Proceeding Paper

Spectroscopic Investigation, Component Analysis and DFT Calculations of Tautomeric Forms of Substituted Dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one [†]

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Abstract: Chromenopirazolopyrimidinone (CPP) was synthesized using microwave radiation in the absence of solvent by a multicomponent reaction of 4-hydroxy-2H-chromen-2-one with 5-amine-1H-pyrazole and benzaldehyde. CPP exists as a mixture of tautomeric forms, the structure of which was examined by means of spectroscopic methods and quantum chemistry. As was shown by MILCA algorithm, UV-Vis spectra obtained in different solvents of various polarity contain bands of all tautomeric forms. Decomposition of the absorption spectra of CPP in various solvents on the individual components of the absorption showed the presence of two tautomers. The molar ratios of tautomeric forms depend on polarity of solvent used.

Keywords: Chromenopirazolopyrimidinone; MILCA algorithm; decomposition; tautomers; UV-Vis spectra

1. Introduction

The scientific community faces the challenge of creating complex organic compounds from simple substratesusing environmental criteria, safety and efficiency of the synthetic procedures [1]. Multicomponent reactions, such as the Biginelli reaction is a well-known method of the synthesis of azaheterocycles [2].

One option of the multicomponent synthesis conditions is the microwave exposure in the absence of solvent, with the addition of a Lewis acid catalyst [3]. Currently, microwave activation (Microwave Assisted Organic Synthesis, MAOS) is one of the most rapidly growing trends in organic chemistry. First of all, this is due to multiple (101–103 times) shortening of the chemical reactions.

The combined experimental and theoretical study of the spectral characteristics of molecular systems is one of the most important modern tools for solving problems of current organic chemistry. Such an approach is relevant for a correct description of the composition and structure of the reaction mixtures of biologically active compounds, especially when it comes to mobile tautomeric equilibrium.

Thus, by combining the techniques of FT-IR, NMR, UV-Vis spectroscopy, and by comparison of experimental data with the calculated values the chromenopirazolopyrimidinone (CPP), a product of multicomponent reaction of 4-hydroxy-2H-chromen-2-one with 5-amine-1H-pyrazole and benzaldehyde, was characterized. CPP exists as a mixture

of tautomeric forms, the structure of which was examined by means of an integrated approach using spectroscopic methods and quantum chemistry.

2. Materials and Methods

2.1. Synthesis of 7-phenyl-7, 12-dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one (CPP)

CPP was synthesized using microwave radiation in the absence of solvent by a multi-component reaction of 4-hydroxy-2H-chromen-2-one with 5-amine-1H-pyrazole and benzaldehyde. Yield 1.5 g (78%), m.p. 125–127 °C; FTIR, cm^{-1} 3251, 3101, 3099, 3032, 2956, 1660, 1604, 1569, 1518, 1473, 1451, 1409, 1359, 1340, 1230, 1195, 1114, 1076, 1049 1033, 1002, 980, 961, 950, 903, 854, 836, 808, 754, 725, 706, 697, 660, 618, 601, 589, 551, 529, 484, 470, 443, 407. Anal. calc. for $C_{19}H_{13}N_3O_2$, %: C, 72.37; H, 4.16; N, 13.33; found: C, 72.42; H, 4.17; N, 13.26.

2.2. NMR Spectra

NMR spectra were recorded on a Varian 400 spectrometer at 20–25 °C. Operating frequency for the ^{1}H NMR spectra was 400 MHz, and for ^{13}C NMR spectra—100 MHz respectively. Internal standard was TMS, and solvents were $CDCl_3$, dimethyl sulfoxide-d_6.

2.3. LDI-MS

Laser desorption and ionization (LDI) mass spectrum was collected with AB Sciex 5800 TOF/TOF (USA) in reflector mode with positive ion detection using solid-state Nd: YAG laser (355 nm) and accelerating voltage of 20 kV.

2.4. FT-IR Spectra

FT-IR spectrum was recorded in Nicolet 6700 spectrometer (Thermo Scientific, USA) by using KBr pellet (wavenumber range of 4000–400 cm^{-1}) with spectral resolution of 4 cm^{-1}.

2.5. UV-Vis Spectra

The UV-Vis spectra of the CPP solutions were recorded on a Shimadzu-1800 spectrophotometer using 1-cm cuvettes; the scanning step being 1 nm. Solvents and reagents were of special grade. Working solutions were prepared by dissolving exactly weighed samples of the compounds ($C = 5 \times 10^{-5}$ M) in an appropriate solvent.

2.6. Decomposition of UV-Vis Spectra

The computer program of the MILCA algorithm and examples of solving practical tasks are available as independent executables with a MATLAB interface (The Math-Works, Natick, MA, USA).

2.7. Quantum Chemical Calculations

Quantum chemical calculations were performed using density functional theory (DFT) using the hybrid functional Lee-Yang-Parr three-parameter Becke B3LYP, split-valence basis set functions 6-311G, with the inclusion of p-orbitals of the hydrogen atom and d-orbitals for more heavy atoms as well as with the addition of polarization functions (B3LYP/6-311G++ (*d,p*)).

3. Results and Discussion

3.1. Synthesis of CPP

Using microwave radiation and the absence of solvent for a similar reaction results in a product CPP that can exist in three forms—as 7-phenyl-7,12-dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one (**1a**), as 7-phenyl-7H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-ol (**1b**), and as 7-phenyl-7,9-dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one (**1c**).
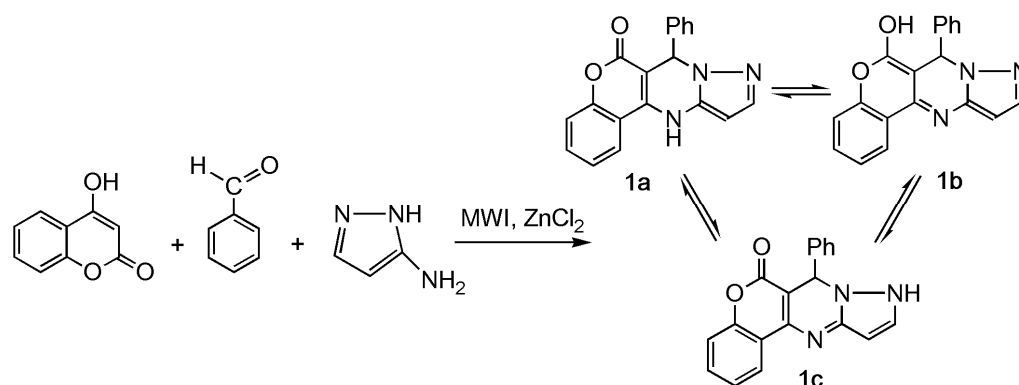
Scheme 1. Synthesis of 7-phenyl-7, 12-dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one (CPP) **1a–c**.

Theoretically, tautomeric system 1 can be represented by three forms: a, b, c. Forms a, and b are realized due keto-enamine-enol-imine tautomerism, and the c form is the result of pyrazole moiety tautomerism.

3.2. NMR Spectroscopic Data

^{1}H NMR spectrum indicates the presence of CPP in two tautomeric forms: a, b due to the implementation of keto-enamine-enol-iminetautomerism.

In the ^{1}H NMR spectrum of the mixture of tautomers a, b singlets NH, OH groups are at 10.58 ppm and 12.31 ppm respectively. Tertiary—proton H7′ singlet appears in 4.97 ppm and the proton H7—at 5.23 ppm The aromatic portion includes 22H and presented in a doublet 8.31 ppm (H1,1′, *J* = 8 Hz); 7.60 (t., N3,3′, *J* = 8 Hz); 7.42–7.32 (m., N2,2′, N4,4′); 7.28–7.25 (m. N10,10′ N11,11′); 7.24–7.06 (m., 10H)

To confirm the presence in the ^{1}H NMR spectrum signals tautomeric forms **1a** use nuclear Overhauser effect (NOE 1D), discovers a spatial interaction ortho-proton H1 chromenone fragment and NH group proton of the pyrimidine moiety.

HSQC spectrum definitively confirmed the existing of two tautomeric forms **1a**, **b** in DMSO-d$_6$ solution. It was evidenced by the presence of C7/C7 H7 and ′/H7′ cross peaks.

3.3. LDI-MS Data

For the considered tautomeric mixture of 1a-c was recorded as a mass spectrum by laser desorption and ionization (LDI). LDI method is different from the "classical" methods of ionization, such as electron impact or chemical ionization, a higher probability of occurrence of photochemical processes, resulting in often arise ions dimer molecules peregruppirovochnye and other more complex cluster ions. In general LDI mass spectrometry are more complex, however, they can more accurately interpret the structure (Figure 1).
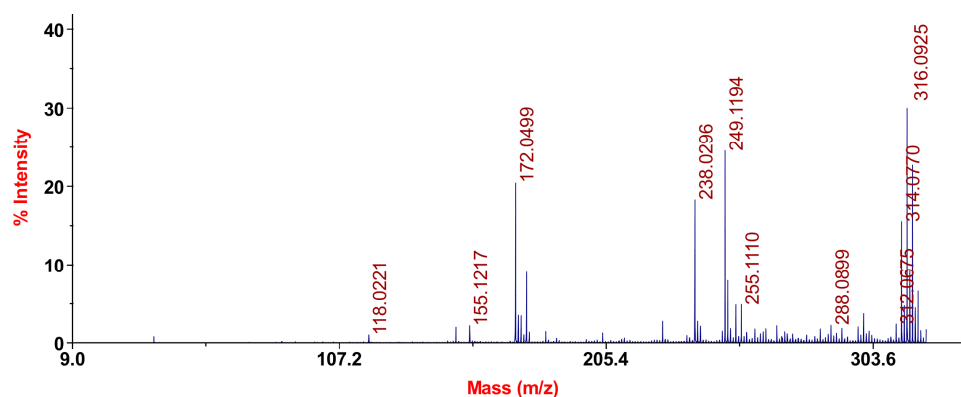

Figure 1. Mass spectrum chromenopirazolopyrimidinone (CPP) **1a–c**.

For three tautomeric forms **1a–c** would expect similar mass-spectrometric patterns as isomeric structure capable of producing isobaric ions.

The mass spectrum of **1a–c** LDI chromenonepirazolopirimidina no molecular ion peak, but there are signals of protonated [M + H]$^+$ and deprotonated [M − H]$^+$ forms of molecules with mass-charge number respectively *m/z* 314 and 316. The instability characteristic of the molecular ion fused aromatic systems, and are often observed in their mass spectra, including rows and heterocyclic compounds.

3.4. FT-IR Spectra and Vibrational Analysis

The highest frequency vibrations 3400–3250 cm^{-1} were assigned to the NH stretching vibrations of forms 1 and 3, and for tautomeric form 2 the highest frequency band was the OH stretching vibration band.

In aromatic heterocycles C-H ring vibrations are expected to occur in the region of 3000–3030 cm^{-1} and usually the bands are strong. In our case these bands appear at 3129 cm^{-1} (HATR) and 3101 cm^{-1}, while corresponding scaled calculated frequencies for the third tautomer were at 3131 and 3104 cm^{-1}.

Stretching vibration of lactone carbonyl group C=O in various cyclic esters differing by the number of atoms in the cycle and the degree of saturation can be observed as a very strong band in FT-IR spectra at 1770–1720 cm^{-1}.

3.5. UV-Vis Spectra and Their Decomposition

To confirm the assumptions about the form of existence synthesized by us pyrimidine recorded UV spectra of a mixture of tautomers 1a-c in solvents of different polarity.

In the UV spectra of the mixture chromenopirazolopirimidines **1a–c** exhibits two intense bands with λ_{max} 260–273, 335–345 nm (Figure 2, Table 1)

Table 1. This is a table. Values of absorption maxima in UV spectra depending on the solvent of the compound **1a–c**.

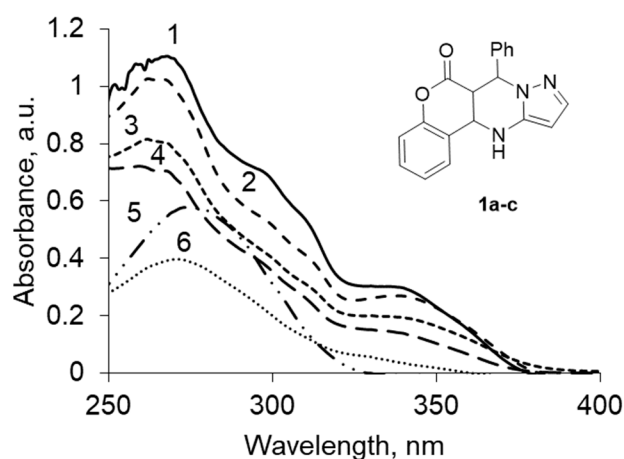| Solvent, λ_{max}, nm (lg ε)				
Dioxane	**Chloroform**	**Acetonitrile**	**Ethanol**	**Hexane**
268 (1.11)	271(0.40)	260 (0.72)	266 (1.02)	273 (0.58)
335 (0.32)	-	335 (0.15)	345 (0.30)	-

Figure 2. UV spectrum chromenopirazolopyrimidinone (CPP) **1a–c** at concentrtation, 5 × 10^{-5} M) in: (1) dioxane, (2) ethanol, (3) methanol, (4) acetonitrile, (5) hexane, (6) chloroform.

Bands with λ_{max} 260–273 nm (k = 0.39–1.15) are forbidden by symmetry n-π * transitions observed in the spectra of compounds in which the supporting structure of the unshared electron pair of the heteroatom connected multiple π-bonds with neighboring

atoms. Recognize strip n → π * transition is possible on hypsochromic shift when changing the nonpolar solvent to polar.

Bands with λ_{max} 335–345 nm (k = 0.13–0.36) for the characteristic most of the enol form. Bathochromic shift of the absorption bands in the transition from non-polar to polar solvents can be attributed them to the π-π * transitions enimine fragment.

In all tested solvents prevalent keto-enamine form 1a, but varying the ratio trend in tautomeric forms depending on the degree of polarity of the solvent.

3.6. Decomposition of UV-Vis Spectra

Using chemometrics methods—independent components, implemented in the MILCA algorithm, we have shown the possibility of quantitatively studying tautomeric equilibria with overlapping absorption bands of tautomers in an individual solvent. The essence of self-similar separation of curves is the decomposition of the matrix of spectra of a multicomponent system X of size M × N (N is the number of counts by wavelength, M is the number of spectra of the mixture) into a matrix of spectra of individual components S of size K × N and a matrix of their relative concentrations A of size M × K (K is the number of components in the system) (Equation (1)):

$$\mathbf{X} = \mathbf{A} \cdot \mathbf{S} \tag{1}$$

Decomposition of the absorption spectra of Compound **1a–c** in various solvents on the individual components of the absorption showed the presence of two tautomeric forms (Figure 3) And allowed to calculate the percentage of each of them in various solvents (Table 2).

The analysis is carried out regardless of the number of components, their spectral characteristics of individual compounds, as well as the quantitative composition of the mixture. Thus, the work of the algorithm consists in isolating the individual spectral contours of the ketone and enol forms and subsequent calculation of the relative content of the enol form in solutions of solvents of different polarity.
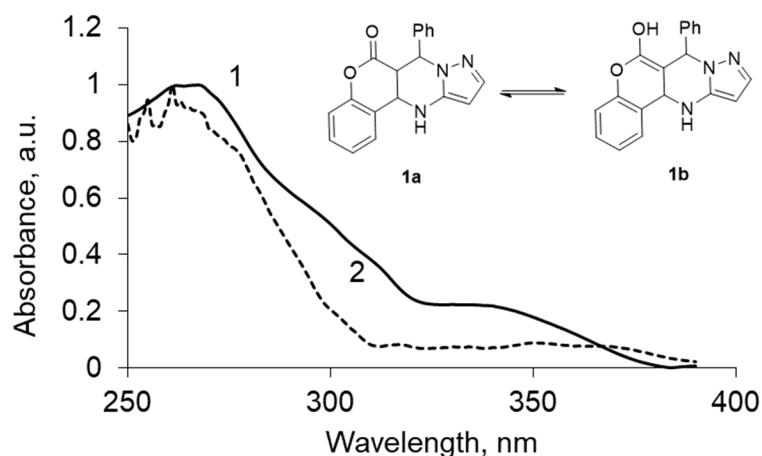

Figure 3. UV spectrum of the two tautomeric forms obtained by treating the source of the UV spectrum program MILCA, for chromenopirazolopyrimidinone (CPP) 1: (1) keto-enamine form (**1a**), (2) enol-imine form (**1b**).

Table 2. Ratio between keto-enamine and enol-imine forms of **1a–c** depending on the solvent.

Solvent	Keto-Enamine 1a	Enol-Imine 1b
Dioxane	81	19
Ethanol	98	2
Methanol	86	14
Acetonitrile	99	1

| Hexane | 100 | 0 |
| Chloroform | 100 | 0 |

3.7. Quantum Chemical Calculations

To compare the thermodynamic stability of all three putative tautomeric forms **1a–c** in vacuum and in two solvents, polar methanol and low-polar hexane, we carried out quantum chemical calculations with full geometry optimization. Calculation data indicate that the most thermodynamic stable structure is 1a regardless the medium. The **1b** tautomer is 15–19 kcal/mol less stable, so, one can speculate that in solvent this form will be significantly less represented in tautomeric equilibrium. These data are in harmony with UV-vis spectra and their deconvolution by MILCA algorithm results. Considering solvents of different polarities, we can conclude that all three tautomers prefer the more polar methanol, where their energies tend to a minimum.

4. Conclusions

Thus, the existence of all three tautomeric forms is possible: in the solid state (mass and IR) form **1c** predominates; in solution (NMR, UV), form 1a predominates.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Calculated energies of tautomeric forms **1a–c**, Data S3: Data of DFT calculations, Data S3: Decomposition results of MILCA.

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