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## pH Dependent structural interconversion of 2-(2-hydroxy-benzylidene)-cyclohexan-1-one: crystal structures and spectroscopic investigation

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## Abstract

The behavior of 2-(2-hydroxy-benzylidene)-cyclohexan-1-one (BC) in the acidic and basic media was investigated. In acidic conditions a new dimeric structure 4'a-methoxy-2,2',3,3',4',4'a,9',9'a-octahydro-1H,1'H-4,9'-bixanthene (XTC) was isolated. The structures of BC and XTC were confirmed by single crystal X-ray diffraction and NMR. In basic conditions, unprotonated specie (BC<sup>-</sup>) was characterized by NMR, UV-Vis and fluorescent spectroscopy; BC could function as pH sensor.

Keywords: xanthene derivative; crystallographic structure; pH sensor; deuteration.

## **1. Introduction**

Di-substituted cyclohexanone derivatives, curcumin analogues, have been studied so far due to their toxic and cytotoxic activities [1-7]. Recently, our research group has opened a new direction of research and application of xanthylium derivatives on photochemistry at molecular level [8,9]. All practical applications of photochromic compounds are based on the chemical transformations they can be submitted to by the actions of different stimuli, namely, light, pH, temperature, etc.[10-16]. The study of curcumin analogues based on cyclohexanone are done most on symmetrical structures; from the point of view of photochromic investigation, the complexity of asymmetrical systems (see Fig. 1) due to the transient species

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from the network of the chemical reaction open the way to a diversity of applications in optoelectronics and biomedicine.



Fig. 1. Structure of asymmetrical di-substituted benzylidene cyclohexanone derivatives

2-(2-Hydroxy-benzylidene)-cyclohexan-1-one (BC) is a very useful intermediate for the synthesis of a variety of functional systems (photochromic, dyes, therapeutics) [17-22]. The presence of phenolic group, the conjugated double bond system and the active methylene group from the alpha position of ketone moiety turn this compound into attractive candidate for various types of chemical reactions [23]. Herein we present the BC behavior in acidic and basic conditions and the characterization of the isolated compounds.

#### 2. Experimental

#### 2.1. Instrumentation

The NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer (500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C) at 298 K. NMR assignments were done on the basis of 1D and 2D spectra. The HPLC-MS results were obtained using an Agilent 6500 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS. The sample was separated on an Zorbax SB-C18 reverse phase column (4.6 mm x 250 mm, 5 µm particle size) using a 20 µL injection volume, solvent flow 0.6 mL/min and isocratic elution. The mobile phase consisted of 100% acetonitrile filtered and degassed under vacuum before use. The detector UV-VIS DAD was monitored at 282 nm. After the chromatographic detector, the system was fitted with a flow splitter, which directed 10% of the flow directly to the electrospray ion source (ESI) mass spectrometer. The ESI-Q/TOF MS conditions were set as follows: electrospray ionisation (positive ion mode), drying gas (N<sub>2</sub>) flow rate 7.0 L/min; drying gas temperature 325°C; nebulizer pressure 15 psig, capillary voltage 4000 V; fragmentation voltage 200 V. The fullscan mass spectra of the investigated compounds were acquired in the m/z range of 100–1000. The mass scale was calibrated using the standard calibration procedure and compounds provided by the manufacturer. Data were collected and processed using MassHunter Workstation Software Data Acquisition for 6200/6500 Series, version B.01.03. The samples were dissolved in acetonitrile and then diluted up to a concentration of 100 µg/mL. Only positive the ions were analysed, because, in ESI-MS, the formation of the molecular ion occurs in positive mode of operating.

UV-Vis absorption spectra were recorded on Cary 60 UV-Vis spectrophotometer. Fluorescence spectra were recorded on Perkin Elmer LS-55 spectrometer on basic aqueous solutions using  $\lambda_{ex} = 364$  nm and  $\lambda_{em} = 443$  nm, the excitation slit was set at 10 and the emission slit at 5 and the scanning speed was 100 nm/min; for the acetonitrile solution of

XTC  $\lambda_{ex} = 362$  nm and  $\lambda_{em} = 439$  nm with 15 and 5 slits respectively. The fluorescent spectra were recorded using front-face illumination of XTC on front surface powder accessory with 1% attenuation and 15/7.5 slits, and  $\lambda_{ex} = 397$  nm and  $\lambda_{ex} = 443$  nm. The pH of solutions was measured with a Wissenschaftlich-Techniche Werkstatten 330I pH/ion meter. The pH of the solutions was adjusted by addition of NaOH or universal buffer based on sodium citrate [24].

X-ray diffraction: Intensity data were collected at 298 K with Oxford Diffraction SuperNova diffractometer using hi-flux micro-focus Nova Cu Ka radiation. The crystal was placed at 47 mm from the CCD detector and 566 frames were measured each for 10/50 s over 1\_ scan width. Data were processed with the CrysAlis PRO software [25]. The structure was solved by direct methods using Olex2 [26] software with the SHELXS<sup>27</sup> structure solution program and refined by full-matrix least-squares based on  $F^2$  with SHELXL-97 [27]. Atomic displacements for non-hydrogen atoms were refined using an anisotropic model. C-bound hydrogen atoms were placed at idealized positions. Positions of hydrogen atoms for the O-H groups were located on electron density difference map and refined accounting for the hydrogen bonds parameters. The O-H distances were constrained to 0.86 Å. All hydrogen atoms were refined using riding model with Uiso(H) = 1.2 eq of their carrier atoms. Crystal data and further details of data collection and structure refinement are given in Table 1. CCDC 1456381 for **BC** and 1506218 for **XTC** contains the supplementary crystallographic data for this contribution. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.ca.ac.uk).

#### Table 1

Crystallographic data, details of data collection and structure refinement parameters for BC and XTC.

BC	XTC
$C_{26}H_{28}O_4$	$C_{27}H_{28}O_3$
404.48	400.49
100	220
monoclinic	triclinic
$P2_{1}/n$	<i>P</i> -1
17.070(2)	8.0572(8)
7.2073(9)	11.6405(9)
17.140(3)	11.7201(9)
90	85.318(6)
104.201(17)	83.526(8)
90	74.506(8)
2044.3(5)	1051.00(16)
4	2
1.314	1.266
0.699	0.081
$0.092 \times 0.072 \times 0.028$	$0.25 \times 0.1 \times 0.08$
	<b>BC</b> $C_{26}H_{28}O_4$ 404.48 100 monoclinic $P2_1/n$ 17.070(2) 7.2073(9) 17.140(3) 90 104.201(17) 90 2044.3(5) 4 1.314 0.699 0.092 × 0.072 × 0.028

$\theta_{\min}, \theta_{\max}(\circ)$	13.39 to 117.834	3.502 to 50.054
Reflections collected	4853	13371
Independent reflections	2817 [ $R_{int} = 0.0626$ ]	3711 [R <sub>int</sub> = 0.0811]
Data/restraints/parameters	2817/2/273	3711/0/272
$\mathrm{GOF}^c$	1.070	1.045
$R_1^a(I>2\sigma(I))$	0.0808	0.0581
$wR_2^{b}$ (all data)	0.1847	0.1255
Largest diff. peak/hole/e Å <sup>-3</sup>	0.22/-0.26	0.19/-0.21

 ${}^{a}R_{1} = \Sigma ||F_{o}| - |F_{c}|| \Sigma |F_{o}|.$   ${}^{b}wR_{2} = \{\Sigma [w(F_{o}^{2} - F_{c}^{2})^{2}] / \Sigma [w(F_{o}^{2})^{2}] \}^{1/2}.$   ${}^{c}$  GOF =  $\{\Sigma [w(F_{o}^{2} - F_{c}^{2})^{2}] / (n-p) \}^{1/2}$ , where *n* is the number of reflections and *p* is the total number of parameters refined

#### 2.2. Materials and methods:

Salicylaldehyde, 99%, cyclohexanone, 99% were of analytic grade and were purchased from Sigma-Aldrich. Other reagents, sodium hydroxide (powder), chloroform, petroleum ether, acetonitrile, perchloric acid 70% and methanol were purchased from Merck and were used without further purification.

#### 2.3. Synthesis of compounds:

2-(2-hydroxy-benzylidene)-cyclohexan-1-one (see Scheme 1) was obtained following the literature [3] with a slight modification. A solution of salicylaldehyde (5.2 mL, 0.05 mol) in 4% aqueous NaOH (150 mL) was added dropwise to a mixture of 4% aqueous NaOH (50 mL) and cyclohexanone (25.9 mL, 0.25 mol) under stirring. The reaction mixture was stirred at room temperature for 40h and then neutralized with HCl 6N (40 mL). The HCl solution was added dropwise to avoid heating. The product was extracted into CHCl<sub>3</sub> (100 ml), washed with water (4 X 100 ml) and dried over Na<sub>2</sub>SO<sub>4</sub>. The chloroform was evaporated and the resulting dark brown oil was extracted with hot petroleum ether. Upon cooling a yellow precipitate was filtered off, while from the mother liquor yellow crystals were formed. ( $\eta = 24\%$ , m.p. = 126-128 °C).



Scheme 1. Synthesis of BC

**FT-IR** (KBr) cm<sup>-1</sup>: 3370 (br,s), 2937 (w), 2871 (w), 1655 (m), 1561 (s), 1249 (s), 1145 (m), 750 (m).

<sup>1</sup>**H-NMR** (500 MHz, CD<sub>3</sub>OD, δ ppm): 7.65 (s, 1H, H<sup>7</sup>), 7.27 - 7.16 (m, 2H, H<sup>9,11</sup>), 6.87 - 6.82 (m, 2H, H<sup>10,12</sup>), 2.78 (t, 2H, H<sup>2</sup>, J = 6.7 Hz), 2.51 (t, 2H, H<sup>5</sup>, J = 6.7 Hz), 1.97 - 1.89 (m, 2H, H<sup>3</sup>), 1.79 - 1.72 (m, 2H, H<sup>1</sup>)

<sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD, δ ppm): 204.7 (C6), 158.2 (C13), 137.3 (C4), 133.2 (C7), 131.5 (C9, C11), 123.9 (C8), 120.3 (C10), 116.6 (C12), 41.4 (C5), 30.2 (C2), 25.1 (C1), 24.8 (C3).

4'a-Methoxy-2,2',3,3',4',4'a,9',9'a-octahydro-1H,1'H-4,9'-bixanthene (XTC) – Fig. 2. 50 mg of BC were dissolved into 20 mL of methanol, then 5 mL of  $HClO_4$  70 % w solution were added while stirring. The solution immediately turned red and after 2 hours of stirring at room temperature, a white precipitate was obtained and isolated by filtration. Suitable single crystals for X-ray diffraction were obtained in about 24 hours by slow evaporation of a methanol/chloroform solution of XTC. m.p. = 156-158°C



Fig. 2. Structure of XTC

<sup>1</sup>**H-NMR** (500 MHz, CDCl<sub>3</sub>,  $\delta$  ppm): 7.1 (m, 1H, H<sup>24</sup>), 7.07 (m, 1H, H<sup>26</sup>), 7.03 (m, 1H, H<sup>9</sup>), 6.90 (m, 1H, H<sup>7</sup>), 6.81 - 6.83 (m, 4H, H<sup>8,10, 23,25,</sup>), 6.05 (s, 1H, H<sup>5</sup>), 4.41 (d, 1H, H<sup>14</sup>, J = 11.7 Hz), 3.25 (s, 3H, H<sup>21</sup>), 2.45 (m, 2H, H<sup>3</sup>), 2.37, 1.46 m (m, 2H, Hx), 1.93, 1.85 (m, 2H, H<sup>1</sup>), 1.79 (m, 1H, H<sup>15</sup>), 1.77, 1.27 (m, 2H, Hx), 1.75, 1.42 (m, 2H, Hx), 1.68, 1.57 (m, 2H, H<sup>2</sup>), 1.68, 1.45 (m, 2H, Hx).

<sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>, δ ppm): 153.2 (C11), 152.7 (C22), 147.5 (C12), 131.1(C4), 128.5 (C26), 128.0 (C9), 127.1 (C24), 126.6 (C27), 125.2 (C7), 122.3 (C6), 121.7 (C23), 120.9 (C25),117.6 (C5), 116.8 (C8), 114.6 (C10), 112.2 (C13), 99.5 (C20), 48.0 (C21), 41.6 (C15), 35.7 (C14), 31.4 (C18), 30.7 (C3), 27.2 (C16), 25.7 (Cx), 24.5 (C1), 22.6 (Cx), 21.8 C2).

**ESI-MS**: calcd. for C<sub>27</sub>H<sub>28</sub>O<sub>3</sub> m/z = 400.2038 [M<sup>+</sup>], found m/z = 400.1974 [M<sup>+</sup>]

Sodium 2-((2-oxocyclohexylidene)methyl)phenolate (BC<sup>-</sup>Na<sup>+</sup>) 20 mg of BC were dissolved into 1 mL of methanol then 40 $\mu$ L of NaOH 40% wt. solution were added. Similar conditions were used for deuterated solvents and the structure for the product is presented in Fig. 3.



**Fig. 3.** Stucture of BC<sup>-</sup>Na<sup>+</sup> in NaOD and methanol-*d4* 

<sup>1</sup>**H-NMR** (500 MHz, 1 ml CD<sub>3</sub>OD + 40μL NaOD, δ ppm): 8.01 (s, 1H, H<sup>7</sup>), 7.14 (dd, 1H, H<sup>9</sup>, J = 7.7, 1.8 Hz), 7.02-6.99 (m, 1H, H<sup>11</sup>), 6.63 (dd, 1H, H<sup>12</sup>, J = 8.3, 1.1 Hz), 6.40-6.37 (m, 1H, H<sup>10</sup>), 2.82-2.80 (m, 2H, H<sup>5</sup>), 1.90-1.87 (m, 2H, H<sup>3</sup>), 1.76-1.72 (m, 2H, H<sup>4</sup>).

<sup>13</sup>**C-NMR** (CDCl<sub>3</sub>, δ ppm): 205.3 (C1), 170.7 (C13), 138.9 (C7), 132.8 (C6), 132.3 (C11), 131.4 (C9), 126.1 (C8), 121.7 (C12), 113.9 (C10), 40.5 (weak – C2), 30.5 (C5), 25.3 (C3), 24.5 (C4).

**ESI-MS**: calcd. for  $C_{13}H_{13}O_2 m/z = 201.0921 [M<sup>-</sup>]$ , found m/z = 201.0386 [M<sup>-</sup>]

#### 3. Results and discussions

According to X-ray crystallography both **BC** and **XTC** compounds have a molecular crystal structure, as shown in Fig. 4 and 5, respectively. The asymmetric part of the unit cell of **BC** is consisted of two crystallographic independent but chemically equivalent neutral entities, denoted as molecule **A** and **B**. As expected, the geometric parameters of two molecules are very close, which is evidenced by the values of bond distances and angles summarized in Table 1S and 2S (see Supplementary Information).



Fig. 4. View of the asymmetric part in the crystal structure of BC. Thermal ellipsoids are drawn at 50% probability level.



Fig. 5. X-ray molecular structure of **XTC**. Thermal ellipsoids are drawn at 40% probability level.

The interaction between the independent molecules **A** and **B** in the crystal is realized via two intermolecular H-bonds, where O1A-H and O1B-H act as donor towards O2B and O2A as acceptor of protons, respectively. It determines the formation of the supramolecular *zig-zag* like chains running along 101 direction. A view of one-dimensional supramolecular chain is shown in Fig. 6.



Fig. 6. One-dimensional H-bonded chain in the crystal structure of BC.

H-bonds parameters: O1B–H···O2A [O1B–H 0.86 Å, H···O2A 1.97 Å, O1B···O2A (-0.5 – x, 0.5 – y, -0.5 + z) 2.774(4) Å, O1B–H···O2A 155.4°], O1A–H···O2B [O1A–H 0.86 Å, H···O2B 1.90 Å, O1A···O2B 2.736(4) Å, O1A–H···O2B 164.9°].

The BC in acidic condition in the first step turns to a red solution due to the formation of cationic form  $BC^+$  and after two hours it converts to a white solid product (XTC).  $BC^+$  is the unstable form and in the presence of perchloric acid turns into metastable mixture of HX and  $BC^+$ . These two unstable species react with the formation of dimeric structure HTX, stabilized by addition of a nucleophile (methoxy group). Kanitz and co-workers [28] previously reported the formation of a different dimer structure resulting from dimerization of two HX molecules in the acidic conditions. The mechanism of the reaction for XTC form is depicted in Scheme 2.





Scheme 2. The proposed mechanism for the formation of XTC

The XTC structure identified by use of single crystal X-ray diffraction is confirmed by NMR spectroscopy but, the attribution signals to each proton of <sup>1</sup>H-NMR spectrum is very complex. In the low field they are two sets of signals attributed to the absorption by the aromatic protons: 7.12-7.01 ppm corresponding to 3H, 6.91- 6.79 ppm corresponding to 5H. The <sup>1</sup>H-<sup>13</sup>C 2D HSQC NMR spectrum (correlates <sup>13</sup>C with directly attached protons) indicated the exact correlation, as a cross peak, between each proton and its corresponding carbon- first set: H from 7.10 ppm with C from 127.1 ppm; H from 7.07 ppm with C from 128.5 ppm; H from 7.03 ppm with C from 128.0 ppm; - second set: H from 6.90 ppm with C from 125.2 ppm; H from 6.86 ppm with C from 120.9 ppm; H from 6.85 ppm with C from 116.8 ppm; H from 6.83 ppm with C from 121.7 ppm; H from 6.81 ppm with C from 114.6 ppm. Based on <sup>1</sup>H-<sup>13</sup>C 2D HMBC NMR spectrum (correlates long range coupling <sup>1</sup>H-<sup>13</sup>C over two and three bond) it was obtained the next correlations: H from 7.1 ppm with C from 152.7 and 128.5 ppm, respectively; H from 7.07 ppm with C from 152.7, 127,1 and 35.7 ppm, respectively, H from 7.03 ppm with C from 153.2 and 125.2 ppm, respectively; H from 6.90 ppm with C from 153.2, 128.0 and 117.6 ppm, respectively; H from 6.86 ppm with C from 125.6 and 116.8 ppm, respectively; H from 7.85 ppm with C from 153.2, 128.0, 122.3 and 114.6 ppm, respectively; H from 6.83 ppm with C from 152.7, 125.6 and 120.9 ppm, respectively; H from 7.81 ppm with C from 153.2 and 122.3 ppm, respectively. The correlation of the above results had led to the establishment of chemical shift for each atom of carbon and hydrogen (see Experimental section and Supplementary Information).

HPLC analysis revealed the presence of only one peak at about 11 min, for which was extracted the mass spectrum in the positive ionization mode. The ESI-MS spectrum (see Fig. S19) illustrates the presence of the molecular ion of the XTC at m/z = 400.1974. In addition, the base peak at m/z = 369.1804 corresponds to XTC structure without methoxy group, the fragmentation being produced during the ionization process.

BC in the basic condition turned into unprotonated form (BC<sup>-</sup>) (see Scheme 3). In the presence of deuterated solvent, a hydrogen-deuterium exchange reaction takes place; the hydrogen atoms in alpha position to the keto group were exchanged with deuterium atoms (see Fig. 3). This is a well-known method for synthesis of many deuterated aldehydes and ketones with a suitable alpha position to the carbonyl group [29]. Confirmation of deuterated BC<sup>-</sup> specie was proved by NMR experiments. Thus, comparative H-NMR spectra of BC and BC<sup>-</sup> reveals the shifts of all proton signals and the absence of the protons signal from alpha position to keto group (triplet from 2.51 ppm). COSY-NMR spectrum of BC<sup>-</sup> shows faint signals of correlation between proton from position no. 2 and 3, while HSQC-NMT displayed weak correlation signals of C from 40.5 ppm and H from 2.51 ppm.

BC is a chalcone derivative that is expected to have photochromic behavior upon pH variation and light excitation, similar to those registered for 2,6-bis-(2-hydroxybenzylidene)cyclohexan-1-one [8,9]. As a consequence, experiments were carried out in order to establish color change during pH shift from very acidic to very basic environment and identify the chemical species responsible for this behavior (see Scheme 3). We have seen beforehand that in very acidic media, BC turns into the irreversible dimeric structure identified as XTC, so we have focused in studying the photochromic behavior only in basic environment.



Scheme 3. BC behavior along pH variation (only stable species are displayed)

The UV-Vis spectra of BC in aqueous solutions of basic pH are presented in Fig. 7. The samples in basic pH exhibit yellow color and present a broad absorption band at 394 nm. At pH = 13.26 we identify the most intense absorption band, with  $\varepsilon = 3077.6 \text{ L mol}^{-1} \text{ cm}^{-1}$ . This band, corresponding to the deprotonated form Ct<sup>-</sup> registers a drastic decay along pH drop, expressed by a logarithmic function. Fig. 8 shows the linear dependence between ln A (394 nm) and pH on the range 6-14, which could recommend BC to be used as pH sensor in basic environment.



Fig. 7. UV-Vis spectra of 2-(2-hydroxy-benzylidene)-cyclohexan-1-one at basic pH values  $(2x10^{-4} \text{ mol/L in aq. solution})$ 



Fig. 8. Linear variation of ln A (394 nm) versus pH in basic environment

The excitation and emission spectra of 2-(2-hydroxy-benzylidene)-cyclohexan-1-one in different pH solutions, ranging from 6.96 to 13.75 are presented in Fig. 9. The compound exhibits fluorescent properties in mild basic conditions. The fluorescence intensity decreases with the increase of pH, while at pH values above 11 the fluorescence is quenched.



**Fig. 9.** Superimposed excitation (left) and emission (right) spectra of 2-(2-hydroxy-benzylidene)-cyclohexan-1-one at different pH values  $(2x10^{-4} \text{ mol/L in aqueous solution})$ 



**Fig. 10.** Fluorescent behaviour on XTC solid sample: left on powder and right on monocrystals; top - samples before irradiation and bottom after irradiation with UV light

The maximum emission intensity was obtained at pH = 7.28 and the corresponding Stokes shift is  $\Delta \lambda = 69$  nm.

XTC displays fluorescent behavior in solution and even in solid state (see Fig. 10). By irradiation at  $\lambda = 365$  nm using the laboratory UV lamp it shows obvious fluorescent properties, which is confirmed by fluorescence spectroscopy (see Fig. 11: a) on solid, b) in solution)



**Fig. 11.** Fluorescence behaviour of XTC on solid state - left, in acetonitile  $(10^{-5} \text{ mol } \text{L}^{-1})$  – right.

#### 4. Conclusions

2-(2-hydroxy-benzylidene)-cyclohexan-1-one (BC) was synthesized in good yield and its X-ray diffraction structure was assessed. BC shows pH dependent photochromic behavior and it could be used as a pH sensor on basic pH range (pH =  $6 \div 14$ ). In very acid environment it turns into a dimeric form HTX stabilized by addition of methoxy group – XTC form. The structure of XTC was identified by single crystal X-ray diffraction and confirmed by NMR and mass spectrometry. Good fluorescence properties were found for the BC on the 6 -11 pH range and for the XTC in solid state and in solution.

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## Highlights

- Synthesis and characterization of 2-(2-hydroxy-benzylidene)-cyclohexan-1one (BC) and 4'a-methoxy-2,2',3,3',4',4'a,9',9'a-octahydro-1H,1'H-4,9'bixanthene (XTC);
- Crystal structures of BC and XTC;
- UV-Vis and fluorescence spectroscopic investigation;
- Mechanism formation of XTC from BC;
- Deuteration of  $\alpha$ -position to keto group.