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Spectroscopy and photophysics of alloxazines studied in their ground and first excited singlet states

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Abstract

The acid–base properties of alloxazine (All) and its methyl derivatives have been studied in their ground and first excited singlet states. The concept of an effective electronic valence potential was applied to predict the changes in basicity and acidity of heteroatoms upon excitation and substitution. Changes in the acid–base properties of N(1) and N(10) nitrogen atoms are particularly important from the point of view of the excited state proton transfer in alloxazines from N(1) to N(10) to form isoalloxazinic structures. A good linear correlation was obtained between the calculated electronic potentials of N(1) and N(3) nitrogen atoms and the experimental pK_a values for ground and excited state deprotonation.

Keywords: Photophysics; Alloxazines; Ground states; Effective valence electron potentials

1. Introduction

Alloxazines (Alls), products of decomposition of flavins, have been the subject of study because of their possible biological importance and their interesting photophysical and photochemical properties [1–16]. Experimental studies [2–4,12–14] and theoretical calculations [8,9,17] have revealed that a substitution in the alloxazine molecule leads to a change in the electron density distribution that strongly influences spectral, photophysical and photochemical properties and alters the acid–base parameters. Isoalloxazines and alloxazines are closely related compounds, representing two classes of nitrogen heterocycles with active centers at N(10), N(5), N(3) and N(1), and at both carbonyl oxygens at C(2) and C(4). The spectroscopic and photophysical properties of isoalloxazines are relatively well known. The main features of spectroscopy and photophysics of alloxazines, quite different from those of isoalloxazines, are also understood fairly well, but the results are very scattered and mostly limited to lumichrome and its derivatives, and some details need further investigation. Recently, we have undertaken studies in order to describe the spectroscopy and photophysics of alloxazines in a more systematic way [13–16,18].

Alloxazines unsubstituted at the N(1) position can undergo excited state proton transfer from N(1)–H to N(10) to the corresponding isoalloxazinic form. The excited state reaction occurs in the presence of compounds having proton donor and acceptor groups, able to form *correct* hydrogen bonds with alloxazine molecules [1–5]. In this context, the acid–base properties of both the N(1) and N(10) nitrogen atoms in the alloxazine molecule are especially important. The experimentally observed changes in the pK_a of deprotonation were rather poorly reproduced by semi-empirically calculated net charges [19]. A better correlation was found between the deprotonation enthalpy and the ground state pK_a values [19].

It was previously shown [8,9,17,19] that there is no simple relation between the pK_a values of aza-aromatic compounds and the calculated electronic population of the protonated or deprotonated centres. In the present investigation, we apply the concept of an effective valence electronic potential [20–23]. The effective potential corresponds to local ionization energy and depends also on contributions from atoms not involved in the proton transfer directly. Effective valence electronic potentials have been applied with success in the prediction of acid–base properties of

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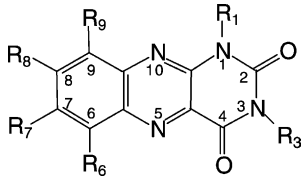

Fig. 1. Structure of the alloxazines studied and atom numbering.

aza-aromatics in their ground and excited states, see, e.g. [20].

The present study was undertaken to provide a more systematic insight into the effect of substituent and its position on photophysics of alloxazines. In this respect, we studied alloxazine and its derivatives, from mono- to trimethyl substituted, having the methyl group at different positions in the molecule. Other substituents were represented by two cyanoalloxazines (7-cyanoalloxazine, 7CNAll, and 8-cyanoalloxazine, 8CNAll) and 5-deazalumichrome (5-deaza-7,8-dimethylalloxazine, 5DLc). In 5DLc, a CH group replaces the N(5) nitrogen atom. Apart from experimental results, we report theoretical predictions of the changes in the acid–base properties of heteroatoms caused by substitution, especially at the two nitrogen atoms N(1) and N(10), both in the ground and lowest singlet excited state. The structures of the alloxazines studied in this paper and the numbering of atoms are shown in Fig. 1.

In this study, we have chosen a set of alloxazines, which included on purpose 7CNAll, 8CNAll and 5DLc, as we expected that their quite different photophysical properties would also be reflected in the calculations, which indeed turned out to be the case. The addition of 7CNAll, 8CNAll and 5DLc to the set containing alloxazine and its monomethyl derivatives, extends the range of observed changes in both photophysical and acid–base properties of studied compounds, which is quite limited for the monomethyl derivatives per se. To avoid making any arbitrary selections, we also included the results for dimethylalloxazines, as these compounds were available, and their acid–base properties known. In fact, the results that exclude dimethylalloxazines produce better correlations, although at a cost of making what we perceive an arbitrary selection of results that we were not willing to pay. The dimethyl derivatives, in general, turned out to be more complex systems resisting simple interpretations—here we have to consider a superposition of effects of the two methyl groups, see for instance the case of 7,8- and 6,9-dimethylalloxazines. The introduction of dimethylalloxazine derivatives did not significantly affect the linear regression parameters, the only significant consequence were the higher standard deviation values.

2. Materials and methods

Alloxazine, lumichrome and the solvent 1,2-dichloroethane, all from Aldrich, were used as received. The alloxazine derivatives were available from previous work.

Fluorescence decays were measured in 1,2-dichloroethane using excitation at 355 nm and time-correlated single-photon counting detection on an IBH model 5000U fluorescence lifetime spectrometer. Time-resolved fluorescence measurements of lumichrome in 1,2-dichloroethane were also conducted with a model C-700 fluorometer from PTI. The system utilizes a nanosecond flash lamp for excitation and a stroboscopic detection system [24]. Steady-state fluorescence spectra were obtained with a Jobin Yvon-Spex Fluorolog 3-11 spectrofluorometer, and UV-Vis absorption spectra on a Varian Cary 5E spectrophotometer.

The electronic ground state geometry optimizations for neutral and anionic forms of the alloxazines studied were performed by the semi-empirical AM1 method. All structures optimized were positively identified as global energy minima. In the next step, the net charges (q) and effective valence electron potentials (W_{2p} for oxygen and nitrogen atoms) for neutral and anionic forms were computed using the INDO/S-CI-1 method for the ground state S_0 and for the excited states. Net charges are given in units of electrons; '+' means a deficiency of electrons and '−' means an excess of electrons as compared with the isolated atomic state. Thereafter, to make abbreviations simpler we decided to use only the W symbol throughout the manuscript. In the CI procedure, 200 single excited configurations were taken into account. The precise location of the low-lying n, π^* state is unknown, but the semi-empirical calculations predict its existence for both neutral and anionic forms of alloxazines; the respective transition has very low oscillator strength and is not observed experimentally. The UV-Vis absorption and emission bands of alloxazines are all assignable to the allowed electric-dipole π, π^* transitions. The first allowed transition observed in both neutral and anionic forms of alloxazines has π, π^* character. In further discussions, only the $S_1(\pi, \pi^*)$ excited state will be considered. All computations were repeated for the electronic excited state approximated by a single pure HOMO → LUMO excitation.

Effective valence electron potentials were computed using a program provided by Prof. J. Waluk of the Polish Academy of Sciences, Warsaw, as a supplement to the INDO/S-CI-1 method.

The effective valence electron potentials, W, are a function of atomic charges and interatomic distances. The more positive the value of W, the higher the basic and nucleophilic character of a given region of the molecule, and vice versa, the more negative the value of W, the more acidic and electrophilic the character of a given part of the molecule [20–23]. The concept of the effective valence electron potentials is described in detail in [22]. The effective potential W_μ^A of an atomic orbital μ on atom A is calculated by the formula

$$W_\mu^A = W_\mu^A(q_A) + \sum_{B \neq A} W_\mu^A(q_B)$$

where q_A is the gross atomic population on atom A, q_B the populations on all other centres. The effective potential

is thus computed as a sum of one-center $W_\mu^A(q_A)$ and two-center $W_\mu^A(q_B)$ contributions. The functional forms of $W_\mu^A(q_A)$ and $W_\mu^A(q_B)$ are as follows:

$$W_\mu^A(q_A) = -(a - bq_A)^{3/2}$$

$$W_\mu^A(q_B) = \gamma_{AB}(q_B - Z_B^{core})$$

Here, γ_{AB} represents the Coulomb repulsion between a valence electron on atom A and a valence electron on atom B; Z_B^{core} is equal to the number of valence electrons in the neutral atom B. The details of the respective calculations are given in [22].

All the reported results, both experimental and calculated, are listed to the respective uncertainty values and the significant digit notation is used.

3. Results and discussion

3.1. Spectroscopy and photophysics in 1,2-dichloroethane

A summary of photophysical parameters of alloxazine and its methyl derivatives in their lowest excited singlet states is given in Table 1. The results presented allow us to selectively observe the effect of the methyl substituent on the alloxazine photophysical properties. In every alloxazinic compound examined here, the non-radiative relaxation dominates the excited state decay. Especially, interesting is that all characteristics of the excited singlet state—ϕ_F, τ_F, k_r and $\sum k_{nr}$—are essentially identical for the 7,8-dimethylalloxazine (7,8MAll) (lumichrome) and its 1- and 3-methyl and 1,3-dimethyl derivatives. On the other hand, there is an evident effect of the methyl group position on the spectral and photophysical properties of alloxazines. The introduction of the methyl group into the alloxazine molecule at positions 6, 7, and 9 increases fluorescence quantum yields and fluorescence lifetimes. The introduction of the methyl group at the position 8 causes little if any effect on the fluorescence quantum yield, and a small increase of the fluorescence lifetime as compared to alloxazine. Among monomethyl-substituted alloxazines, the shortest lifetimes are observed for 7- and 8-methylalloxazine, and the longest—for 6- and 9-methylalloxazine. The position of methyl substituents affects very markedly the directions of the transition moments of alloxazines [9]. Quantum chemical calculations predict that methyl substitution in positions C(7) and C(8) leads to orientation of the first π, π^* transition moment more or less parallel to the long axis of the molecule. In contrast, methyl substitution at C(6) and C(9) leads to a polarization direction closer to the short molecular axis. Significant substituent effects on photophysics of dimethyl substituted alloxazines are observed as predicted, with the most evident effect in the case of 6,9-dimethylalloxazine (6,9MAll) compared to 7,8MAll.

Introduction of the cyano group at C(7) or C(8) changes the spectral and photophysical properties in comparison with the corresponding methyl derivatives. For example, both cyano derivatives display shorter fluorescence lifetimes than their methyl analogues (see Table 1). The idea of using cyano-substituted alloxazines comes from the fact that in these the excited state proton transfer also occurs in presence of methanol, which was never observed for methyl-substituted alloxazine derivatives [7].

As reported by Koziołowa et al. [11], 5DLc undergoes excited state proton transfer with high efficiency. For example, in comparison to 3,7,8-trimethylalloxazine (3,7,8MAll), 5DLc in 1,2-dichloroethane in presence of acetic acid undergoes excited state proton transfer with higher efficiency.

Table 1
Spectroscopic and photophysical data for the singlet states of alloxazines in 1,2-dichloroethane[a]

No.	Compound	λ_2 (nm)	λ_1 (nm)	λ_F (nm)	ϕ_F	τ_F (ns)	k_r ($\times 10^8$ s^{-1})	$\sum k_{nr}$ ($\times 10^8$ s^{-1})
1	Alloxazine (All)	322	374	434	0.023	0.19	1.2	51
2	6-Methylalloxazine (6MAll)	336	375	455	0.029	0.87	0.33	11
3	7-Methylalloxazine (7MAll)	323	384	442	0.034	0.49	0.69	20
4	8-Methylalloxazine (8MAll)	348	368s	425	0.024	0.32	0.75	30
5	9-Methylalloxazine (9MAll)	336	380	450	0.028	0.74	0.38	13
6	6,7-Dimethylalloxazine (6,7MAll)	335	380	468	0.038	2.2	0.17	4.4
7	6,8-Dimethylalloxazine (6,8MAll)	350		463	0.017	1.19	0.14	8.3
8	6,9-Dimethylalloxazine (6,9MAll)	344	380	491	0.094	9.5	0.10	0.95
9	7,9-Dimethylalloxazine (7,9MAll)	333	379	465	0.041	1.6	0.26	6.1
10	8,9-Dimethylalloxazine (8,9MAll)	351	375	462	0.017	1.0	0.17	9.6
11	7,8-Dimethylalloxazine (7,8MAll)	334	382	440	0.026	0.61	0.43	16
12	1,7,8-Trimethylalloxazine (1,7,8MAll)	334	382	440	0.026	0.62	0.41	16
13	3,7,8-Trimethylalloxazine (3,7,8MAll)	334	381	439	0.024	0.65	0.37	15
14	1,3,7,8-Tetramethylalloxazine (1,3,7,8MAll)	335	383	440	0.026	0.62	0.41	16
15	5-Deaza-7,8-dimethylalloxazine (5DLc)	320	352, 370s	413		3.3		
16	7-Cyanoalloxazine (7CNAll)	322	372, 386s	420	0.022	0.18	1.2	5.4
17	8-Cyanoalloxazine (8CNAll)	320	380, 398s	430	0.016	0.16	1.0	6.1

[a] λ_1, λ_2 are the positions of the two lowest-energy bands in the absorption spectra, λ_F the fluorescence emission maximum, ϕ_F the fluorescence quantum yield, τ_F the fluorescence lifetime, k_r the radiative rate constant and $\sum k_{nr}$ the sum of non-radiative rate constants.

Table 2
Acid–base properties of nitrogen atoms of alloxazines molecules in the ground state, S_0

No.	Compound	$W^{N(10)}(S_0)$ (eV)	$q^{N(10)}(S_0)$	$W^{N(1)}(S_0)$ (eV)	$q^{N(1)}(S_0)$	$W^{N(3)}(S_0)$ (eV)	$q^{N(3)}(S_0)$	$W^{N(5)}(S_0)$ (eV)	$q^{N(5)}(S_0)$	$pK_a(S_0)$[a]
1	All	−12.813	−0.352	−14.729	−0.187	−14.699	−0.175	−12.937	−0.260	8.11 ± 0.03
2	6MAll	−12.755	−0.354	−14.706	−0.187	−14.679	−0.175	−12.937	−0.261	8.18 ± 0.03
3	7MAll	−12.760	−0.350	−14.668	−0.187	−14.650	−0.175	−12.846	−0.264	8.20 ± 0.03
4	8MAll	−12.725	−0.356	−14.671	−0.187	−14.639	−0.175	−12.866	−0.260	8.23 ± 0.05
5	9MAll	−12.811	−0.352	−14.707	−0.187	−14.685	−0.175	−12.894	−0.261	8.27 ± 0.04
6	6,7MAll	−12.712	−0.352	−14.651	−0.187	−14.632	−0.175	−12.850	−0.264	8.48 ± 0.02
7	6,8MAll	−12.668	−0.358	−14.655	−0.186	−14.626	−0.176	−12.873	−0.259	8.32 ± 0.05
8	6,9MAll	−12.755	−0.354	−14.686	−0.187	−14.666	−0.175	−12.896	−0.261	8.48 ± 0.03
9	7,8MAll	−12.674	−0.354	−14.625	−0.187	−14.606	−0.175	−12.791	−0.263	8.28 ± 0.03
10	7,9MAll	−12.760	−0.350	−14.649	−0.188	−14.638	−0.175	−12.804	−0.265	8.39 ± 0.05
11	8,9MAll	−12.732	−0.355	−14.652	−0.187	−14.627	−0.176	−12.827	−0.261	8.44 ± 0.05
12	3,7,8MAll	−12.617	−0.355	−14.558	−0.189	−14.407	−0.129	−12.721	−0.264	8.58 ± 0.05
13	5DLc	−12.281	−0.400	−14.395	−0.187	−14.404	−0.175	–	–	8.80 ± 0.03
14	7CNAll	−13.215	−0.352	−15.053	−0.185	−14.976	−0.174	−13.376	−0.256	7.39 ± 0.03
15	8CNAll	−13.223	−0.349	−15.033	−0.185	−14.974	−0.174	−13.352	−0.259	7.61 ± 0.03

$W^N(S_0)$: effective valence electron potentials for nitrogen atoms in the ground state; $q^N(S_0)$: net charges for nitrogen atoms in the ground state.
[a] Apparent pK_a of deprotonation in the ground state [3], the accuracy of the pK_a experimental determination was ±0.2.

It was proposed that the replacement of the N(5) by carbon in the benzene ring of alloxazine restricts the electron density redistribution involved in the phototautomerism to the N(1)–C(10)–N(10) region, and eliminates the contribution of the remaining conjugated π-system [11].

3.2. Acid–base properties in the ground state

The analysis of net charge values reveals that in the ground state these values decrease in the order C(2)=O, C(4)=O, N(10), N(5), N(3), N(1); with the values of electronic valence effective potentials decreasing in the similar order: C(4)=O, C(2)=O, N(10), N(5), N(3), N(1). Modification of the alloxazine molecule by substitution changes the charge density distribution on the atoms studied (see Table 2 and Fig. 2A). In particular, the introduction of an electron-withdrawing cyano group at the positions 7 and 8 significantly decreases the values of the effective valence electron potentials at N(1) and N(10) nitrogen atoms in the ground state. As a result, 7CNAll and 8CNAll are predicted to have the most acidic N(1)–H proton among the compounds studied. In contrast, the introduction of weakly electron-donating methyl groups alters only slightly the acid–base properties. Replacement of the N(5) nitrogen atom by a carbon atom in 5-deazalumichrome (5DLc) results in an increase of W values for N(10) and N(1) nitrogen atoms. These changes result in prediction of the highest basicity of the N(10) and the lowest acidity of the N(1) nitrogen atoms of 5DLc among the derivatives studied in the ground state.

The experimental apparent pK_a values of deprotonation in the ground state were determined by Koziołowa [3] for all the alloxazines studied in this work. The pK_a values of deprotonation in the ground state were estimated spectrophotometrically in buffers of constant ionic strength of 0.05 within the 4.5–12.7 pH range and in NaOH solutions of suitable concentration for higher pH values. There are two possible sites of deprotonation in an alloxazine molecule, namely N(1)–H and N(3)–H positions. The deprotonation of the alloxazine molecule proceeds in two steps: creation

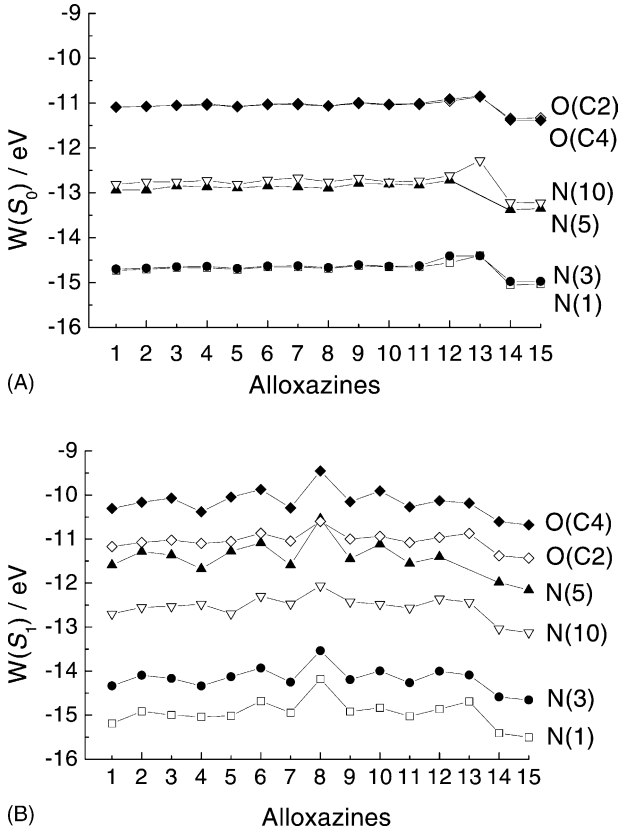

Fig. 2. The effective valence electron potentials of heteroatoms of alloxazine derivatives in the ground state (A) and in the first excited state (B). See Table 2 for compound numbers.

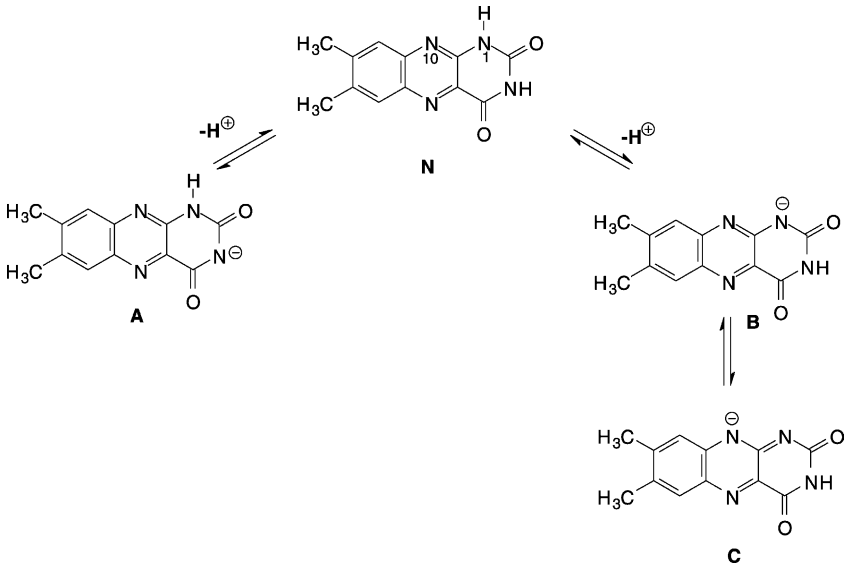

Fig. 3. Scheme of deprotonation of lumichrome, with formation of monoanions. N—neutral form, A—anion N(3) with alloxazinic structure, B—anion N(1) with alloxazinic structure, C—anion with isoalloxazinic structure.

of a monoanion with a pK_a of about 8, and creation of a dianion with a pK_a >12. It was proved that for lumichrome the dissociation of one proton leads to two forms, characterized by different absorption, emission and excitation spectra and different fluorescence lifetimes [10]. The dissociation of N(1)–H leads to a redistribution of electron density in the anion formed and yields the monoanion of the isoalloxazinic structure. In contrast, the dissociation of the N(3)–H group leads to a another form, the $N(3)^-$ monoanion of the alloxazinic structure (Fig. 3). It was shown that in the ground state, the microscopic pK_a values for N(1) and N(3) are almost identical (microscopic pK_a values for the two monoanions of 7,8MAll differ only by 0.12) [3,12].

There is an increase of pK_a upon methyl substitution as compared to unsubstituted alloxazine, the dimethyl substituted alloxazines exhibiting higher pK_a values of deprotonation than the monomethyl or unsubstituted alloxazines. The substitution of a cyano group at the benzene ring of an alloxazine molecule at the positions 7 and 8 leads to a decrease of pK_a values by about one unit, resulting in stronger acidic properties of the cyano derivatives. In contrast, 5-deazalumichrome has the highest pK_a value among the derivatives studied.

The net charges and effective valence electron potentials for the N(1) and N(3) nitrogen atoms are very similar for every compound studied. The net charges of N(1) and N(3) nitrogen atoms for some derivatives in the ground state do not change with substitution, while the effective valence electron potentials demonstrate differences, which nicely reproduce the corresponding changes of the p$K_a(S_0)$ values. A comparison of the experimental pK_a values of deprotonation, net charges and effective valence electron potentials illustrates the benefits of using the latter parameter to determine the acid–base properties of the alloxazines studied (see Table 2, Figs. 4 and 5). The effective valence electron potentials are well correlated with pK_a values for all compounds studied in their ground states, and this correlation (the respective correlation coefficients $r = 0.962$ for N(1) and $r = 0.953$ for N(3)) is much better than that using net charges ($r = 0.764$ for N(1)).

3.3. Acid–base properties in the first singlet excited state

Excitation to the first singlet state $S_1(\pi, \pi^*)$ produces changes in the electron distribution in a lumichrome molecule, and a predicted increase in basicity at the N(5), N(10), N(3) and C(4)=O positions, and a decrease in basicity at the N(1) and C(2)=O positions. As a result of these changes, the net charge values in the excited state decrease in the order: C(2)=O, C(4)=O, N(5), N(10), N(3), N(1). A similar order is predicted by the effective valence electronic potentials (with the highest W value for C(4)=O). An analogous relation holds also for differently substituted alloxazines (Fig. 2, Table 3). In the excited state, the N(5) position becomes the most basic among the nitrogen atoms and may play an important role in the excited state protonation and creation of hydrogen bonds in the excited state. Mimicking the ground state behavior, the introduction of a cyano group into the benzene ring of an alloxazine molecule at the positions 7 and 8 leads to a decrease in basicity of the heteroatoms in the first excited singlet state (see Fig. 2B). In contrast, 6,9MAll has the highest heteroatom basicity in the excited state among the derivatives studied.

The differences between the acidities of N(1)–H and N(3)–H hydrogen atoms increase considerably in the first singlet excited state. The pK_a values for deprotonation of alloxazines at N(1) in the first singlet excited state are considerably lower (by 2–6 units) than those in the ground

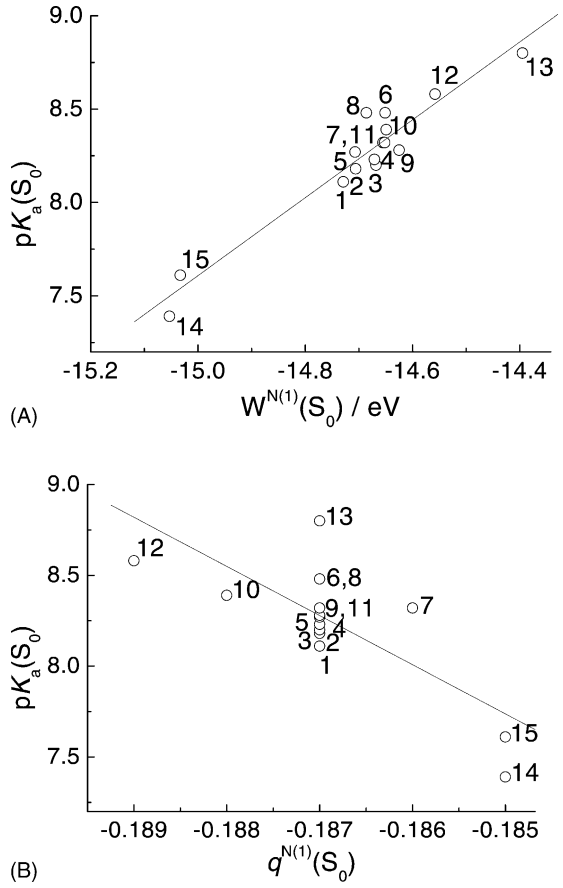

Fig. 4. Correlation between $W^{N(1)}(S_0)$ and apparent pK_a of deprotonation in the ground state, $r = 0.962$, S.D. $= 0.0997$, p$K_a = 38.9 + 2.1 \times W^{N(1)}(S_0)$ (A) and the relation between $q^{N(1)}(S_0)$ and apparent pK_a of deprotonation in the ground state, $r = 0.764$ (B); INDO/S-CI procedure used in calculations. See Table 2 for compound numbers.

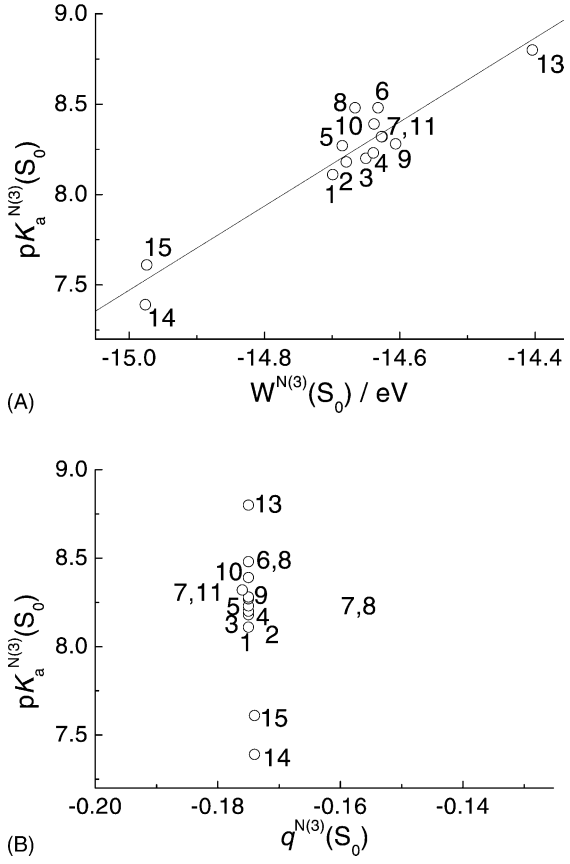

Fig. 5. The relation between $W^{N(3)}(S_0)$ and apparent pK_a of deprotonation in the ground state, $r = 0.953$, S.D. $= 0.117$, p$K_a = 42.38 + 2.33 \times W^{N(3)}(S_0)$ (A) and the relation between $q^{N(3)}(S_0)$ and apparent pK_a of deprotonation in the ground state (B); INDO/S-CI procedure used in calculations. See Table 2 for compound numbers.

Table 3
Acid–base properties of nitrogen atoms of alloxazine molecules in the first excited singlet state, S_1

No.	Compound	$W^{N(10)}(S_1)$ (eV)	$q^{N(10)}(S_1)$	$W^{N(1)}(S_1)$ (eV)	$q^{N(1)}(S_1)$	$W^{N(3)}(S_1)$ (eV)	$q^{N(3)}(S_1)$	$W^{N(5)}(S_1)$ (eV)	$q^{N(5)}(S_1)$	p$K_a^{N(1)}(S_1)$[a]	p$K_a^{N(3)}(S_1)$[a]
1	All	−12.702	−0.436	−15.189	−0.124	−14.335	−0.179	−11.592	−0.436	2.3	7.5
2	6MAll	−12.551	−0.426	−14.912	−0.132	−14.094	−0.180	−11.285	−0.456	4.7	7.6
3	7MAll	−12.530	−0.441	−14.997	−0.130	−14.166	−0.180	−11.363	−0.463	3.7	7.4
4	8MAll	−12.479	−0.449	−15.039	−0.131	−14.336	−0.179	−11.678	−0.461	2.7	7.5
5	9MAll	−12.696	−0.428	−15.017	−0.130	−14.128	−0.180	−11.279	−0.458	4.0	7.6
6	6,7MAll	−12.298	−0.439	−14.679	−0.139	−13.930	−0.180	−11.087	−0.463	5.9	7.7
7	6,8MAll	−12.473	−0.433	−14.944	−0.134	−14.251	−0.179	−11.592	−0.448	6.1	7.7
8	6,9MAll	−12.062	−0.445	−14.177	−0.155	−13.536	−0.180	−10.541	−0.482	6.3	7.8
9	7,8MAll	−12.425	−0.444	−14.919	−0.134	−14.192	−0.180	−11.451	−0.458	3.6	7.5
10	7,9MAll	−12.480	−0.438	−14.831	−0.135	−13.996	−0.180	−11.118	−0.462	5.7	7.7
11	8,9MAll	−12.566	−0.436	−15.027	−0.131	−14.264	−0.180	−11.554	−0.451	6.1	–
12	3,7,8MAll	−12.358	−0.446	−14.861	−0.134	−14.002	−0.133	−11.403	−0.457	3.8	–
13	5DLc	−12.434	−0.409	−14.689	−0.146	−14.090	−0.179	–	–	4.5	8.8
14	7CNAll	−13.037	−0.431	−15.407	−0.125	−14.587	−0.178	−11.984	−0.453	2.7	–
15	8CNAll	−13.125	−0.427	−15.504	−0.123	−14.657	−0.178	−12.160	−0.441	1.0	–

$W^N(S_1)$: effective valence electron potentials for nitrogen atoms in the first excited singlet state; $q^N(S_1)$: net charges for nitrogen atoms in the first excited singlet state.

[a] pK_a of deprotonation of N(1)–H and N(3)–H respectively, in the first excited singlet state [3], the accuracy of the excited state pK_a experimental determination was ±0.2.

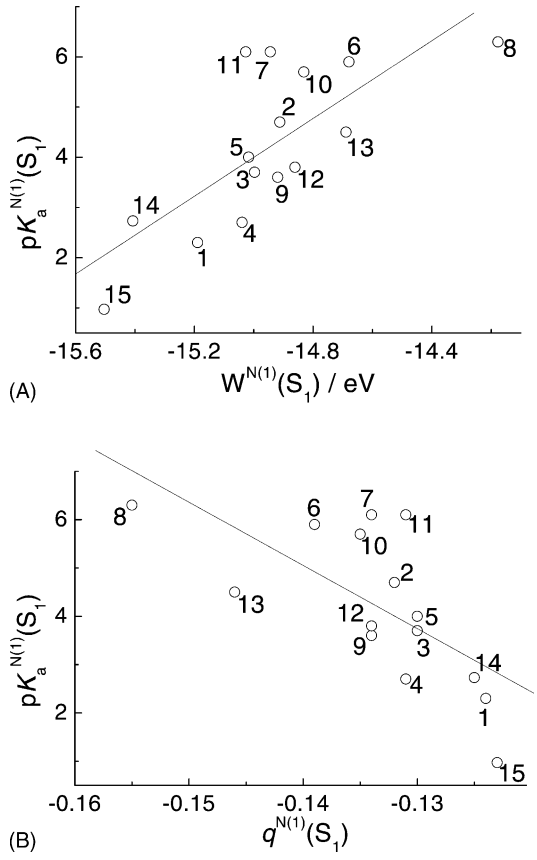

Fig. 6. The relation between $W^{N(1)}(S_1)$ and pK_a of deprotonation of N(1) in the first excited singlet state, $r = 0.748$, S.D. = 1.11, $pK_a^{N(1)}(S_1) = 62.20 + 3.87 \times W^{N(1)}(S_1)$ (A) and the relation between $q^{N(1)}(S_1)$ and pK_a of deprotonation of N(1) in the first excited singlet state, $r = 0.673$ (B); INDO/S-CI procedure used in calculations. See Table 2 for compound numbers.

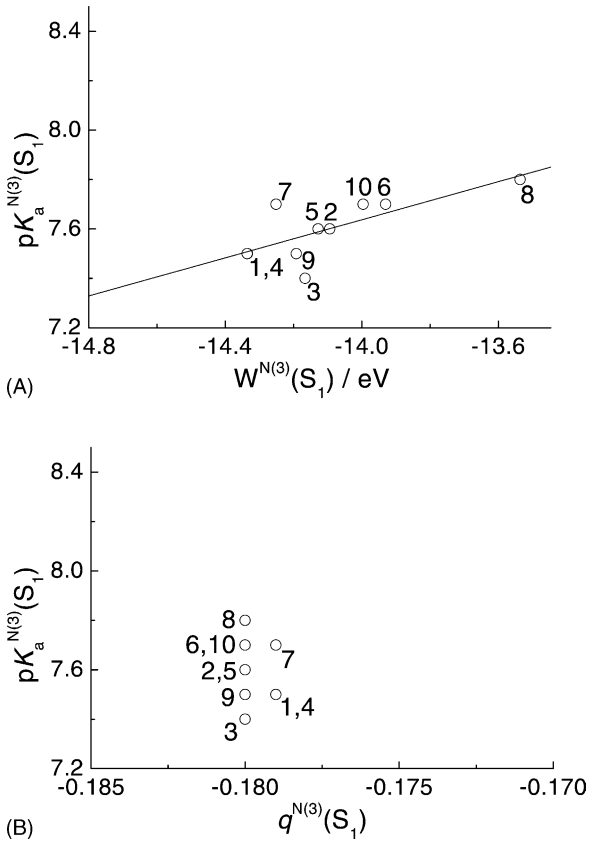

Fig. 7. The relation between $W^{N(3)}(S_1)$ and pK_a of deprotonation of N(3) in the first excited singlet state, $r = 0.732$, S.D. = 0.090, $pK_a^{N(3)}(S_1) = 13.03 + 0.38 \times W^{N(3)}(S_1)$ (A) and the relation between $q^{N(3)}(S_1)$ and pK_a of deprotonation of N(3) in the first excited singlet state, $r = 0.184$ (B); INDO/S-CI procedure used in calculations. See Table 2 for compound numbers.

state [3]. The excited state pK_a values were calculated from the Förster cycle using absorption and emission spectral data. The differences between the pK_a values of alloxazines studied are larger in the S_1 state. In the first excited singlet state, the quantitative correlation between the effective valence electron potentials and the pK_a of deprotonation is relatively poor ($r = 0.748$ for N(1) and $r = 0.732$ for N(3)) (see Figs. 6 and 7). Such poor correlation might have various reasons, of experimental and theoretical origin. Experimentally, the excited state pK_a values cannot be determined with a precision equal to that of the ground state values. Theoretically, it is much more difficult to describe the excited electronic states theoretically, thus, the resulting uncertainties of the W values could additionally impair the correlations.

Much better results are obtained when, in the INDO/S calculations, only HOMO → LUMO single excitations are taken into account (see Table 4). The improved correlations are shown on Fig. 8. The problem shown here relates to the disadvantages of the INDO approximation. Spectroscopically parameterized INDO scheme, e.g. INDO/S was thought to reproduce mainly transition energies and their intensities. Some weakness of the INDO/S-CI method have

Table 4
Acid–base properties of nitrogen atoms N(1) and N(10) of alloxazines molecules in the first excited state, S_1, calculated for single HOMO → LUMO transition

No.	Compound	$W^{N(1)}(S_1)$ (eV)	$q^{N(1)}(S_1)$	$W^{N(3)}(S_1)$ (eV)	$q^{N(3)}(S_1)$
1	All	−15.419	−0.096	−14.034	−0.181
2	6MAll	−14.461	−0.134	−13.38	−0.181
3	7MAll	−15.050	−0.112	−13.822	−0.181
4	8MAll	−15.208	−0.110	−14.11	−0.181
5	9MAll	−14.673	−0.128	−13.482	−0.181
6	6,7MAll	−14.486	−0.132	−13.428	−0.181
7	6,8MAll	−13.883	−0.163	−13.11	−0.180
8	6,9MAll	−13.885	−0.162	−13.088	−0.181
9	7,8MAll	−15.016	−0.114	−13.879	−0.181
10	7,9MAll	−14.747	−0.124	−13.563	−0.182
11	8,9MAll	−14.436	−0.137	−13.328	−0.181
12	3,7,8MAll	−14.957	−0.115	−13.686	−0.135
13	5DLc	−14.749	−0.122	–	–
14	7CNAll	−15.439	−0.102	−14.213	−0.179
15	8CNAll	−15.822	−0.092	−14.498	−0.180

$W^N(S_1)$: effective valence electron potentials for nitrogen atoms in the first excited singlet state; $q^N(S_1)$: net charges for nitrogen atoms in the first excited singlet state.

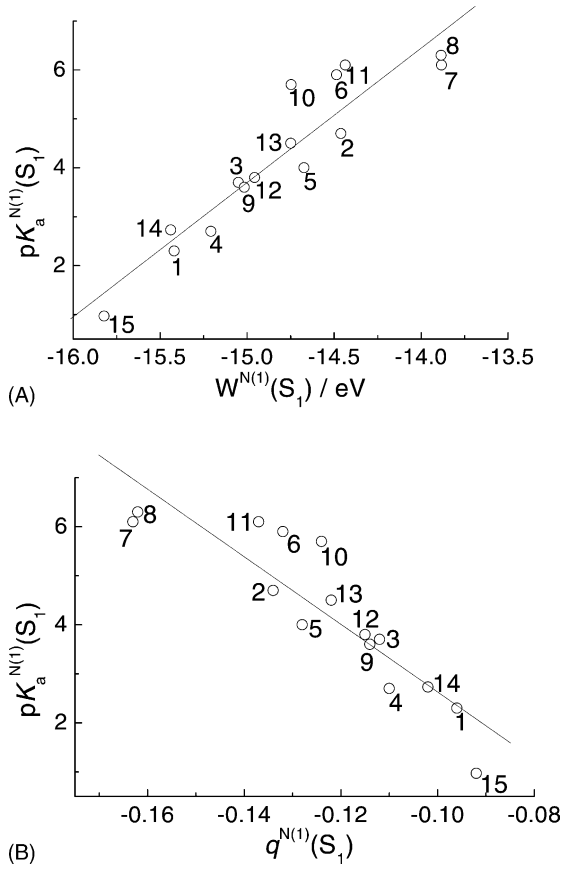

Fig. 8. The relation between $W^{N(1)}(S_1)$ and pK_a of deprotonation of N(1) in the first excited singlet state, $r = 0.931$, S.D. $= 0.61$, $pK_a^{N(1)}(S_1) = 44.89 + 2.75 \times W^{N(1)}(S_1)$ (A) and the relation between $q^{N(1)}(S_1)$ and pK_a of deprotonation of N(1) in the first excited singlet state, $r = 0.894$ (B); only HOMO → LUMO transition used in INDO/S calculations. See Table 2 for compound numbers.

already been shown [19] for a set of monomethyl alloxazines where without a CI procedure, i.e. based purely on a single configuration (HOMO → LUMO), the correlation between the observed absorption band maxima and calculated energies (HOMO → LUMO and HOMO-1 → LUMO energy gap) for some methylalloxazines was much better than those calculated using CI. This is clearly a case where the CI procedure is responsible for some loss of the substituent effect in the excited states. As an example, the correlations shown on the Fig. 8 are much better if compared to the results obtained using the INDO/S-CI procedure, see Fig. 6.

The changes of electron density distribution upon excitation produce an increase in basicity at the N(10) nitrogen atom and of acidity at N(1) (see Fig. 2). These results agree with the decrease in the experimental values of pK_a for the N(1)–H deprotonation in excited alloxazines. For deprotonation at N(3), the pK_a values are only slightly lower than those obtained in the ground state, although the calculations predict an increase in basicity of this atom after excitation. The rather small changes in acidity of the N(3) nitrogen atom after excitation may be due to the lack of conjugation between its p-orbital with the π-electron system of the alloxazinic molecule.

3.4. Excited state proton transfer

Changes in electron density are the driving force for the excited state proton transfer which takes place in alloxazines in presence of acetic acid, water or pyridine [1–4]. In the case of acid-catalyzed phototautomerism, the acetic acid molecule plays the role of a bridge between the proton donor (N(1)–H) and acceptor (N(10)) centres. The overall process consists of two steps: creation of alloxazine–acetic acid complexes of an appropriate structure, and transfer of a proton from N(1) to N(10) nitrogen atoms. The first step can be influenced by the ground and/or excited state properties, while the second depends on the excited state properties. Formation of alloxazine–acetic acid complexes depends on the hydrogen-bonding ability of the N(1) and N(10) nitrogen atoms and thus should be related to the calculated acid–base properties of these two atoms.

The calculated highest acidity of the N(1) nitrogen atom in the cyano derivatives is confirmed by the observation that these compounds phototautomerize in presence of alcohol, which was not observed for methyl analogues of alloxazine nor for other alloxazines [7]. It seems that in the alcohol-catalyzed proton transfer reaction a hydrogen bond in the ground state is created between N(1)–H hydrogen atom of alloxazine and the oxygen atom of the alcohol, thus, the acidity of the N(1)–H bond plays a crucial role in this process.

5-Deazalumichrome, as was shown [11], only phototautomerizes in presence of acetic acid (but not alcohol), however, its ability to undergo proton transfer is markedly higher than that of its analogue lumichrome and other compounds studied. This may be due to the high basicity of the N(10) nitrogen atom of the ground state 5-deazalumichrome. As a result, this compound can form stronger hydrogen bonds with proton donor compounds such as acetic acid in the ground state, and can consequently undergo excited state proton transfer with higher efficiency. Spectral evidence for the interaction between 5-deazalumichrome and acetic acid in the ground state was indeed reported [11]. As the structure of alloxazine–acetic acid complexes in the ground state is not known, the results of the calculations provide support for the occurrence of some interaction in the ground state, in which the N(10) nitrogen atom plays a crucial role.

6,9MAll is the only compound among those studied which, according to the calculations, shows a decreased acidity of the N(1)–H, hydrogen atom in the excited state. This compound, in contrast to the others studied, was shown to undergo excited state proton transfer in presence of pyridine with a very low efficiency [3]. In the pyridine-catalyzed phototautomerism, the crucial role is played by the N(1)–H group, so an unusually low acidity of this atom in the excited state could explain the lack of proton transfer in this compound.

Considerable work has been done to study the mechanism of the excited state proton transfer reaction in lumichrome–acetic acid and other complexes [2,6,13,25–27], however, there still remain several discrepancies in the reported results concerning the mechanism and kinetics of the alloxazine–isoalloxazine tautomerism. Moreover, there exists a general lack of information on the excited state proton transfer reaction studied by direct time-resolved techniques for other substituted alloxazines. Many of the compounds presented in this study have never been examined in such a context. We expect that the theoretical results presented, along with the spectral and photophysical data, should help the development of future work aimed at elucidating the role of ground and excited states acid–base properties of substituted alloxazines.

4. Conclusions

In this paper, we have successfully applied the concept of effective electron valence potentials to explain the experimentally observed changes in the acid–base properties of alloxazines upon substitution. Earlier, good correlations between pK_a and calculated potentials were reported for aza-aromatic compounds in the ground state [20,21,23]. The same correlations, however, were much less pronounced for the excited state [21]. We believe this might result from a reduced precision of the results of the excited state calculations as compared to the ground state. Also, the experimental investigation of the excited state properties is much more complicated than that of the ground state.

The effective valence electron potentials, overall, exhibit a larger variability within the group of compounds studied as compared to the net charges, both in the ground state and in the excited state. This demonstrates higher sensitivity of this property to the details of the molecular structure and consequently its higher predictive ability for the experimentally measurable quantities.

The studies of differently substituted alloxazines gave us an opportunity to estimate some intramolecular properties, which can influence proton transfer in this class of compounds. The results presented show that the ability to undergo the excited state proton transfer is determined, among others, by the ability of ground–state alloxazines to form hydrogen bonds with molecules promoting this process. This ability is well predicted by the values of the calculated effective valence electron potentials, which quantitatively reflect changes of the acid–base properties of alloxazines upon substitution.

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