Spectrochimica Acta Part A 71 (2008) 1274–1280

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Spectrometric study of tautomeric and protonation equilibria of *o*-vanillin Schiff base derivatives and their complexes with Cu(II)

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ARTICLE INFO

Article history:
Received 9 January 2008
Received in revised form 14 March 2008
Accepted 19 March 2008

Keywords:
Schiff bases
o-Vanillin
Aminopyridine
Spectrophotometry
Spectrofluorimetry
Tautomeric constants
Protonation constants
Copper(II) complexes

ABSTRACT

Electronic absorption and emission properties of a series of Schiff bases derived from 2-hydroxy-3-methoxybenzaldehyde and 2-aminopyridine, 2,3-diaminopyridine, 2,6-diaminopyridine, or 3-aminomethylpyridine were studied in solvents of different polarities. The interconversion of the enolimine to the ketoamine tautomeric form was observed for compound **1**, 6-methoxy-2-(3-pyridylmethyliminomethyl)phenol, and the corresponding equilibrium constant was estimated in several solvents. Protonation constants of all the investigated compounds were determined spectrophotometrically in the methanol/water 1/4 system. The effect of copper(II) ions on absorption and on the emission spectra of these ligands was examined in the buffered dioxane/water 1/1 system (pH 5.8). Strong complexation of Cu(II) and formation of a 1:1 complex were observed for the bis-Schiff base derived from 2,3-diaminopyridine. The complex of copper(II) with compound **1** was isolated and characterized by elemental analysis, magnetic susceptibility measurement, UV–vis and IR spectrometry.

1. Introduction

Schiff bases have been extensively studied as they possess many interesting features, including photochromic and thermochromic properties [1], proton transfer tautomeric equilibria [2], biological and pharmacological activities [3–6], as well as suitability for analytical applications [7,8]. Due to synthetic flexibility and simple preparation procedure these compounds have received a great deal of attention as suitable ligands for coordination [9,10] and determination of various metal ions [7,11–14]. Many Schiff base metal complexes with a variety of biological activities have been described in the literature [15–20].

The Schiff bases of salicylaldehyde and aminopyridines have been characterized in detail in solid state and in solution [21–25], and proposed as highly sensitive spectrometric and spectrofluorimetric reagents for Cu(II) [26,27]. However, analogous heteroaromatic Schiff bases derived from 2-hydroxy-3-methoxybenzaldehyde (*o*-vanillin) have not been investigated so thoroughly. The mono- and bis-Schiff bases of *o*-vanillin and 2,3-diaminopyridine have been used as ionophores in a Cu(II) selective electrochemical sensor [28]. These compounds as well as their metal complexes have been found to possess biological activity [19]. Antibacterial activity has also been reported for the ruthenium(II) complex of the Schiff base of *o*-vanillin and 2-aminopyridine [20].

The Schiff bases derived from salicylaldehyde and amino-, aminoalkyl- and diaminopyridines are unstable in solution, and are involved in various equilibria like keto-enol or in ring-chain tautomeric interconversion, hydrolysis or some others [25,27]. Therefore, their successful application requires a detailed study of their characteristics. In this work a series of known Schiff bases derived from amino- or diaminopyridines and 2-hydroxy-3-methoxybenzaldehyde were prepared. A new Schiff base was synthesized from 3-aminomethylpyridine (Scheme 1). The spectroscopic characteristics, tautomeric properties, and protonation constants of the prepared compounds in the methanol/water mixture are presented and compared to those of analogous Schiff bases of salicylaldehyde. The effect of copper(II) ions on the absorption and emission spectra of these compounds is also discussed.

2. Experimental

2.1. Chemicals

2-Amino-, 2-aminomethyl-, 2,3-diamino-, 2,6-diaminopyridine (purum), and *o*-vanillin (puriss) were purchased from Fluka

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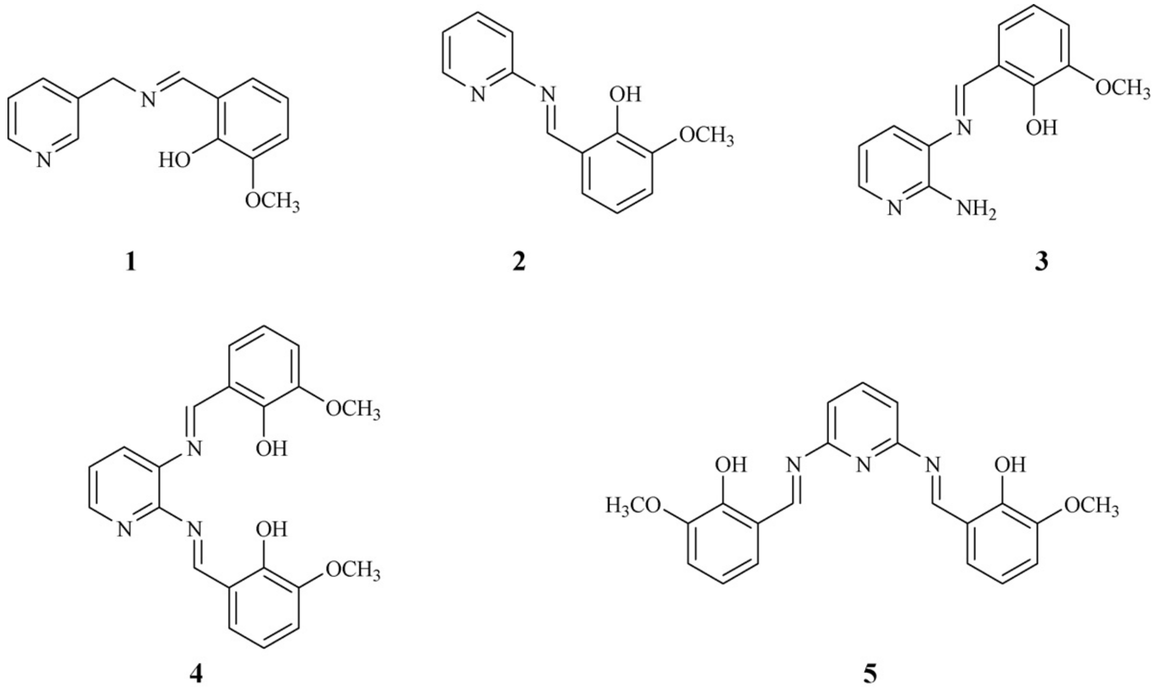
doi:10.1016/j.saa.2008.03.029



Scheme 1.

and used as supplied. $BeSO_4$ (The British Drug Houses Ltd.), $Al(NO_3)_3$ and $Cu(CH_3COO)_2$ (Merck) were p.a. purity grade. The solvents used for UV–vis and spectrofluorimetric measurements were p.a. (diethyl ether, dichloromethane, chloroform, dimethylformamide) or for luminescence spectroscopy (methanol and dioxane).

2.2. *Preparation of Schiff bases and Cu(II) complex*

For the preparation of 6-methoxy-2-(3-pyridylmethyliminomethyl)phenol (compound **1**) 3-aminomethylpyridine (0.50 g, 4.6 mmol), an equimolar quantity of *o*-vanillin, and a few drops of piperidine were heated to reflux in absolute ethanol (20 ml) for 2 h. After the solvent evaporation a dark yellow-brown viscous liquid was redistilled under a vacuum (reaction yield 89%).

Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.41; H 5.82; N 11.56%; found: C, 68.24; H 5.83; N 11.56%.

IR, ν/cm^{-1}: ν(C=N) 1625; ν(C=N)$_{\text{pyridine}}$ 1585; ν(C–N) 1460; ν(C–O) 1265, 1250; ν(substituted benzene) 730.

^{1}H NMR (DMSO, TMS, δ ppm): 13.41 [(OH), s, 1H]; 3.78 [(OCH_3), s, 3H]; 7.07 [(Ar–H), d, 1H, J = 7.94 Hz]; 6.85 [(Ar–H), t, 1H]; 7.08 [(Ar–H), d, 1H, J = 7.94 Hz]; 8.75 [(CH=N), s, 1H]; 4.86 [(N–CH_2), s, 2H]; 7.77 [(Py–H), d, 1H, J = 7,78 Hz]; 7.41 [(Py–H), dd, 1H]; 8.52 [(Py–H), d, 1H, J = 4.98 Hz]; 8.60 [(Py–H), s, 1H].

6-Methoxy-2-(2-pyridyliminomethyl)phenol (compound **2**), 6-methoxy-2-(2-amino-3-pyridyliminomethyl)phenol (compound **3**), *N*,*N*′-bis(2-hydroxy-3-methoxyphenylmethylidene)-2,3-pyridinediamine (compound **4**), and *N*,*N*′-bis(2-hydroxy-3-methoxyphenylmethylidene)-2,6-pyridinediamine (compound **5**) were prepared according to refs. [19,20,29]. The purity of the ligands was checked by ^{1}H NMR, IR (KBr), and elemental analysis.

The copper(II) complex with compound **1** was prepared by the following procedure: 2 mmol of 3-aminomethylpyridine was dissolved in 5 ml absolute ethanol, mixed with 2 mmol of *o*-vanillin, and refluxed for 1 h. Ethanol solution of 1 mmol $Cu(CH_3COO)_2$ was added and the resulting mixture was refluxed for another 2 h. The product was filtered, washed with ethanol, chloroform and ether, and dried under vacuum.

Yield: 56%. mp 221–222 °C.

Anal. Calcd. for $Cu(C_{14}H_{13}N_2O_2)_2$: C, 61.58; H, 4.80; N, 10.26%; found: C, 61.54; H, 4.82; N, 10.23%.

IR (KBr), ν/cm^{-1}: ν(C=N) 1608; ν(C=N)$_{\text{pyridine}}$ 1590; ν(C–N) 1460; ν(C–O) 1325, 1235; ν(substituted benzene) 740.

UV–vis ($CHCl_3$), $\lambda_{\max}$/nm ($\varepsilon \times 10^{-4}$/dm^3 mol^{-1} cm^{-1}): 284 (3.14), 385 (0.83).

2.3. *Apparatus and methods*

UV–vis spectra were recorded on a Varian Cary 3 spectrometer. Fluorescence spectra were measured on a PerkinElmer LS50 spectrofluorometer. Measurements of IR spectra were performed with a PerkinElmer 1725X FT-IR spectrometer. A JOEL JNM-FX 100 spectrometer was used for recording NMR spectra. For pH measurements a Radiometer pH/mV meter with a Radiometer combined glass-calomel electrode GK2401C was used. Magnetic susceptibility measurements of Cu(II) complex were carried out at 298 K on a Gouy balance. Data were corrected for diamagnetic contributions, which were estimated from Pascal constants.

For the determination of tautomeric constants, the solutions of compounds **1**–**5** in diethylether, dichloromethane, chloroform, dimethylformamide, dioxane, methanol, and in dioxane/water or methanol/water mixtures were prepared. The protonation constants were determined in the methanol/water 1/4 system. The buffer system was a universal buffer mixture. The pH meter was calibrated and the protonation constants were determined according to the procedures described in detail elsewhere [25]. The concentrations of Schiff bases in the measuring solutions were 1×10^{-4} mol dm^{-3} (compounds **1**–**3**) and 5×10^{-5} mol dm^{-3} (compounds **4** and **5**). For the spectrofluorimetric measurements the respective concentrations were 1×10^{-5} mol dm^{-3} and 5×10^{-6} mol dm^{-3}. Each solution was bubbled with nitrogen for 2 min before measurement. The effect of metal ions on absorption and on the emission spectra of ligands **1**–**5** was investigated in the buffered dioxane/water system (acetate buffer, pH 5.8). Spectroscopic measurements were performed at 25 °C.

3. Results and discussion

3.1. Absorption and emission properties of compounds 1–5

The UV–vis spectral data of compounds **1**–**5** in protic and aprotic solvents of different polarities are summarized in Table 1. As the slow hydrolysis of the investigated Schiff bases occurred in water containing solvents, the spectra were recorded immediately after the preparation of solutions. The bands in the spectral region from 314 nm to 373 nm are associated with the excitation of the whole molecule while those in the region from 260 nm to 297 nm are attributed to the excitation of the aldehyde part of the molecule [25]. The bands which appear at shorter wavelengths (<235 nm) are not considered because of interference of solvent absorption. In non-polar and/or aprotic solvents compounds **1**–**5** are present predominantly in the enolimine form, as confirmed by NMR spectra taken in dimethyl sulphoxide. The signals of the hydroxyl and methine protons for Schiff bases **1**–**5** appear at (12.2–13.4) ppm and (8.9–9.6) ppm, respectively. Their shapes (singlets) and positions (low fields) are characteristic of the enolimine form with a strong intramolecular OH···N=C bond. Similar behaviour has been observed in analogous Schiff bases derived from salicylaldehyde [23,24].

In the case of compound **1** the band at ≈420 nm in methanol and in the dioxane/water mixture is assigned to the ketoamine form [25]. To investigate the ketoamine–enolimine tautomeric equilibrium in more detail, a series of solvents with different polarities were chosen (Table 2). Assuming that the shift of the UV–vis bands of compound **1** to shorter wavelengths (e.g. for enolimine from 334 nm in diethylether to 328 nm in methanol/water 1/4 and for ketoamine from 422 nm in methanol to 416 nm in methanol/water 1/4) is due to the solvent effect and that the molar absorptivities are not significantly affected by variation of solvent, the molar fractions of the enolimine and ketoamine forms can be deduced and the

Table 2
UV–vis spectral data of compound **1** in different solvents

Solvent	λ/nm ($\varepsilon \times 10^{-4}$/dm^3 mol^{-1} cm^{-1})
Diethyl ether	334 (0.25) 264 (1.52) 223 (2.31)
Dichloromethane	334 (0.25) 273 (1.04)
Chloroform	336 (0.25) 265 (1.51)
Dimethylformamide	334 (0.25) 270 (1.33)
Dioxane	332 (0.26) 264 (1.53)
Dioxane/water 1/1	421 (0.10) 329 (0.20) 263 (1.33)
Methanol	422 (0.07) 329 (0.22) 263 (1.46)
Methanol/water 4/1	422 (0.10) 330 (0.20) 262 (1.35)
Methanol/water 1/1	421 (0.18) 331 (0.17) 262 (1.17)
Methanol/water 1/4	416 (0.27) 328 (0.14) 261 (0.93)

Table 1
UV–vis spectral data of compounds **1**–**5**

Solvent	λ/nm ($\varepsilon \times 10^{-4}$/dm^3 mol^{-1} cm^{-1})				
	1	**2**	**3**	**4**	**5**
Diethyl ether	334 (0.25) 264 (1.52) 223 (2.31)	361 (0.69) 315 (2.02) 278 (1.04) 226 (2.07)	371 (1.08) 276 (1.42) 228 (2.49)	[a]	[a]
Chloroform	336 (0.25) 265 (1.51)	370 (0.51) 319 (1.77) 279 (0.87)	365 (1.08) 279 (1.32)	339 (2.13) 287 (2.42)	363 (1.84) 290 (2.37)
DMF	334 (0.25) 270 (1.33)	370[b] 318 (1.80) 282 (0.98)	371 (1.10) 277 (1.42)	321 (1.10)	360 (1.86) 290 (2.36)
Dioxane	332 (0.26) 264 (1.53)	361 (0.63) 317 (1.92) 279 (0.97) 227 (2.04)	373 (0.96) 276 (1.31) 229 (2.33)	337 (2.31) 284 (2.50) 228 (3.85)	360 (1.89) 290 (2.51) 229 (3.79)
Dioxane/water 1/1	421 (0.10)[c] 329 (0.20) 293[b] 263 (1.33) 227 (1.33)	315 (1.24) 273 (0.88) 225 (2.05)	363 (1.09) 276 (1.33) 225 (2.38)	339 (2.16) 282 (2.21) 226 (3.76)	350 (1.56) 273 (1.82) 225 (3.67)
Methanol	422 (0.07)[c] 329 (0.22) 297 (0.26) 263 (1.46) 220 (2.23)	314 (1.46) 279[b] 225 (1.79)	362 (1.08) 276 (1.31) 221 (2.29) 207 (1.95)	343 (1.36) 280 (1.87) 220 (3.19)	355 (1.04) 270 (1.51) 221 (3.29)

[a] Insoluble.

[b] Shoulder.

[c] Bands corresponding to the absorption of the ketoamine form.

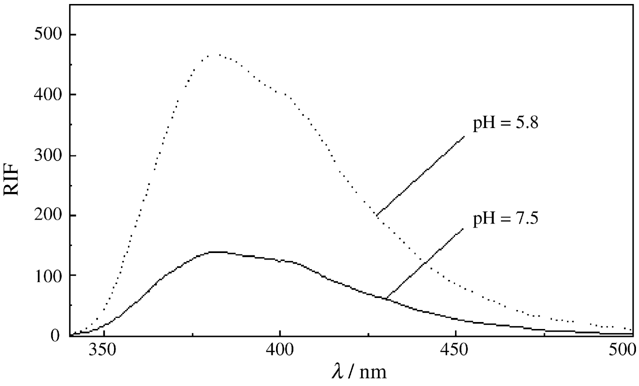


Fig. 1. Emission spectra of compound **5** at different pH values. $c(\mathbf{5}) = 5.0 \times 10^{-6}\ \mathrm{mol\,dm^{-3}}$; solvent: dioxane/water 1/1; $\lambda_{ex} = 333$ nm; slit with = 2.5 nm.

tautomeric constants, defined as $K_t = [\text{ketoamine}]/[\text{enolimine}]$ can be calculated (Tables 2 and 3). As can be seen, an increase in solvent permittivity gives rise to an increase in the value of K_t. The exception is dimethylformamide, an aprotic polar solvent.

The Schiff bases of salicylaldehyde with amino- and diaminopyridines are not fluorescent in non-polar solvents such as hexane, diethyl ether and chloroform. Weak fluorescence has been observed in polar solvents: methanol, DMF and dioxane/water mixtures [27]. Like salicylaldehyde derivatives the compounds **1**–**5** derived from *o*-vanillin are not fluorescent in most organic solvents (diethyl ether, chloroform, dimethylformamide, methanol). In the dioxane/water 1/1 mixture fluorescence was observed for compounds **2** and **5** immediately after the preparation of solutions and relative intensity of emission increased with time or as a result of lower pH. For compounds **1**, **3**, and **4** no fluorescence was observed for freshly prepared solutions. However, solution spectra of compounds **3** and **4** recorded about 24 h after preparation showed weak fluorescence. As an example, the emission spectra of compound **5** at different pH values are given in Fig. 1.

The increase in fluorescence intensity at lower pH can be explained by the protonation of pyridine nitrogen which influences the electronic density of the whole molecule. In water containing solvents slow hydrolysis of compounds **1**–**5** took place, so the increase in fluorescence with time was probably due to the formation of starting fluorescent aminopyridines.

3.2. Protonation constants of compounds 1–5

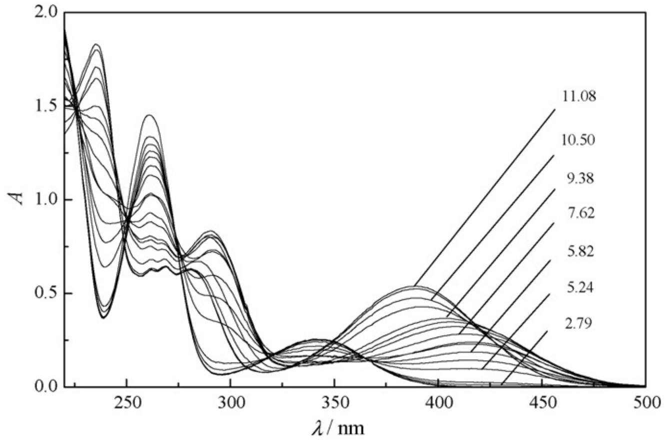


Fig. 2. UV–vis spectra of compound **1** in methanol/water 1/4 mixture at different pH values.

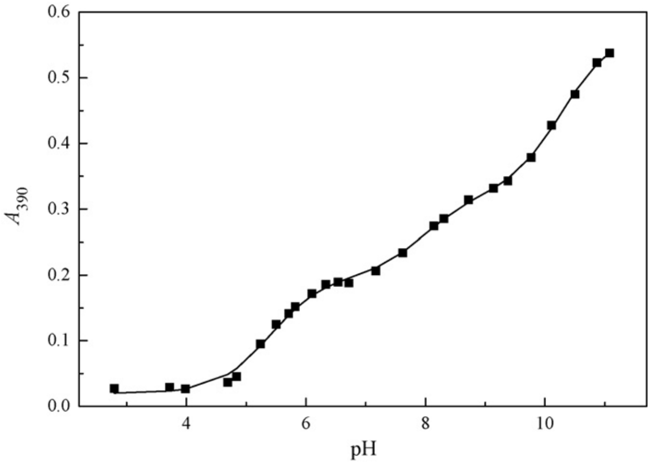


Fig. 3. Dependence of absorbance of compound **1** at 390 nm on pH (■, measured; —, calculated).

Difficulties in obtaining reliable protonation constants of Schiff bases **1**–**5** are due to their poor solubility (it is necessary to work at low concentrations) as well as slow hydrolysis in mixed organic solvent/water solutions. For that reason the batch spectrophotometric titration was used in this work as the most convenient experimental method. The protonation constants were determined in the methanol/water 1/4 mixture. To gain rough information about the rate of hydrolysis, it was estimated that, depending on the compound, this reaction in unbuffered $MeOH/H_2O$ mixture was almost completed in 10 min or more. Therefore, the spectra were recorded immediately after the preparation of solutions, or more precisely, after addition of the methanolic ligands solutions into the buffered aqueous media. As an example, the dependence of the absorption spectra of compound **1** on pH is shown in Fig. 2 and the corresponding plot of the measured and calculated absorbances is given in Fig. 3. The average values of protonation constants from three determinations are listed in Tables 4 and 5. It should be noted that,

Table 3
Tautomeric constants of compound **1** in different solvents and relative permittivities of pure solvents at 20 °C [30]

Solvent	K_t	ε_r
Diethyl ether	0	4.27
Dichloromethane	0	8.93[a]
Chloroform	0	4.81
Dimethylformamide	0	38.25
Dioxane	0	2.22
Dioxane/water 1/1	0.22	
Methanol	0.12	33.0
Methanol/water 4/1	0.21	
Methanol/water 1/1	0.45	
Methanol/water 1/4	0.78	

[a] The value at 25 °C.

Table 4
Protonation constants of compounds **1** and **1a** in methanol/water 1/4 mixture

Protonation constant	Compound **1**	Compound **1a**[a]
$\lg K_1$	10.19 ± 0.06	10.38
$\lg K_1$(A)	9.94	10.07
$\lg K_1$(B)	9.83	10.09
$\lg K_2$	8.01 ± 0.02	8.47
$\lg K_2$(A)	7.65	8.18
$\lg K_2$(B)	7.76	8.16
$\lg K_3$	5.40 ± 0.01	5.47

[a] Ref. [25].

Table 5
Protonation constants of compounds **2**–**5** and **2a**–**5a** in methanol/water 1/4 mixture

Compound	lg K_1	lg K_2	lg K_3
2	9.34 ± 0.03	7.48 ± 0.05	
2a[a]	8.82	6.92	
3	9.67 ± 0.004	5.73 ± 0.004	3.98 ± 0.03
3a[a]	10.01	5.92	4.12
4	9.45 ± 0.02	6.89 ± 0.04	4.31 ± 0.07
4a[a]	9.71	6.90	4.40
5	9.81 ± 0.03	6.52 ± 0.05	
5a[a]	8.75	7.09	

[a] Ref. [25].

because of hydrolysis reaction, some uncertainty is embedded in those values.

The protonation sequence of compound **1** is presented in Scheme 2. As the absorption bands of the pure enolimino and ketoamino forms can be deduced from the UV–vis spectra of compound **1** in solvents of different polarities (Table 2), the individual constants K_1(A) and K_1(B) as well as K_2(A) and K_2(B) can be obtained by means of the following equations [25]:

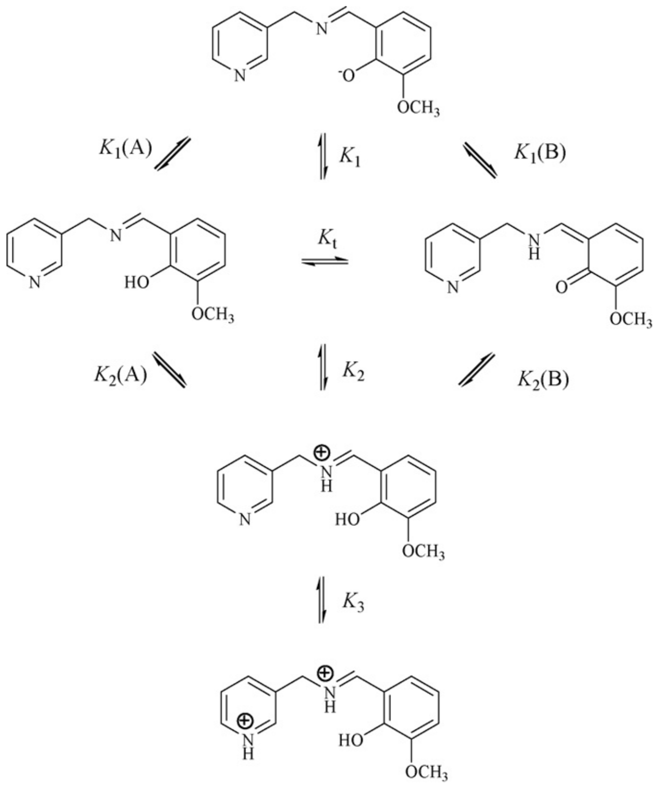
$$K_t = \frac{[\text{ketoamine}]}{[\text{enolimine}]}$$

$$K_t = \frac{K_1(B)}{K_1(A)} = \frac{K_2(A)}{K_2(B)}$$

$$K_1 = K_1(A) + K_1(B)$$

$$K_2 = K_2(A) + K_2(B)$$

The calculated values of the macroscopic and individual constants are presented in Table 4. For comparison, the corresponding values for the Schiff base of salicylaldehyde with 3-aminomethylpyridine (**1a**) are given as well [25].

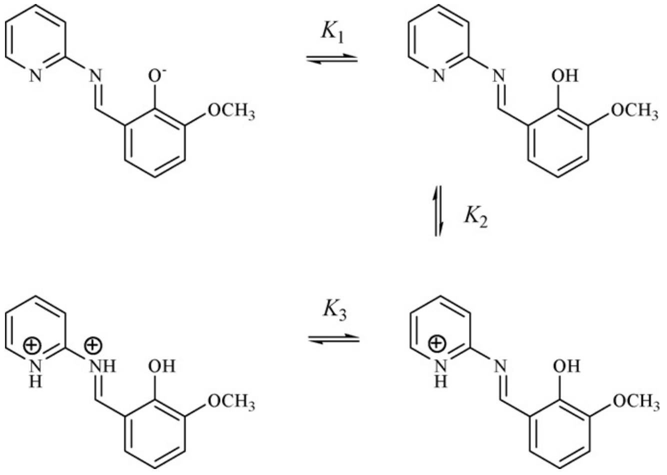


Scheme 2.

The introduction of the methoxy group in the aldehyde part of the Schiff base has a remarkable effect on the crystallization properties of compound **1** as compared to **1a** (at room temperature compound **1** is a viscous liquid while compound **1a** crystallizes easily) [24]. However, the influence on protonation constants is less prominent. The small difference between the lg K_1 values of compounds **1** and **1a** (10.19 and 10.38) can be explained by an inductive effect of the –OCH_3 group. The effect is more pronounced in the case of pure starting materials, e.g. *o*-vanillin and salicylaldehyde (the corresponding lg K values, determined by the same procedure, are 8.02 and 8.59). The equilibrium constant assigned to the protonation of azomethine nitrogen is considerably lower for compound **1** (lg $K_2 = 8.01$) than for compound **1a** (lg $K_2 = 8.47$). Such an effect of the –OCH_3 group is unexpected as the group is situated in the *meta* position to the imine group and no resonance effect is possible. The protonation of pyridine nitrogen is not influenced by substitution because resonance through the whole molecule is disrupted by the methylene group on the pyridine ring at position 3. The corresponding lg K values for compounds **1** and **1a** are 5.40 and 5.47, and are comparable to the unsubstituted pyridine (lg $K = 5.34$).

The protonation pathway for compounds **2**–**5** is shown in Scheme 3 and the calculated constants are listed in Table 5. For comparison, the protonation constants of salicylaldehyde analogues (**2a**–**5a**) are also given [25]. The protonation sequence for compounds **2**–**5** is changed with respect to compound **1** due to the imino nitrogen directly bounded to the pyridine ring at position 2. The resonance involving imino and electron-withdrawing pyridine nitrogen atoms causes a drastic decrease in the basicity of the imino group and an increase in the basic character of the pyridine nitrogen [25]. For that reason the protonation constant K_3 for compounds **2** and **5** (derived from 2-amino and 2,6-diaminopyridines) cannot be determined by the method applied.

The protonation constants of OH groups in compounds **2a** and **5a** (Table 5) are similar to those of salicylaldehyde (lg $K = 8.59$) [25]. That was explained by resonance stabilization of the planar conjugate bases of pyridyliminomethylphenols which is similar to that of the salicylaldehyde anion. As compound **5** in solid state is also planar molecule [29] the K_1 value was predicted to be lower than the one observed. The same could be concluded for compound **2**. The unexpectedly high K_1 values of the two Schiff bases could be tentatively explained by the assumption that compounds **2** and **5** are present in solution in non-planar conformations. As a consequence, delocalization of the free electron pair through the entire molecule is diminished or is no longer possible.



Scheme 3.

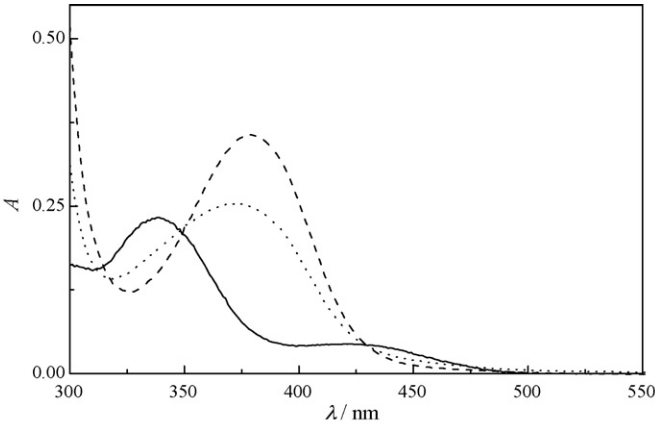


Fig. 4. UV–vis spectra of free compound **1** and in presence of Cu(II) in dioxane/water 1/1 mixture. $c(\mathbf{1}) = 1.0 \times 10^{-4}$ mol dm^{-3}; — **1**; . . . c(Cu(II))/c(**1**) = 1; - - - c(Cu(II))/c(**1**) = 10.

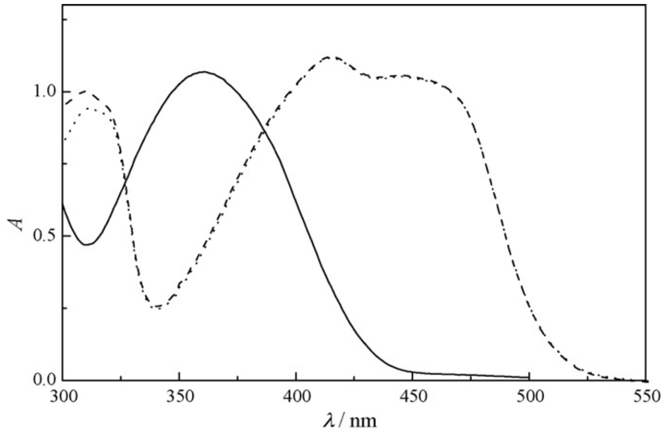


Fig. 5. UV–vis spectra of free compound **3** and in presence of Cu(II) in dioxane/water 1/1 mixture. $c(\mathbf{3}) = 1.0 \times 10^{-4}$ mol dm^{-3}; — **3**; . . . c(Cu(II))/c(**3**) = 1; - - - c(Cu(II))/c(**3**) = 10.

The lower K_1 values of compounds **3** and **4** than of the analogues **3a** and **4a** (Table 5) can be attributed to the inductive effect of the $-OCH_3$ group. Although two hydroxyl groups in compound **4** have a different basicity, the difference is too small to allow determination of the protonation constant of each group. The K_1 value listed in Table 5 is an average value of constants of both groups. The protonation of pyridine nitrogen is not influenced by the presence of the $-OCH_3$ group. For example, the lg K_2 of compound **4** is 6.89 which agrees very well with the salicylaldehyde analogue (lg $K = 6.90$). The protonation constant K_3, determined only for compounds **3** and **4**, is related to the imino group at position 3 of the pyridine ring. As with compound **1** the protonation of azomethine nitrogen is not influenced by the presence of the $-OCH_3$ group so the corresponding equilibrium constants are similar to those of analogous Schiff bases derived from salicylaldehyde (Table 5).

3.3. *Influence of metal ions on spectroscopic characteristics of compounds **1**–**5***

Copper(II) is known to form stable complexes with the Schiff bases derived from salicylaldehyde and aminopyridines [26]. In this work its influence on the absorption spectra of compounds **1**–**5** was investigated in the buffered dioxane/water 1/1 mixture (acetate buffer, pH 5.8). In order to get a qualitative insight into the complexation affinities of the ligands towards copper(II) ion, UV–vis spectra were recorded at two different Cu(II):ligand molar ratios, i.e. 1:1 and 10:1. In the case of compounds **2** and **5**, the equimolar addition of Cu(II) did not cause any significant change in the free ligands spectra. Upon addition of a tenfold excess of metal ion, only a low intensity shoulder appeared in the visible region of the spectrum (≈400 nm). On the contrary, after addition of an equimolar amount of Cu(II) in the solution of compound **1** a new band emerged with a maximum at ≈379 nm, and an upward trend in absorbance was observed upon increasing the molar ratio to 10:1 (Fig. 4). That can be attributed to the moderate complexation affinity of compound **1** towards copper(II) under the conditions used. According to the structural characteristics, number, and arrangement of the donor groups, the most efficient coordination was expected for compounds **3** and **4**. Indeed, the most prominent spectral changes were observed upon addition of Cu(II) into solutions of these Schiff bases. The bands which can be attributed to the metal–ligand complex formed (≈425 nm and ≈453 nm for compound **3**; ≈433 nm for compound **4**) were quite intensive, and, in addition, no significant difference between the spectra of solutions with 1:1 and 10:1 (Cu(II):ligand) ratios was observed (Fig. 5). These findings strongly suggest that among the compounds investigated ligands **3** and **4** form the most stable complexes with copper(II). Therefore, complexation of Cu(II) with compound **4** having four binding sites was studied in more detail spectrophotometrically (Fig. 6a and b). As can be seen in Fig. 6b, an almost linear rela-

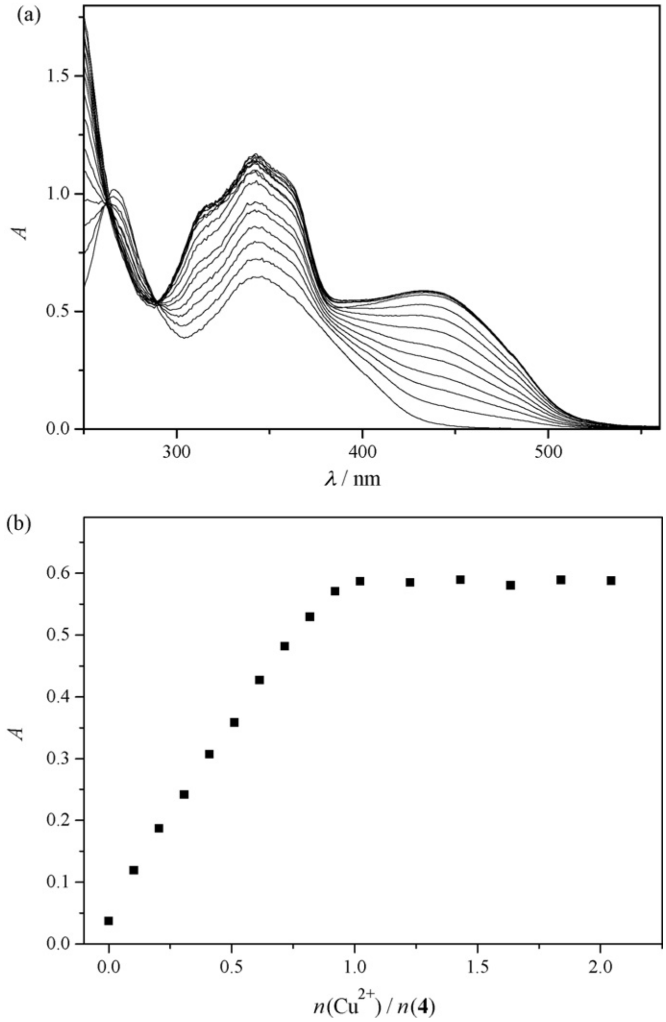


Fig. 6. (a) Spectrophotometric batch titration of compound **4** ($c = 1 \times 10^{-4}$ mol dm^{-3}) with $Cu(CH_3COO)_2$. (b) Dependence of absorbance at 433 nm on n(Cu^{2+})/n(**4**) ratio.

tionship of absorbance vs. $n(\mathrm{Cu}^{2+})$ was observed up to the ratio $n(\mathrm{Cu}^{2+})/n(\mathbf{4}) \approx 1$ (n denotes total amount), followed by a break in the titration curve. That indicates a rather strong complexation under conditions used, and formation of a complex of 1:1 stoichiometry. In order to check whether the hydrolysis of compound **4** in the complex also occurs (as it does in the free ligand solution), spectra of an equimolar reaction mixture were recorded during 15 h. No significant changes were observed indicating that hydrolysis of the carbon-nitrogen double bond was suppressed by binding to copper through the azomethine nitrogen atom.

The reaction of analogous Schiff bases derived from salicylaldehyde with Al(III) and Be(II) ions in the dioxane/water system induced small shifts in the excitation and emission maxima, and an increase in fluorescence intensities [27]. However, in the case of fluorescent compounds **2** and **5**, the addition of those ions in equimolar amount and in excess had no influence on the emission spectra under the conditions used (dioxane/water 1/1; acetate buffer, pH 5.8). The presence of Cu(II) ions slightly reduced the fluorescence intensity, in agreement with the data obtained by UV–vis measurements.

*3.4. Isolation of Cu(**1**)$_2$ complex*

The Cu(II) complexes with compounds **3** and **4** have been prepared and described in the literature [19]. These compounds form Cu(II) complexes with 1:1 stoichiometry, as they act as tridentate and tetradentate ligands. The mono Schiff base **3** derived from 2,3-diaminopyridine coordinates copper(II) through azomethine nitrogen, phenolic oxygen and free amino group, while the fourth coordination site is occupied by an anion. In the case of bis Schiff base **4** there are four donor sites (two azomethine nitrogens and two phenolic oxygens) involved in Cu(II) coordination.

In the present work a complex of copper(II) with compound **1** was isolated from ethanol solution. The infrared spectra of the free compound **1** showed strong bands at $1625\,\mathrm{cm}^{-1}$ and $1265\,\mathrm{cm}^{-1}$, assigned to the azomethine group and phenolic C–O stretching. Upon complexation a negative shift to $1608\,\mathrm{cm}^{-1}$ and a positive one to $1325\,\mathrm{cm}^{-1}$ were observed indicating coordination of compound **1** through azomethine nitrogen and phenolic oxygen [19,20,26,31].

At 298 K the magnetic moment of the complex was $1.58\mu_\mathrm{B}$ which was close to the spin-only value expected for Cu(II) compounds with $S = 1/2$.

Contrary to **3** and **4**, Schiff base **1** acted as bidentate ligand forming the copper(II) complex of 1:2 stoichiometry as confirmed by CHN analysis.

4. Conclusion

A series of Schiff bases were prepared: 6-methoxy-2-(3-pyridylmethyliminomethyl)phenol (compound **1**), 6-methoxy-2-(2-pyridylimino-methyl)phenol (compound **2**), 6-methoxy-2-(2-amino-3-pyridyliminomethyl)phenol (compound **3**), *N*,*N*′-bis (2-hydroxy-3-methoxyphenylmethylidene)-2,3-pyridine-diamine (compound **4**) and *N*,*N*′-bis(2-hydroxy-3-methoxyphenylmethylidene)-2,6-pyridinediamine (compound **5**). In non-polar and aprotic solvents all Schiff bases appeared predominantly in the enolimine form. A shift to the ketoamine tautomer was observed only for compound **1** in polar protic solvents and the corresponding tautomeric constants were determined. Protonation constants for all compounds were determined spectrophotometrically in the methanol/water 1/4 system. Fluorescence was observed in the case of compounds **2** and **5** in the dioxane/water 1/1 mixture. The effect of copper(II) ions on the absorption spectra of the Schiff bases in the dioxane/water 1/1 system was most pronounced for compounds **3** and **4** singling them out as potential ionophores in optical sensors. The Cu(**1**)$_2$ complex was isolated and coordination of the Schiff base to the metal ion through azomethine nitrogen and phenolic oxygen was proposed according to the data obtained by IR spectroscopy.

Acknowledgements

This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (projects 119-1191342-2960 and 119-1191342-1083). We thank Prof. Marina Cindrić for performing magnetic measurements.

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