

Network reaction of 2,6-bis(2-hydroxybenzylidene)cyclohexanone by external stimuli



Ana-Maria Pana^a, Valentin Badea^b, Radu Bănică^c, Alina Bora^a, Zoltan Dudas^{a,d},
Liliana Cseh^{a,*}, Otilia Costisor^a

^a Institute of Chemistry Timisoara of Romanian Academy, 24 Mihai Viteazul Bvd, 300223 Timisoara, Romania

^b "Politehnica" University Timisoara, Faculty of Industrial Chemistry and Environment Engineering, 6 Vasile Pârvan Bvd, 300223 Timisoara, Romania

^c National Institute for Research and Development in Electrochemistry and Condensed Matter – Timisoara, 1 Plautius Andronescu str., 300569 Timisoara, Romania

^d MTA Wigner Research Center for Physics, XII, Konkoly Thege Miklós Street, No. 29-33, 1121 Budapest, Hungary

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ABSTRACT

The pH-dependent photochromic behaviour of a curcumin analogue, 2,6-bis(2-hydroxybenzylidene)cyclohexanone (HBC) has been investigated. The identification of stable and unstable species from network reaction was done by combination of NMR, UV–vis and fluorescence spectroscopy. The structure of stable species was established by ¹³C NMR, ¹H NMR and 2D NMR spectra: DQF-COSY, HSQC and HMBC. The results indicate that HBC could be used as pH sensor on a 1–5 scale. Experimental results were correlated by theoretical calculations using ZINDO/S/CI semi-empirical methods. HBC exhibits fluorescent properties both in acidic and neutral media and in basic environment the fluorescence is quenched.

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1. Introduction

The network of chemical reactions involving anthocyanins and related compounds has been shown to exhibit versatile applications besides its ubiquitous use by Nature to obtain most of the red and blue colours in flowers and fruits [1]. Further photochromic systems switching from a variety of colours in solution, [2] in micelles, [3] or gels, [4] and model systems capable of behaving as optical memories [5] or even mimicking elementary properties of neurons [6] have been described.

All practical applications of these compounds are based on the chemical transformations that they can be submitted to by the actions of different stimuli, namely, light, pH, temperature, etc. [7–11]. An illustrative example regarding the chemical reaction network is presented in Scheme 1; herein the trans-chalcone suffers several transformations leading to several species [12–15].

Curcumin analogues are compounds similar in structure to the chalcones, whose photochromic behaviour has been described beforehand. To the best of our knowledge the curcumin analogues have been studied so far for their cytotoxic

and antioxidant activity [16,17] but we envision a new direction of research and applications for these compounds based on their network of chemical reactions when submitted to different stimuli. Considering the above, we have synthesized and characterized 2,6-bis(2-hydroxybenzylidene)cyclohexanone (HBC), a curcumin analogue. Our main goal was to identify the species involved in the network of chemical reactions of 2,6-bis(2-hydroxybenzylidene)cyclohexanone, which has a similar behaviour when submitted to different stimuli to the flavylum cation [10]. Theoretical calculations have been carried out by AM1 and ZINDO/S/CI semi-empirical methods aiming to correlate the experimental and theoretical UV spectral characteristics of the title compound.

2. Experimental

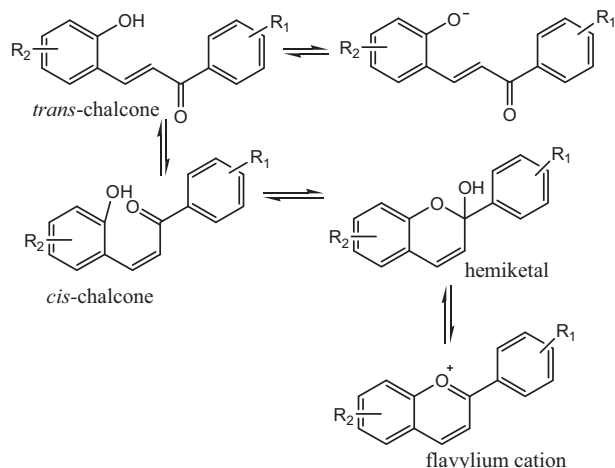
2.1. Measurements and methods

All reagents and solvents used were of analytical grade.

The NMR spectra were measured in methanol-d₄ on a Bruker Avance III 500 spectrometer (500.13 MHz for ¹H, 125.77 MHz for ¹³C) at 298 K. The centre of the solvent signal was used as an internal standard, which was related to TMS with δ 3.31 ppm for ¹H and δ 49.15 ppm for ¹³C. Chemical shifts δ are reported in ppm,

* Corresponding author. Tel.: +40 256 491818; fax: +40 256 491824.

E-mail addresses: lili.cseh@yahoo.com, lilianacseh@gmail.com (L. Cseh).



Scheme 1. Structural transformations of the flavylum-type compounds [10].

coupling constants are reported in Hz and following abbreviations are used for splitting pattern: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), and m (multiplet). NMR assignments were done on the basis of ^1H , ^{13}C , DEPT 135 and 2D NMR spectra: DQF-COSY, standard multiplicity-edited HSQC (HSQCETGPIISP) and standard HMBC (HMCETGPL3ND). The NMR measurements: (i) the basic pH of the HBC solution in deuterated methanol was reached by adding a solution of a 40 wt% NaOD in D_2O ; and (ii) the acidic pH of HBC deuterated methanol was reached by adding a few drops of a solution of DClO_4 in D_2O . Analytical data were obtained by a Perkin Elmer model 240C elemental analyzer for C, N, and H.

UV–vis absorption spectra were recorded on a Perkin-Elmer Lambda 12 spectrophotometer. Fluorescence spectra were recorded on Perkin Elmer LS-55 spectrometer on solution using $\lambda_{\text{ex}} = 361/365 \text{ nm}$ and $\lambda_{\text{em}} = 439/443 \text{ nm}$; the excitation slit was set at 15 and the emission slit at 7.5 and the scanning speed was 100 nm/min. The pH of solutions was measured with a Wissenschaftlich-Technische Werkstätten 330I pH/ion metre. UV–vis and luminescence measurements: the HBC solutions were

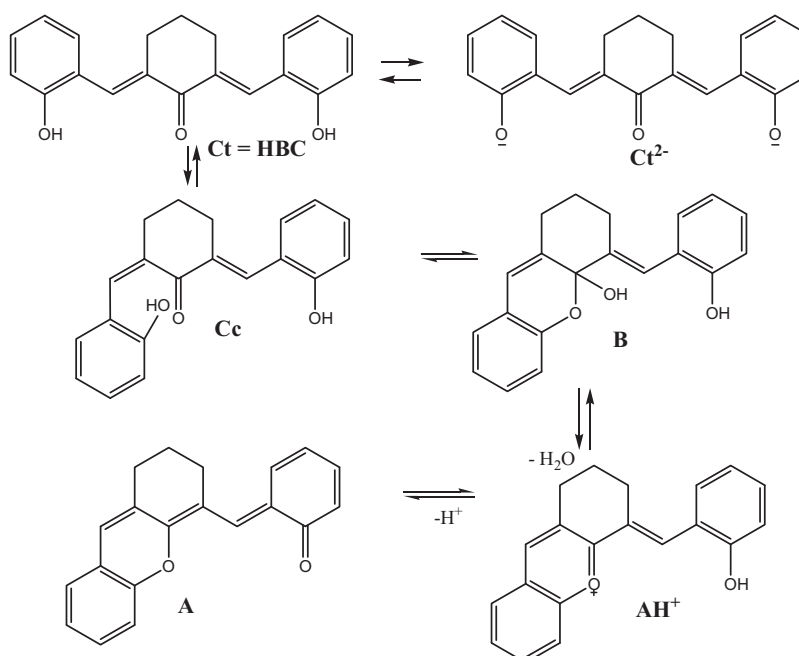
prepared using distilled water and absolute methanol. The pH of solutions was adjusted by addition of HCl, NaOH or universal buffer based on sodium citrate [18].

Quantum chemical molecular simulations were carried out by the AM1 (Austin Model 1) [19,20] and ZINDO/S (Zerner's Intermediates Neglect of Differential Overlap/Spectroscopy) [21,22] semi-empirical methods implemented in the HyperChem programme [23]. Starting structure of the investigated compound was created with the aid of the HyperChem package and the ground state geometry was optimized employing the AM1/RHF (RHF stands for Restricted Hartree–Fock) method. A root-mean-square (RMS) gradient in the energy of $0.005 \text{ kcal } \text{\AA}^{-1} \text{ mol}^{-1}$ was used, as a criterion for choosing an optimized conformation along with the Polak–Ribiere conjugate gradient algorithm [20,23]. The theoretical UV spectrum was computed for the AM1 optimized structure by the ZINDO/S-CI method considering singly excited electronic configurations (CI). This method was parameterized to reproduce electronic spectrum. The ZINDO/S-CI simulations were realized by employing the default parameters. The CI matrix included seven occupied and seven unoccupied molecular orbitals (99 singly excited configurations).

2.2. Chemical synthesis

2.2.1. Synthesis of 2,6-Bis(2-hydroxybenzylidene)cyclohexanone

The synthesis of this curcumin analogue was carried out using a literature protocol, adapted for this case [24]. 4.2 mL of salicylaldehyde (4.88 g, 40 mmol) were dissolved into 5 mL of ethanol, while stirring at room temperature. 2.1 mL of cyclohexanone (1.96 g, 20 mmol) are then added and stirred vigorously. 24 g of a NaOH 20%w. solution are added dropwise, slowly by maintaining the temperature below 40°C . This mixture is left under stirring for 24 h, then 30 mL of distilled water are added, afterwards the mixture is neutralized to pH = 5.5 by using a solution of HCl 6 N. The red-dish precipitate was filtered and recrystallized from a MeOH/water mixture. After dried the product a yellowish powder is obtained, $\eta = 68.62\%$, m.p. = $148\text{--}150^\circ\text{C}$ [lit. $148\text{--}149^\circ\text{C}$]. Elem. anal. calcd for $\text{C}_{20}\text{H}_{18}\text{O}_3$ (306.36): C 78.41%, H 5.92%; found C 78.64%, H 5.76%.



Scheme 2. Structural transformation of the HBC depending on pH conditions.

^1H NMR (500 MHz, CD_3OD), δ (ppm): 7.98 (s, 2H), 7.31 (d, $J=9.0$ Hz, 1H) 7.18 (t, $J=7.7$ Hz, 1H), 6.91–6.77 (m, 2H), 2.83 (dd, $J=8.6, 3.6$ Hz, 2H), 1.84–1.61 (m, 1H).

^{13}C NMR (100 MHz, CD_3OD) δ (ppm): 191.3, 156.6, 135.8, 132.9, 130.0, 122.9, 118.7, 115.1, 28.4, 23.2.

IR (cm^{-1}): 3366, 3061, 2953, 2915, 2838, 1649, 1599, 1577, 1549, 1452, 1335, 1280, 1169, 1158, 1094, 761, 749, 567.

3. Results and discussion

2,6-Bis(2-hydroxybenzylidene)cyclohexanone (HBC) was synthesized in basic conditions, in a good yield.

HBC is a curcumin analogue with a great ability for colour switching along pH modification. This ability can be attributed to the several species involved (Scheme 2) in the interconversion of the compound using different acidic/basic conditions [2], similar to those registered for the flavylum system [25].

In order to highlight the stable species, the NMR experiments were carried out at two extreme pH values ($\text{pH} < 1$ and $\text{pH} > 13$) and neutral pH conditions.

In the basic condition the ^1H NMR spectrum recorded immediately after the deuterated base was added, proved the total transformation of Ct form into the deprotonated base Ct^{2-} . The conversion into the unprotonated base Ct^{2-} occurs practically instantaneous from the moment the base was added. The spectrum was recorded again, after 1 and 10 days; the features were the same, which confirms the stability of this specie in basic conditions [26].

The NMR spectrum of the HBC at $\text{pH} < 1$ was recorded immediately and in time after the acidic conditions was created. In the spectrum recorded immediately was detected the presence of several species and the total conversion of Ct towards the cation AH^+ was reached after 29 h (see Table 1).

Because the compound does not turn instantaneously into the AH^+ compound, the protonated sample in the above conditions was rerun several times in order to observe the moment when it turns completely into the xanthylium derivative. Thus, several spectra were recorded: immediately, after 20 min, then every 2 min for 30 min, every 20 min for 3 h, 29 h, 36 h ($1\frac{1}{2}$ days), and then from time to time for 14 days. In the Fig. 1b, which presents the evolution of the NMR spectra of HBC in time, we can notice that even after 50 min the sample has present the signals corresponding to the neutral form of the compound and that the conversion of Ct into AH^+ specie is complete only after about 29 h. The NMR analysis, run after 15 days, confirms the stability of the cationic form AH^+ in the given conditions [5].

Based on the NMR spectra recorded at different moments of time and temperature of 25°C , the kinetics of the Ct conversion into the protonated adduct AH^+ can be described by the curve presented in Fig. 2.

The conversion of HBC into related species (see Scheme 2) was theoretically investigated in terms of energy released or absorbed reflected by the heat of formation (ΔH). According to semiempirical molecular orbital calculations (AM1), the theoretical stabilization for the AH^+ cation species is $\Delta H = 154.52$ kcal/mol, relatively high positive value compared to those computed for the Ct, and Ct^{2-} species ($\Delta H_{\text{Ct}} = -48.56$ kcal/mol, $\Delta H_{\text{Ct}^{2-}} = -65.49$ kcal/mol, respectively). In this light, the most stable geometry was assigned for the Ct^{2-} comparing with the relatively unstable geometry of AH^+ . Moreover, the values suggest that the conversion between these species is easier for e.g., Ct into Ct^{2-} and for Ct into Cc species than for Ct into AH^+ species because the conformational flexibility of these species is different. The main difference between the Ct and AH^+ species is the increasing rigidity of the AH^+ system; the conformational rigidity of AH^+ system increases the thermodynamic instability.

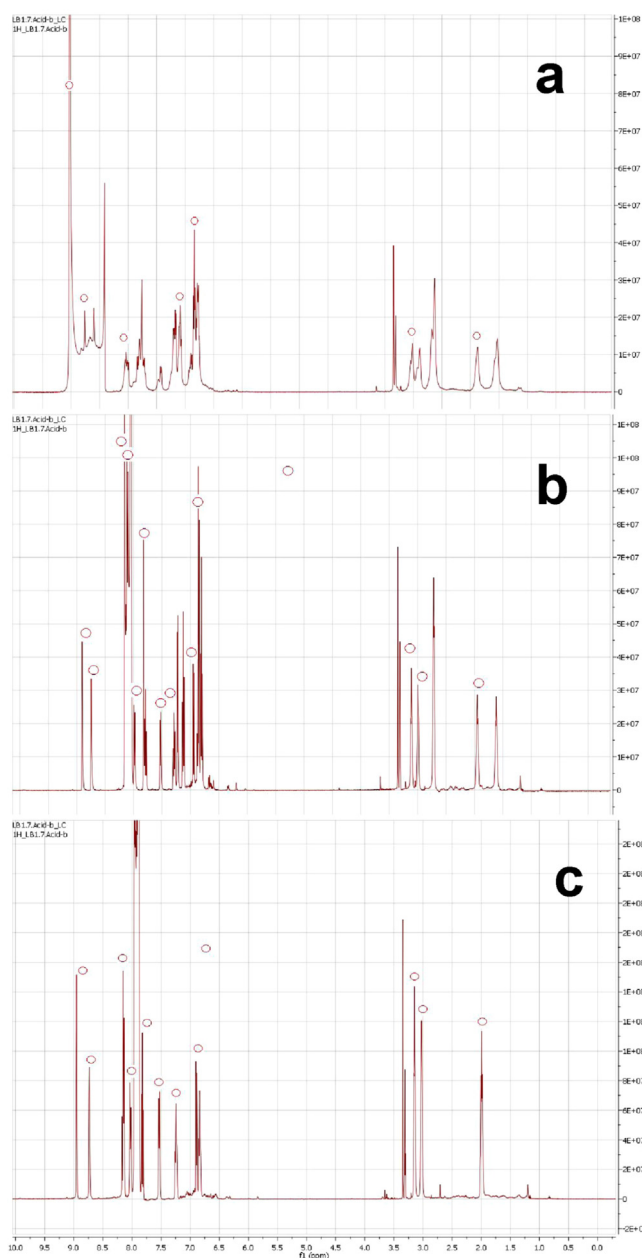
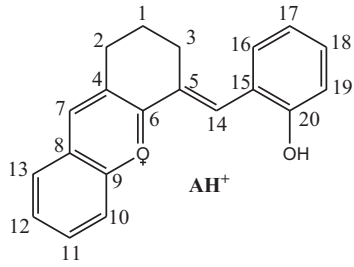
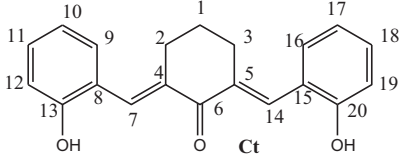
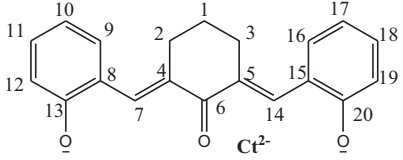


Fig. 1. The evolution of the ^1H NMR spectra of HBC in acidic media, at 25°C ; the signals marked with the red circle correspond to AH^+ ; (a) at $t=0$; (b) at $t=50$ min and (c) at $t=29$ h.

The UV–vis spectrometry allowed in emphasizing the colour shift behaviour of the compound depending on the pH increase. Fig. 3 presents the superimposed UV–vis absorption spectra of the compound in different pH solutions, ranging from 1.54 to 5. The samples in acid pH are reddish and exhibit a broad band with a maximum absorption at 498 nm, corresponding to the formation of the AH^+ cation. As the pH value increases, the solutions became non-coloured, corresponding to the neutral form of the compound. The broad absorption band of 498 nm is not detectable for the neutral form, and it practically disappears as the pH increases to values beyond 2.25. Moreover, as the band from 498 nm increases in intensity and the pH value drops, the absorption band from 258 nm decreases in intensity, proving that the xanthylium cation has been formed and the reddish colour of the solution is the straight consequence. The UV–vis spectra also sustain the information obtained via NMR, because even in acid environment both species, the

Table 1Assignment of ^1H NMR and ^{13}C NMR data for AH^+ , Ct and Ct^{2-} forms in acid, neutral and basic conditions.

Labelling	 AH^+		 Ct		 Ct^{2-}	
	^1H NMR, δ (ppm)	^{13}C NMR (ppm)	^1H NMR, δ (ppm)	^{13}C NMR (ppm)	^1H NMR, δ (ppm)	^{13}C NMR (ppm)
1	1.99 (qu, 2H, $J=6.1$ Hz)	21.9	1.67, m, 2H	24.7	1.69, m, 2H	24.9
2	3.15 (t, 2H $J=6.2$ Hz)	29.2	2.80 (t, $J=5.3$ Hz, 2H)	29.9	2.83, m, 2H	30.0
3	3.04 (t, 2H $J=5.4$ Hz)	28.5	2.80 (t, $J=5.3$ Hz, 2H)	29.9	2.83, m, 2H	30.0
4	–	128.9	–	134.5	–	132.7
5	–	125.6	–	134.5	–	132.7
6	–	174.3	–	193.1	–	194.0
7	8.95, s 1 H	154.4	7.84, s, 1H	137.4	8.32, s, 1H	139.8
8	–	133.6	–	113.8	–	113.8
9	–	156.6	7.20 (t, $J=8.5$ Hz, 1H)	131.5	7.18 (d, $J=7.8$ Hz, 1H)	131.1
10	8.05 (d, 1 H $J=8.5$ Hz)	119.5	6.84 (t, $J=7.5$ Hz, 1H)	124.4	6.99 (t, $J=8.5$ Hz, 1H)	132.3
11	8.15 (m, 1H $J=7.8$ Hz)	139.3	6.92 (d, $J=8.1$ Hz, 1H)	131.5	6.37 (t, $J=7.4$ Hz, 1H)	126.3
12	7.83 (t, 1 H $J=7.5$ Hz)	130.6	6.84 (t, $J=7.5$ Hz, 1H)	120.2	6.65 (d, $J=8.3$ Hz, 1H)	121.6
13	8.15 m, 1H $J=7.8$ Hz)	131.3	–	158.1	–	171.1
14	8.76, s 1H	143.5	7.84, s, 1H	137.4	8.32, s, 1H	139.8
15	–	123.3	–	113.8	–	113.8
16	7.55 d 1H $J=7.5$ Hz)	132.4	7.20 (t, $J=8.5$ Hz, 1H)	131.5	7.18 (d, $J=7.8$ Hz, 1H)	131.1
17	6.87 (t 1H $J=7.6$ Hz)	120.9	6.84 (t, $J=7.5$ Hz, 1H)	124.4	6.99 (t, $J=8.5$ Hz, 1H)	132.3
18	7.28 (t 1H $J=7.6$ Hz)	135.8	6.92 (d, $J=8.1$ Hz, 1H)	131.5	6.37 (t, $J=7.4$ Hz, 1H)	126.3
19	6.91 (d 1H $J=8.3$ Hz)	117.2	6.84 (t, $J=7.5$ Hz, 1H)	120.2	6.65 (d, $J=8.3$ Hz, 1H)	121.6
20	–	160.1	–	158.1	–	171.1

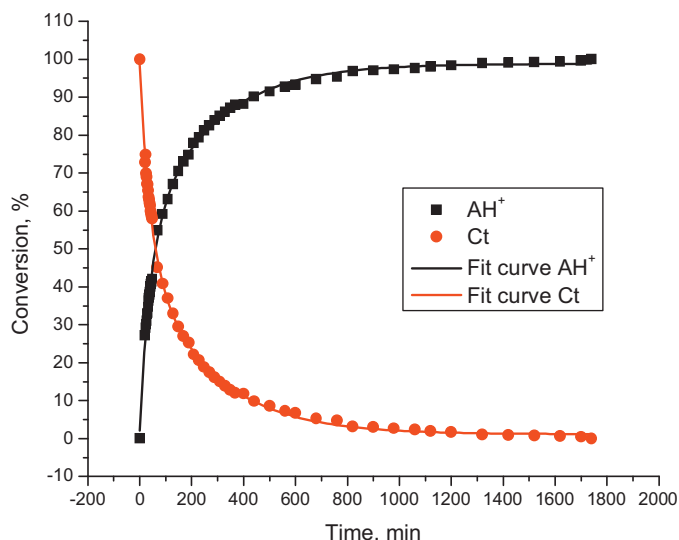


Fig. 2. The conversion of Ct into AH^+ vs. time after addition of $DCIO_4$.

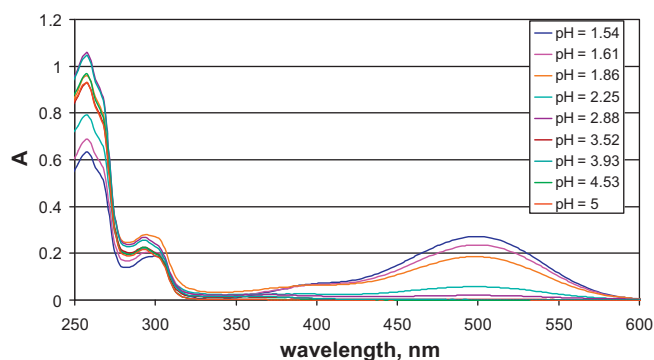


Fig. 3. UV-vis spectra vs. pH for HBC ($5.04 \times 10^{-5} \text{ mol L}^{-1}$ in $H_2O/MeOH$ 3: 2).

neutral and the cationic, are present, expressing different absorption maxima of 498, 298–292 and 258 nm, the last two being found in both cases, while the first corresponds, as stated beforehand, to the cation. Also, the superimposed UV-vis spectra reveal the presence of an isosbestic point at 282 nm corresponding to the conversion of the protonated cationic species AH^+ into the neutral compound. The quinoidal form (A) is not detectable by UV-vis spectrometry, but that does not mean it cannot be formed considering the given conditions [27].

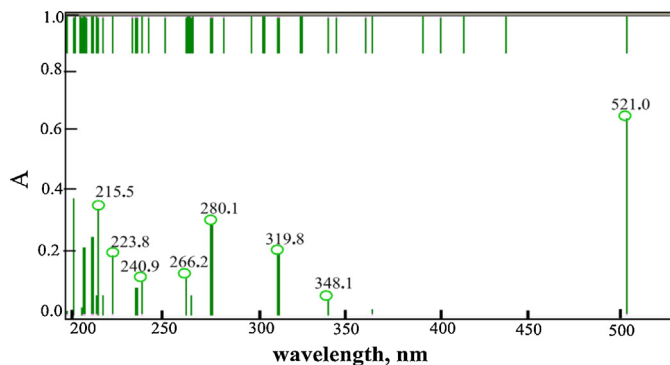


Fig. 5. Electronic absorption spectrum of AH^+ species calculated by the ZINDO/S method (absorption bands highlighted by green sticks with circle).

This colouring behaviour of the sample may lead to its using as a pH sensor on the studied range. Fig. 4 presents the absorbance decay measured at 498 nm depending on the pH shift; the best fitting is registered for an exponential decay. This expression can be turned into a linear dependency, as the following:

$$\ln A = -0.0734 - 1.2378\text{pH} \quad (1)$$

where A is the absorbance of the sample measured at 498 nm; this expression can be applied for pH values ranging between 1 and 5.

To obtain a more comprehensive view about the AH^+ cation specie we studied its electronic spectrum theoretically. The interpretation of the theoretical UV spectrum was based on the ZINDO/S calculations performed on the AM1 geometries. The simulated spectrum is drawn in Fig. 5 by green sticks with circle.

The calculations predicted showed an intensive (oscillator strength is about 0.632) long-wave-length absorption band at 521 nm and three moderately intense bands at 215.53 nm, 280.12 nm and 319.79 nm, respectively. This intensive absorption band is generally an electron shift from HOMO to LUMO orbital (e.g., transition from 54 orbital to 55 orbital). Both orbital are distributed throughout the entire structure of the molecule. Two shortest absorption bands are observed at 240.91 nm and 266.17 nm, respectively. These two bands are characterized by rather weak absorption values (oscillator strength are about 0.117 and 0.126, respectively), and these mostly consist of the electron shift between different orbitals (e.g., transitions from 50 orbital to 55). The results of the ZINDO/S calculations revealed a good correlation between the positions of the bands in the experimental and theoretical spectrum of the compound [28].

The structural and electronic modifications, that accompany the interconversion of photochromic compounds, are reflected on the absorption and emission properties of the compounds [29].

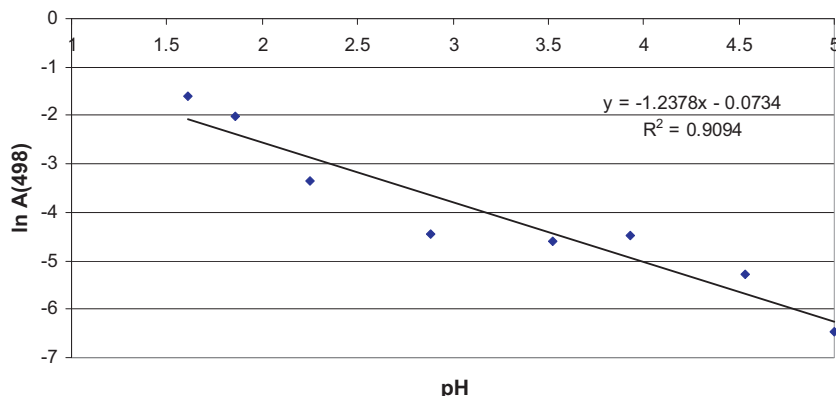


Fig. 4. Logarithm of absorbance vs. pH, for a concentration of $5.04 \times 10^{-5} \text{ mol L}^{-1}$.

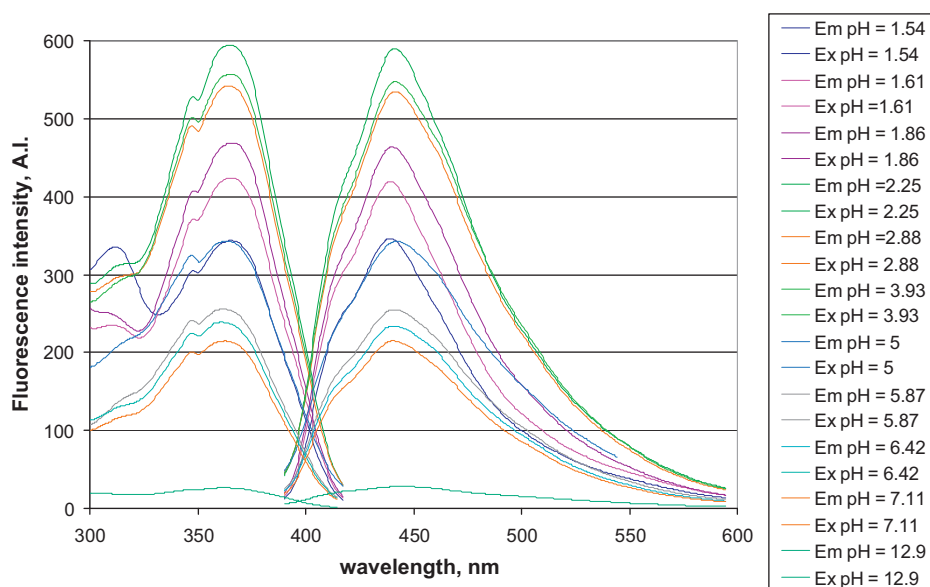


Fig. 6. Superimposed excitation (left) and emission (right) spectra of HBC at different pH values (5.04×10^{-5} mol L⁻¹ in H₂O/MeOH 3:2).

The normalized excitation and emission spectra of the compound depending on the pH increase are presented in Fig. 6. Fluorescence emission/excitation spectra of HBC under acidic, neutral and basic conditions were measured at an excitation wavelength of 361/365 nm and emission at 439/443 nm, respectively [30,31].

On the fluorescence spectra, the maximum emission intensity was obtained for the acid species at pH = 2.25 and the corresponding Stokes shift is $\Delta\lambda = 77$ nm. Fluorescence quenching appears at higher pH, in basic media, effect caused by the deprotonation of the phenolic hydroxyle of HBC.

4. Conclusions

The curcumin analogue, 2,6-bis(2-hydroxybenzylidene)-cyclohexanone has been synthesized in good yield and tested for photochromic behaviour considering the changes in colour due to pH shift. In acid media it functions as a cation, having a reddish colour, while in basic media it is in anionic form, displaying an orange colour. These changes are also registered in UV–vis spectra, the cationic form presenting a λ_{max} of 498 nm, while the anionic form has a λ_{max} of 456 nm. Good correlation of the theoretical and experimental characteristics of the UV–vis spectra was obtained by the method ZINDO/S for the wavelengths of the species studied. The acidic and neutral species of HBC exhibit fluorescent properties.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jphotochem.2014.03.008>.

References

- [1] F. Pina, M. Maestri, V. Balzani, Photochromic flavylum compounds as multistate/multifunction molecular-level system, *Chem. Commun.* 2 (1999) 107–114.
- [2] F. Pina, M.J. Melo, M. Maestri, R. Ballardini, V. Balzani, Photochromism of 4'-methoxyflavylum perchlorate: a write-lock-read-unlock-erase molecular switching system, *J. Am. Chem. Soc.* 119 (1997) 5556–5561.
- [3] A. Roque, C. Lodeiro, F. Pina, M. Maestri, S. Dumas, P. Passaniti, V. Balzani, Multistate/multifunction system: a thermodynamic, kinetic, and photochemical investigation of the 4'-dimethylaminoflavylum compound, *J. Am. Chem. Soc.* 125 (2003) 987–994.
- [4] F. Pina, A. Roque, M.J. Melo, M. Maestri, L. Belladelli, V. Balzani, Multistate/multifunctional molecular-level systems: light and pH switching between the various forms of a synthetic flavylum salt, *Chem. Eur. J.* 4 (1998) 1184–1191.
- [5] V. Petrov, A.J. Parola, F. Pina, Isomerization between 2-(2,4-dihydroxystyryl)-1-benzopyrylium and 7-hydroxy-2-(4-hydroxystyryl)-1-benzopyrylium, *J. Phys. Chem. A* 116 (2012) 8107–8118.
- [6] F. Pina, M. Joao Melo, M. Maestri, P. Passaniti, V. Balzani, Artificial chemical systems capable of mimicking some elementary properties of neurons, *J. Am. Chem. Soc.* 122 (2000) 4496–4498.
- [7] A.M. Diniz, C. Pinheiro, V. Petrov, A.J. Parola, F. Pina, Synthesis and characterization of a symmetric bis-(7-hydroxyflavylum) containing a methyl viologen bridge, *Chem. Eur. J.* 17 (2011) 6359–6368.
- [8] H. Horiuchi, H. Shirase, T. Okutsu, R. Matsushima, H. Hiratsuka, Photochromism of 2-hydroxy-4'-methoxychalcone: a novel photon-mode erasable optical memory system with nondestructive readout ability, *Chem. Lett.* 29 (2000) 96–97.
- [9] K. Tokumura, N. Taniguchi, T. Kimura, R. Matsushima, Primary processes in photochromic reaction of 4-diethylamino-4'-dimethylamino-2-hydroxychalcone in toluene, *Chem. Lett.* 30 (2001) 126–127.
- [10] F. Pina, M.J. Melo, C.A.T. Laia, A.J. Parola, J.C. Lima, Chemistry and applications of flavylum compounds: a handful of colours, *Chem. Soc. Rev.* 41 (2012) 869–908.
- [11] A. Roque, F. Pina, S. Alves, R. Ballardini, M. Maestri, V. Balzani, Micelle effect on the write-lock-read-unlock-erase cycle of 4'-hydroxyflavylum ion, *J. Mater. Chem.* 9 (1999) 2265–2269.
- [12] H. Li, J. Guo, X. Zhang, Z. Chen, A novel colorimetric and fluorescent pH sensor derived from iminocoumarin and thiophene-carboxaldehyde, *Heteroat. Chem.* 23 (2012) 551–559.
- [13] Y. Leydet, A.J. Parola, F. Pina, Hydroxypyridinechromene and pyridinechalcone: two coupled photochromic systems, *Chem. Eur. J.* 16 (2010) 545–555.
- [14] F. Pina, M.J. Melo, H. Santos, J.C. Lima, I. Abreu, R. Ballardini, M. Maestri, Excited state proton transfer in synthetic flavylum salts: 4-methyl-7-hydroxyflavylum and 4',7-dihydroxyflavylum. Example of a four-level molecular device to invert the population of the excited state, *New J. Chem.* 22 (1998) 1093–1098.
- [15] F. Pina, J.C. Lima, A.J. Parola, C.A.M. Afonso, Thermal and photochemical properties of 4',7-dihydroxyflavylum in water-ionic liquid biphasic systems: a write-read-erase molecular switch, *Angew. Chem. Int. Ed.* 43 (2004) 1525–1527.
- [16] Z.Y. Du, Y.F. Jiang, Z.K. Tang, R.Q. Mo, G.H. Xue, Y.J. Lu, X. Zheng, C.Z. Dong, K. Zhang, Antioxidation and tyrosinase inhibition of polyphenolic curcumin analogs, *Biosci. Biotechnol. Biochem.* 75 (2011) 2351–2358.
- [17] J.R. Dimmock, P. Kumar, A.J. Nazarali, N.L. Motaganakalli, T.P. Kowalchuk, M.A. Beazely, J.W. Quail, E.O. Oloo, T.M. Allen, J. Szydowski, E. DeClercq, J. Balzarini, Cytotoxic 2,6-bis(arylidene)cyclohexanones and related compounds, *Eur. J. Med. Chem.* 35 (2000) 967–977.
- [18] Y. Lourie, Aide-memoire de chimie analytique, Editions Mir, Moscou, 1975.

- [19] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, AM1: a new general purpose quantum mechanical molecular model, *J. Am. Chem. Soc.* 107 (1985) 3902–3909.
- [20] J.J.P. Stewart, Optimization of parameters for semiempirical methods: I. Method, *J. Comput. Chem.* 10 (1989) 209–220.
- [21] J. Ridley, M. Zerner, An intermediate neglect of differential overlap technique for spectroscopy: pyrrole and the azines, *Theor. Chim. Acta (Berl.)* 32 (1973) 111–134.
- [22] J.E. Ridley, M.C. Zerner, Triplet states via intermediate neglect of differential overlap: benzene, pyridine and the diazines, *Theor. Chim. Acta (Berl.)* 42 (1976) 223–236.
- [23] HyperChem Release 7.52 for Windows, Hypercube Inc., Gainesville, FL, USA, 2005.
- [24] X. Wang, Z. Han, Z. Wang, K. Ding, Catalytic asymmetric synthesis of aromatic spiroketals by spinphox/iridium(I)-catalyzed hydrogenation and spiroketalization of α,α' -bis(2-hydroxyarylidene) ketones, *Angew. Chem. Int. Ed.* 51 (2012) 936–940.
- [25] H. Wunscher, G. Haucke, P. Czerney, U. Kurzer, Photochromic properties of hydrolyzed benzopyrylium salts – the influence of substitutes, *J. Photochem. Photobiol. A* 151 (2002) 75–82.
- [26] S. Gago, V. Petrov, A.M. Diniz, A.J. Parola, L. Cunha-Silva, F. Pina, Unidirectional switching between two flavylum reaction networks by the action of alternate stimuli of acid and base, *J. Phys. Chem. A* 116 (2012) 372–380.
- [27] R. Matsushima, H. Mizuno, H. Itoh, Photochromic properties of 4'-amino-substituted 2-hydroxychalcones, *J. Photochem. Photobiol. A* 89 (1995) 251–256.
- [28] H. Horiuchi, A. Yokawa, T. Okutsu, H. Hiratsuka, Laser flash photolysis studies of the photo-ring-opening reaction of flav-3-en-2-ol, *Bull. Chem. Soc. Jpn.* 72 (1999) 2429–2435.
- [29] X. Liu, Z. Xu, J.M. Cole, Molecular design of UV-vis absorption and emission properties in organic fluorophores: toward larger bathochromic shifts, enhanced molar extinction coefficients and greater Stokes shifts, *J. Phys. Chem. C* 117 (2013) 16584–16595.
- [30] A. Roque, J.C. Lima, F. Pina, Substitution and solvent effects in the chalcones isomerization barrier of flavylum photochromic systems, *Photochem. Photobiol. Sci.* 6 (2007) 381–385.
- [31] R. Gavara, V. Petrov, F. Pina, Characterization of the 4'-hydroxynaphthoflavylum network of chemical reactions, *Photochem. Photobiol. Sci.* 9 (2010) 298–303.