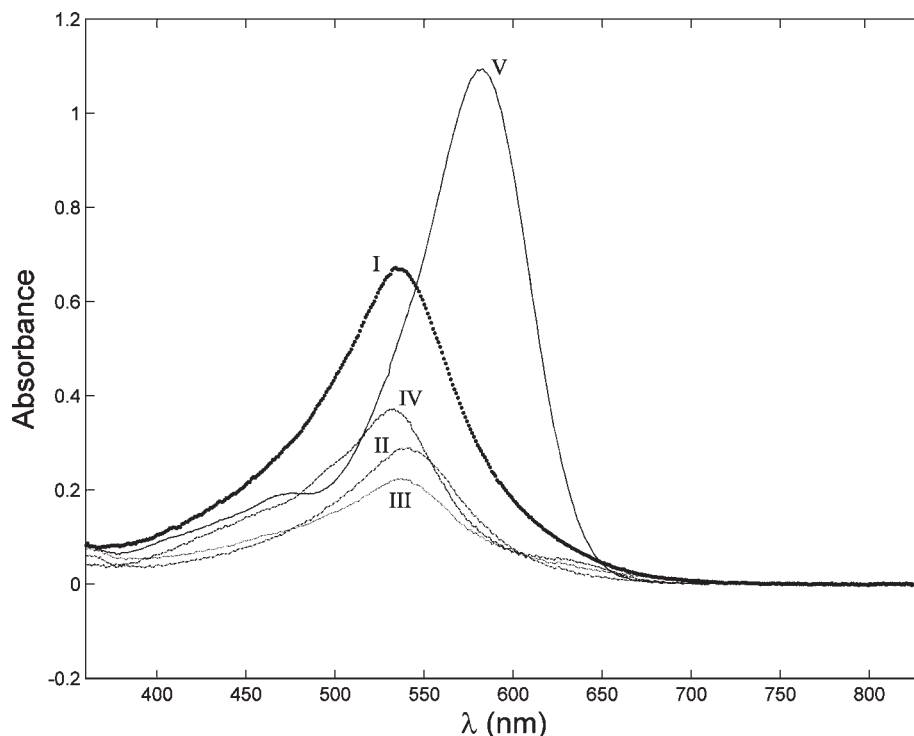


Figure 2. Experimental visible spectra of portisins, the structures of which are presented in **Figure 1**, at pH 2 in water/ethanol 12% (v/v) 18: I, λ_{\max} , 538 nm; II, λ_{\max} , 537 nm; III, λ_{\max} , 537 nm; IV, λ_{\max} , 533 nm; V, λ_{\max} , 583 nm.

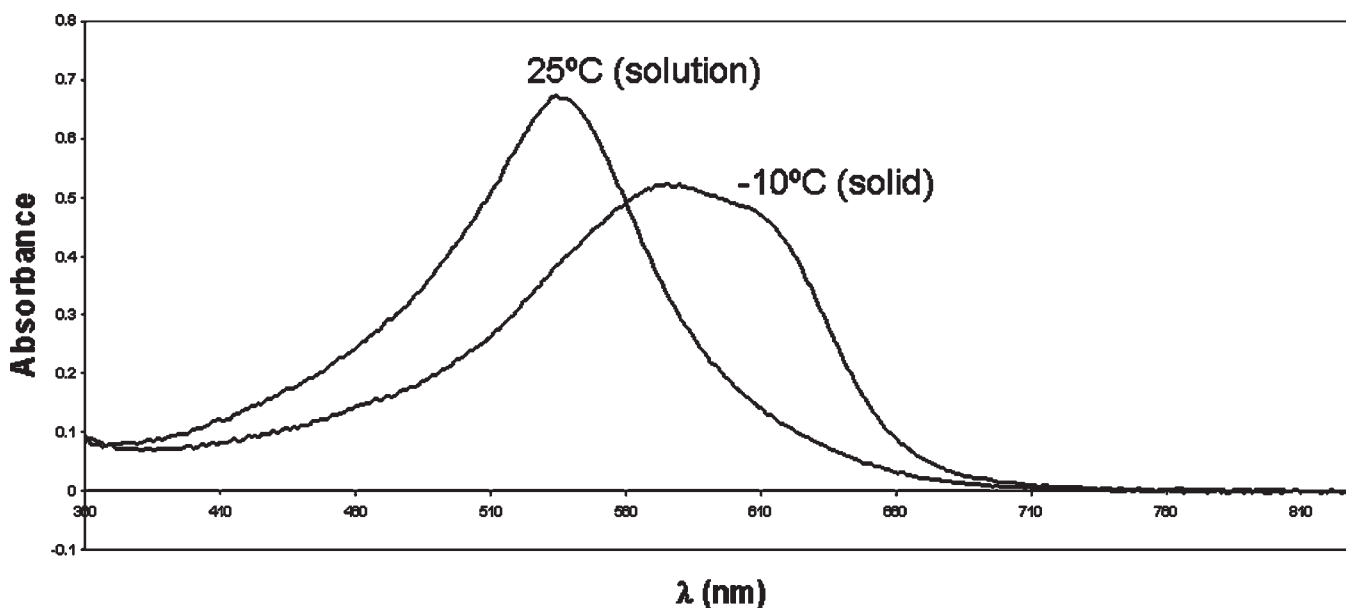


Figure 3. Experimental visible spectra of pigment I, according to the notation presented in **Figure 1**, in water as a function of temperature and phase state.

The interpretation of color at a molecular level, using rigorous theoretical methods, is computationally very expensive in the case of portisins. They can be considered large molecules for CASSFC active space calculations (20), adding the presence of different conformations and different forms depending on pH (21).

In this context, several theoretical studies using more approximate methods have been carried out in this field with success (22, 23). In the present work, the theoretical spectra of portisin pigment models were calculated with Zindo approximation. The spectroscopic properties of the portisin pigments were interpreted from vibrational and electronic points of view.

MATERIALS AND METHODS

For the measurement of the visible spectra of pigments I–V, synthesized as previously described (18), solutions of each pigment (2 mM) were prepared in 12% (v/v) aqueous ethanol at pH 2. Spectroscopic absorbance curves were recorded for all of the solutions from 360 to 830 nm with a 1 nm sampling interval, using a 10 mm path length cell in a Shimadzu UV-3101 spectrophotometer. For the measurement of the visible spectra of pigment I in water at 25 and -10 °C, a 2 mM solution was prepared and put in two 10 mm path length cells, one being left at 25 °C and the other at -10 °C. The pigment solutions were left to equilibrate for 2 h, and then spectroscopic absorbance curves were recorded from 360 to 830 nm using a Shimadzu UV-3101 spectrophotometer.

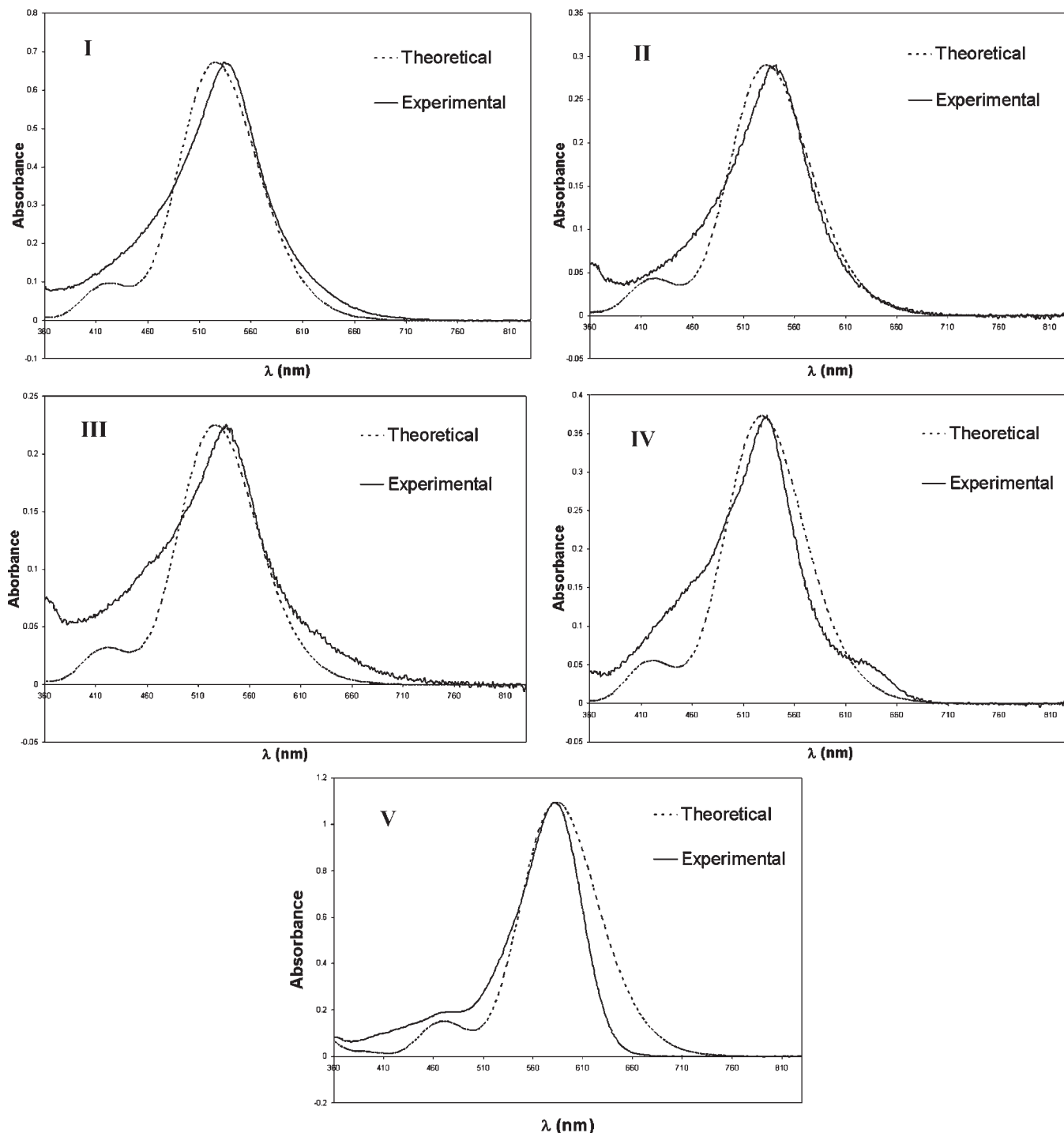


Figure 4. Theoretical and experimental spectra for pigments I–V. The respective structures are presented in **Figure 1**.

Anthocyanins usually occur as glycosides, and the ability to change color is attributed to the aglycon chromophore moiety but not to the sugar moiety (21). Thus, the computational study was concentrated on a model of anthocyanidins with a methyl group replacing the sugar. In this work, five models of vinylpyranoanthocyanin–phenolic pigments were studied (**Figure 1**).

Ring B of the pigment in the vinylpyranoanthocyanin–phenolic pigments is nonplanar in the minima and considered to make a negligible contribution to the color change in the present study. On the other hand, dihedral angle Δ with the double bond in the vinylic group is a well-known isomeric transformation rather than a conformational one. Therefore, conformational scan analyses were performed for Θ and Γ dihedral angles in the vinylic part of the pigments (24). These calculations were performed using the Gaussian 03 program at the B3LYP/6-31G(d)

level. An energy correction, associated with a specific vibrational normal mode, was analyzed. In this context, the corresponding expected value for the molar vibrational energy $\langle E^{\text{vib}} \rangle$ can be calculated as

$$\langle E^{\text{vib}} \rangle = \frac{N_A}{2} hc\bar{\nu} + \frac{N_A hc\bar{\nu}}{\exp(hc\bar{\nu}/k_B T) - 1} \quad (1)$$

where N_A is Avogadro's number, c is the speed of light in a vacuum, and $\bar{\nu}$ is the wavenumber associated with the specific normal mode.

The theoretical UV/VIS spectra were calculated with the Zindo package in the Cache (25) program using the INDO/1 Hamiltonian (26). A $\Delta\lambda_{\text{max}}$ map using a conformational scan in both Θ and Γ dihedral angles was also obtained using the optimized

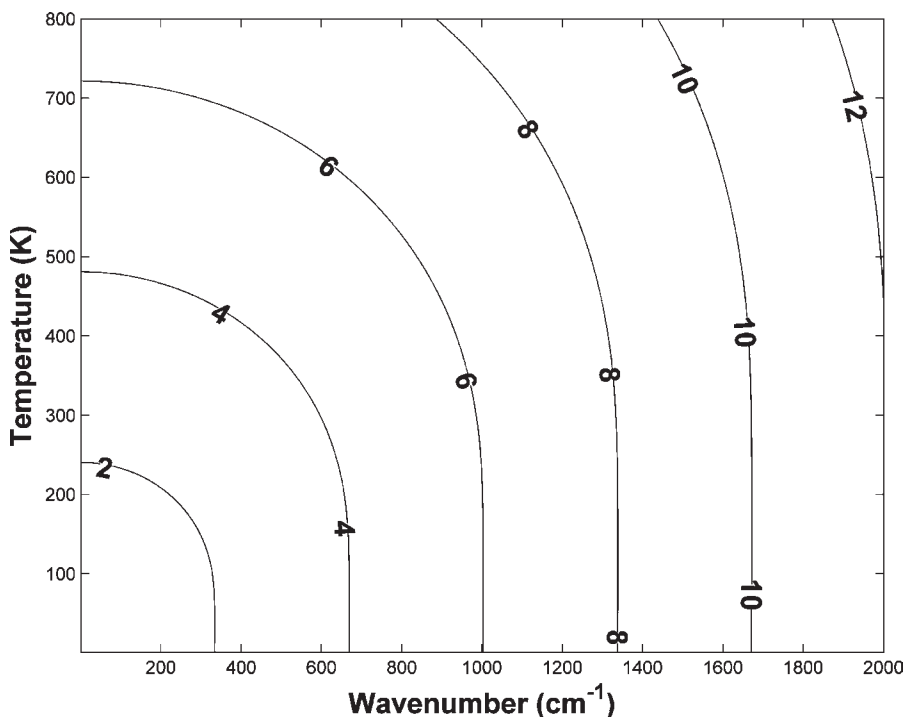


Figure 5. Expected value for the molar vibrational energy (kJ mol^{-1}), associated with a specific normal mode, as a function of wavenumber ($\bar{\nu}$) and temperature. The reduced expected value for the molar vibrational energy with lower temperatures can be surmounted by the increase of the vibrational frequency.

B3LYP/6-31G(d) geometries (24). The $\Delta\lambda_{\text{max}}$ quantity was calculated as

$$\Delta\lambda_{\text{max}} = \lambda_{\text{max}}(\Theta, \Gamma) - \min[\lambda_{\text{max}}(\Theta, \Gamma)] \quad (2)$$

RESULTS AND DISCUSSION

The spectroscopic absorbance curves for the portisin pigments under study recorded in model solutions at 25 °C and frozen in water at -10 °C revealed an interesting color shift. Indeed, the color of all the pigments turned from red to blue. In **Figure 3**, this effect is illustrated for pigment I.

To better understand these color changes of portisin pigments, computational studies were performed in conjunction with the previously obtained data.

In the conformational searches, four planar conformers were found for each molecular model. These correspond to the combination of two minima at 180° and 0°. The associated energetic differences and Boltzmann relative populations at 298.15 K are reported in **Table 1**.

The theoretical and experimental spectra, for the pigments under study, are presented in **Figure 4**. This figure highlights a good conformity between the experimental and theoretical spectra.

The temperature effect in the electronic spectra, presented in **Figure 3**, was analyzed from the vibrational and electronic perspectives. Assuming invariant the vibrational frequencies associated with the normal modes, the average value of vibrational energy should decrease with decreasing temperature. However, the corresponding phase change may originate a more complex behavior. The more ordered crystalline phase can potentially induce stronger interactions between water and the solvatable hydroxyl groups, with the consequent increase of the vibrational frequencies associated with the Θ and Γ torsional modes. The competition between these two opposite effects for a specific normal mode is presented in **Figure 5**. Equation 1

Table 1. Energetic Differences (Kilocalories per Mole) and Relative Boltzmann Populations at 298.15 K Associated with Two Conformers Found for Each Molecular Model

		I	II	III	IV	V
Γ	$E_{180} - E_0$	0.85	0 ^a	0.67	0 ^a	0 ^a
	N_0/N_{180}	0.24	1.00	0.32	1.00	1.00
Θ	$E_{180} - E_0$	2.40	2.62	2.37	2.24	2.11
	N_0/N_{180}	0.02	0.01	0.02	0.02	0.03

^a The E ring is symmetric and the energy of the two populations is the same.

presented in the previous section was used to calculate the dependency of the average value for the correspondent molar vibrational energy on both temperature and vibrational frequency. From the results obtained, it is clear that the decrease in temperature can be surmounted by the increase of the vibrational frequency. This can induce the increase of the ground state energy, consistent with the observed color change.

On the other hand, it is well-known that the decrease of the electronic delocalization induces the decreasing of the corresponding λ_{max} (27). In this work, this effect was analyzed by the evaluation of the $\Delta\lambda_{\text{max}}$ spectroscopy quantity as a function of the Θ and Γ dihedral angles. The results obtained are presented in **Figure 6**. These results confirm that this effect is also present in the pigments under study. The deviation from the planarity (24), associated with a reducing of the electronic delocalization, induces the decrease of λ_{max} . The change of color has a putative explanation due to the more planar conformer (the one with greater electronic delocalization). The conformer is stabilized when the solution is frozen.

Both vibrational and electronic effects previously discussed can be responsible for the color change of the pigments under study after freezing in water. These results contribute to the proposed conjecture and are in agreement with the behavior reported for stilbene. In fact, stilbene was demonstrated to be planar in solid state and nonplanar in solution (28).

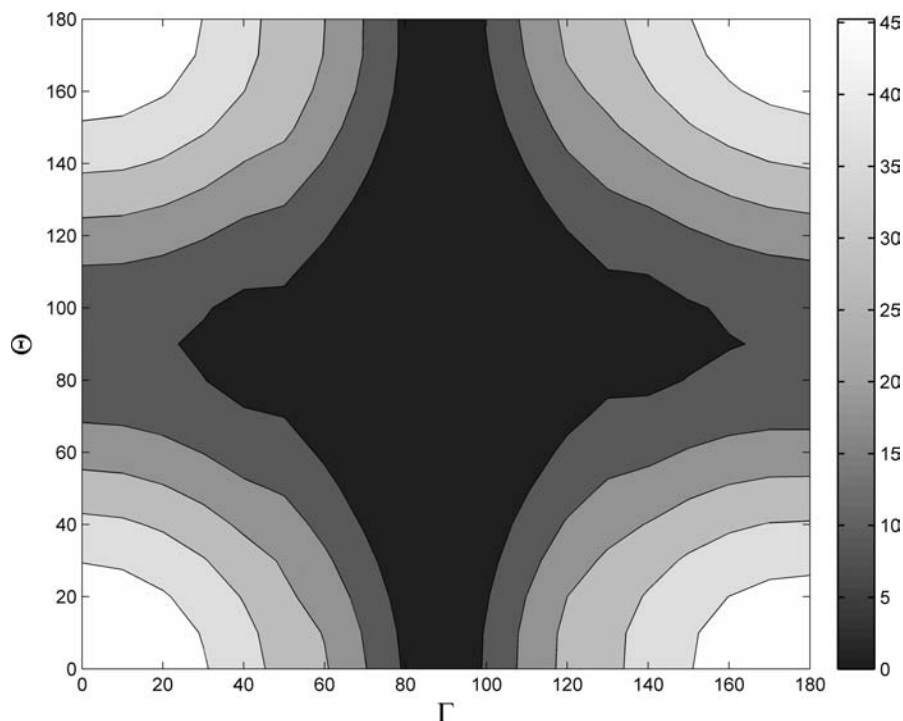


Figure 6. Theoretical UV-vis $\Delta\lambda_{\max}$ map obtained as eq 2 describes it, as a function of different Θ and Γ dihedral angles. It is possible to verify that the four planar conformers have greater λ_{\max} .

In conclusion, the results obtained, by both theoretical and experimental approaches, are in good agreement. An unusual color change for these pigments, from red to blue when frozen in water, was also here reported. This behavior was explained by a reversible physical–chemical change due to electronic and vibrational effects. Future works are planned aiming to use tristimulus colorimetry assays to evaluate adequately this color change in terms of both quantitative and qualitative attributes (e.g., by evaluating the variation of the hue angle, h_{ab}).

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Received for review December 2, 2009. Revised manuscript received February 22, 2010. Accepted February 24, 2010. We thank the Fundação para a Ciência e Tecnologia (FCT) for the doctoral scholarships (SFRH/BD/17900/2004) granted to A.R.F.C. and (SFRH/BD/22622/2005) granted to J.O. and for PTDC/AGR-ALI/65503/2006 grant to support this research.