ACID–BASE EQUILIBRIA OF SOME 7-DIMETHYLAMINO-3-PHENOXAZONE DERIVATIVES

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The acid–base equilibria of some phenoxazine dyes in aqueous–ethanolic solutions were studied spectrophotometrically. The compounds examined were prepared in reagent grade purity, their main spectral characteristics in UV and VIS region were found and their dissociation constants were determined in 41% (m/m) ethanol. For the analytically most interesting derivative, gallocyanine methyl ester (*IV*), the p*K* values were measured also in solutions with different proportions of ethanol. The colour changes accompanying the protolytic reactions and the factors governing the phenomena are discussed.

The phenoxazine dyes studied (*I*–*VIII*) have frequently been employed not only in the dyeing industry, but also in analytical chemistry for the identification and determination of some ions. Among simple amino phenoxazones, this dye group exhibits the most pronounced acid–base, oxidation–reduction, and complexing properties[1–12].

It is particularly the derivative *IV* that has been used as an acid–base indicator[6,14] and for inorganic ion complexation[7–10]. In order to obtain a homogeneous solution of the reagent and the created complex compound, however, an aqueous–ethanolic solvent has to be used, in some cases with as much as 50% ethanol[6,7]. For this reason, the dissociation constants of *III* and *IV* have been measured in that solvent[6,12]; this also enabled the acid–base properties to be compared with those of the parent substances *I* and *II*, for which the medium of 50% ethanol is prerequisite for their maintaining in solution[11].

In all the recent acid–base[6,11] and compexing[7–10] studies, the cell for the pH measurements was adjusted by using standard aqueous buffers. As this way of standardization is of utility for comparison purposes, it has been applied also in the present work. The constants, however, involve then a systematic error, which depends on the proportion of the nonaqueous component and can be determined by calibration of the measuring cell by using standards with different contents of the organic constituent.

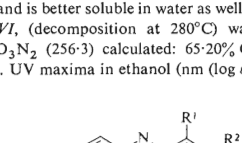
Thus, in the present work the dissociation constants have been calculated based

on the pH found by adjusting the cell to both an "aqueous" and an aqueous-ethanolic acid–base standard; the protolytic equilibria of the dyes under study are discussed.

EXPERIMENTAL

All the dyes studied were prepared in our laboratory, largely by condensation o *p*-nitrosodimethylaniline with the appropriate phenolic components[12,14–16]. The compounds *VI*—*VIII* were synthesized for this study by secondary reactions (decarboxylation or hydrolysis) applying the following procedures:

Compound *VI*: A mixture of 2 g of gallocyanine *II* (ref.[15]) and 2 g of sodium acetate crystals in 200 ml of water was refluxed for 5 h. The unreacted gallocyanine was taken up in sodium carbonate solution, in which the substance *VI* is insoluble. The decarboxylated gallocyanine differs also in other respects: it dissolves in hydrochloric acid to give blue colour (gallocyanine, red colour), and is better soluble in water as well as in organic solvents. 1·55 g of a dark crystalline substance, *VI*, (decomposition at 280°C) was obtained after recrystallization in ethanol. For $C_{14}H_{12}O_3N_2$ (256·3) calculated: 65·20% C, 4·81% H, 10·11% N; found: 64·80% C, 4·87% H, 9·87% N. UV maxima in ethanol (nm (log ε)): 266 (4·43), 572 (4·55).



	R^1	R^2	R^4
I	COOH	H	H
II	COOH	H	OH
III	$COOCH_3$	H	H
IV	$COOCH_3$	H	OH
V	$COOCH_3$	H	OCH_3
VI	H	H	OH
VII	$COOCH_3$	OH	H
VIII	$COOCH_3$	OCH_3	H

Compound *VII*: 500 mg of 1-carbomethoxy-2-aminophenyl-7-dimethylamino-3-phenoxazone[17] was dissolved in 0·8 ml of concentrated H_2SO_4, and 3 ml of water was added dropwise. The mixture was boiled shortly and after cooling down, diluted with water to 300 ml. Substance *VII* separated in fine, metallic-lustrous crystals (m.p. 260—263°C). For $C_{16}H_{14}O_5N_2$ (314·3) calculated: 61·14% C, 4·49% H, 8·91% N; found: 62·03% C, 5·15% H, 8·45% N. UV maxima in ethanol (nm (log ε)): 238 (4·38), 282 (4·00), 550 (4·49).

Compound *VIII*: 500 mg of 1-carbomethoxy-2-aminophenyl-7-dimethylamino-3-phenoxazone[17] was dissolved in 10 ml of methanol, and 1 ml of concentrated H_2SO_4 was added. The reaction mixture was refluxed on water bath for 1·5 h. After cooling down, 200 ml of water and 10 g sodium acetate were added, and the solid formed was collected, washed with water, and dried. To 400 mg of the crude product was added 100 ml of 2M-Na_2CO_3, the insoluble moiety (substance *VII*) was filtered out, and the filtrate was made acidic with hydrochloric acid. 210 mg of substance *VII* was obtained (m.p. 260—263°C). The residue on the filter was dissolved in a 1 : 1 acetone–benzene mixture and chromatographed in this system on a silica gel column; 95 mg of substance *VIII* was obtained as dark green, metallic-lustrous crystals, m.p. 214°C. For $C_{17}H_{16}O_5N_2$ (328·3) calculated: 61·19% C, 4·89% H, 8·55% N; found: 62·42% C, 4·91% H, 7·86% N. UV maxima in ethanol (nm (log ε)): 208 (4·17), 244 (4·43), 281 (4·40), 569 (4·65).

The purity of all the dyes was checked by thin layer chromatography on silica gel (substances *I* and *II* in pyridine in 4 : 1 acetone–ammonia mixture, substances *III*, *V*, and *VIII* in 1 : 3 acetone–benzene mixture, substances *IV* and *VII* in 4 : 1 acetone–ammonia mixture, substance *VI* in 7 : 1 acetone–ammonia mixture) and by elemental analysis (C, H, N).

The concentration of the standard solutions for spectrophotometric measurements was $2 \cdot 10^{-4}$ mol l^{-1} in 93% (m/m) ethanol (the percentage content of ethanol was found by density measurement, $\varrho = 0{\cdot}8095$ kg dm^{-3}). The mixed solvents with different ethanol contents were prepared by mixing the corresponding volumes and converting the volume fractions to the weight per cent values.

The dissociation constants were determined spectrophotometrically applying the relation $pK_a = pH - \log[(A - A_1)/(A_2 - A)]$, where A is the absorbance for the chosen pH in the colour change region, and A_1 and A_2 are the absorbances of the horizontal branches of the pH-curves for $pH < pK_a$ and $pH > pK_a$, respectively. The $A = f(\lambda)$ dependences were constructed in two ways:

a) The solutions to be measured were prepared by diluting the standard dye solutions with water, Britton–Robinson buffers, and an amount of 1M–KCl to a defined volume with the desired ethanol content and ionic strength 0·1. In the strongly acidic region, $pH < 1{\cdot}5$, a mixture of 0·1M-HCl and 0·1M-NH_4Cl was used without controlling the ionic strength. The absorption spectra were measured on a Unicam 1800 recording UV spectrophotometer (Pye, Cambridge) in 1 cm cells, the pH was measured on a PHM 4d instrument (Radiometer, Copenhagen) using a G 200B-K 100 electrode system adjusted by means of an aqueous hydrogenphthalate buffer.

b) Unbuffered dye samples containing ethanol in various proportions and with the ionic strength 0·1 adjusted with 1M-KCl were acid–base titrated either with 0·1M-HCl or with 0·1M-NaOH titrant solutions. The cell compartment of the UV spectrophotometer was adapted for sample titration and simultaneous pH and absorbance measurements. An electrode and cell holder after Karlíček[18] was manufactured at the workshop of the Faculty of Pharmacy, Hradec Králové. Glass cells of the thickness 3·5 cm and volume 100 ml were used. An ABU-12 automatic burette in conjunction with a PHM 26 pH-meter (both Radiometer, Copenhagen) served for the acidity adjustment under potentiometric pH control. The electrode system of a Beckman 40 498 glass electrode and a Radiometer K 401 calomel electrode was adjusted by using an aqueous–ethanolic buffer[19], in which the glass electrode was immersed for 30—50 min (according to the ethanol content) prior to each measurement. After the addition of the titrant, the sample was bubbled with bulb nitrogen[7] whose flow rate was controlled by means of a needle valve; no absorbance corrections for the volume changes during the titrations were necessary.

The dissociation constants were calculated by using an equation corrected for the mean activity coefficient[20], $K_a = a_{H^+}([A]/[HA]) \cdot f_{\pm}$, whose value was obtained from the Debye–Hückel relation; the requisite relative permittivities ε were derived graphically from the dependence on the weight concentration of ethanol[21].

From the set of 15—20 calculated K_a values for different wavelengths, from which remote data were excluded by applying the T-criterion, the arithmetic mean was obtained and the corresponding confidence interval[22] was calculated for α 0·05. The dissociation constants pK_a for the various ethanol contents and for the two different ways of adjusting the electrode cell are given in Table I.

The SCF PPP quantum-chemical calculations of the electronic spectra of the dyes *II*, *IV*, and *VII* were performed on an M-4030 computer (Taras Shevchenko Kiev State University, Kiev, USSR).

TABLE I

Dissociation constants of 7-dimethylamino-3-phenoxazone derivatives in aqueous–ethanolic solutions

Derivative	Calibration[a]	Ethanol concentration % (m/m)	pK_1[b]	pK_2	pK_3	Ref.
I	A	41·0	2·97 ± 0·04	4·59 ± 0·02	—	11
II	A	41·0	3·03 ± 0·02	4·64 ± 0·03	9·25 ± 0·02	11
III	A	41·0	3·51 ± 0·06			12
	C	41·0	3·57 ± 0·06			
IV	A	41·0	3·40 ± 0·04	8·53 ± 0·04		6
		31·9	3·47 ± 0·03	8·30 ± 0·05		
		23·5	3·53 ± 0·02	8·22 ± 0·02		
		15·5	3·63 ± 0·01	8·10 ± 0·02		
		7·7	3·68 ± 0·04	8·00 ± 0·09		
	C	41·0	3·14 ± 0·01	8·32 ± 0·01		
		31·9	3·39 ± 0·02	8·24 ± 0·02		
		23·5	3·50 ± 0·05	8·18 ± 0·03		
		15·5	3·62 ± 0·03	8·08 ± 0·02		
		7·7	3·68 ± 0·02	8·00 ± 0·04		
V	A	41·0	3·09 ± 0·03			6
VI	C	41·0	2·72 ± 0·06	8·59 ± 0·06		
VII	C	41·0	1·60 ± 0·06	6·60 ± 0·06		
VIII	C	41·0	2·17 ± 0·06			

[a] A aqueous buffer, C citrate buffer; [b] the equilibrium type is disregarded in the order.

RESULTS AND DISCUSSION

The compounds under study involve in their molecules mutually conjugated acidic and basic groups, one of which exerts a positive mesomeric effect, the other, a negative mesomeric effect. Owing to the extended conjugation and the associated enhanced delocalization of the nonbonding and π electrons, the absorption maxima lie in the long-wavelength region of the visible spectrum. The absorption maxima of substances *I*–*VIII* in ethanolic solutions are found in the region of 550–610 nm.

The results of the quantum-chemical calculations of the electronic structures and spectra of the phenoxazone dyes in the ground and the two lowest excited states. published by Pilipenko and coworkers[23], indicate that for a 3-phenoxazone molecule the positions 1 and 2 are suitable for introducing electron acceptor groups, the positions 4, 6, 8, 9, and 7, for introducing electron donor groups, the position 4 being dominant. These conclusions are borne out by the electronic spectra of substances

II, *IV*, and *VI*, containing a strong electron donor group (—OH) in the position 4. The absorption maxima of the two acid–base species (Table II) corresponding to the deprotonation of the phenolic hydroxy group are well separated ($\Delta\lambda$ 100 – 110 nm)

TABLE II

Absorption characteristics of 7-dimethylamino-3-phenoxazone derivatives in 41% (m/m) ethanol. ε in 1 mol^{-1} cm^{-1}

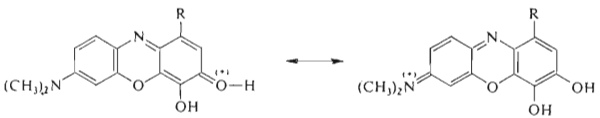
Derivative	Species												Ref.
	1st protonized			neutral			1st deproto-nized			2nd deproto-nized			
	λ_{max} nm	$\varepsilon . 10^{-3}$ (pH)	λ_{iso} nm	λ_{max} nm	$\varepsilon . 10^{-3}$ (pH)	λ_{iso} nm	λ_{max} nm	$\varepsilon . 10^{-3}$ (pH)	λ_{iso} nm	λ_{max} nm	$\varepsilon . 10^{-3}$ (pH)		
I	540	18·1 (1·47)	548			533 618	590	34·1 (7·25)					11
II	540	17·6 (1·12)	580	650	17·8 (4·00)	625	605	20·2 (6·59)	528	500	10·5 (10·73)		11
III	535	17·4 (2·00)	550	610	42·6 (5·56)								
IV	540	32·6 (1·56)	568	635	44·6 (6·00)	555	525	20·5 (10·50)					
IV[a]	538	26·0 (2·26)	572	640	41·1 (5·91)	560	522	16·5 (10·76)					6
IV[b]	536	26·3 (2·27)	572	642	40·3 (5·54)	561	532	17·4 (10·70)					
IV[c]	534	24·6 (2·34)	572	642	41·7 (6·20)	560	542	16·6 (10·10)					
IV[d]	534	27·1 (2·30)	571	642	43·7 (5·11)	564	546	18·6 (10·42)					
V	535	37·7 (1·70)	558	625	71·3 (9·24)								
VI	545	56·2 (1·06)	564	600	41·7 (4·60)	525	500	20·4 (9·53)					
VII	582	21·4 (1·12)	617	582	30·2 (4·00)	532	560	19·1 (10·25)					
VIII	560	25·7 (1·50)	550	597	40·7 (4·60)								

[a] 31·9% (m/m) ethanol; [b] 23·5% (m/m) ethanol; [c] 15·5% (m/m) ethanol; [d] 7·7% (m/m) ethanol

and also are intense enough. On the other hand, introduction of the same electron donor group (—OH) into a position suited for an electron acceptor group (position 2 in substance *VII*) acts against the natural electron density distribution over the chromophor, and as a result, the wavelength difference for the maxima of the two acid-base species is small ($\Delta\lambda$ 22 nm) and their intensity is lowered (ε 4 000 and 10 250, respectively).

The adequacy of the SCF PPP calculations was verified by comparing the experimental and calculated wavelengths of the absorption maxima (for instance, for compound *VII*: λ_1^{calc} 559 nm, λ_1^{exp} 582 nm; λ_2^{calc} 414 nm, λ_2^{exp} 426 nm).

The aqueous–ethanolic solutions of *I – VIII* show protolytic phenomena typical of weak acids. At higher acidities of medium, pH < 3, the neutral species is protonized, which is manifested by a pronounced hypsochromic shift of the absorption maximum (as large as 100 nm) and a considerable drop of the colour intensity. Compounds *VII* and *VIII* are exceptions, their protonation being not accompanied by a marked shift of the absorption band. Electron density distribution calculations for the ground and the two lowest excited states of compounds *II* and *IV* indicate that the transition from the ground state to the two excited states is accompanied by a decrease in the electron density at the amine nitrogen in position 7 and increase at carbonyl oxygen in position 3. Since protonation of a molecule takes place at the site possessing the highest electron density, the protonation of molecules *II* and *IV* can be expected to occur at the carbonyl oxygen in position 3. The charge is then transmitted to the amine nitrogen, where upon the conjugated chromophor system shortens.



R = COOH, $COOCH_3$

The hypsochromic shift of the absorption maximum and decrease in the colour intensity are consequences of this reduced conjugation. In this respect the recently suggested concept[6,11,24,25] of protonation of a free or substituted amino group nitrogen in position 7 should be corrected.

An opposite situation is with the compound *VII*. The calculations show that as a result of the introduction of a strong electron donor group (—OH) into position 2, the electron density at the amine nitrogen in position 7 increases considerably on the transition from the ground state to the two lowest excited states, and that at the carbonyl oxygen in position 3 decreases. The amine nitrogen thus becomes the site with the highest electron density in the molecule, so that the protonation of molecule *VII* can be assumed to proceed at this amine nitrogen.

The experimental data concerning the spectral parameters of the acid–base species (λ_{max}, λ_{iso}, ε_{max}) and the protonation equilibria (pK_a values) of the dyes *I*–*VIII* suggest that the protonation mechanism for substances *I*, *III*, *V*, and *VI* is analogous to that for compounds *II* and *IV*, whereas in the case of substance *VIII*, involvig an electron donor methoxy group in position 2, the protonation mechanism is presumably the same as that of substance *VII*. Introduction of electron donor groups into positions 1 and 2 is associated with a shift of the protolytic equilibria by as much as 2 units into the acidic region of the acidity scale (Table I). Esterification of the carboxy group in position 1 brings about a positive inductive effect with a slight electron localization. This effect gives rise to a hypsochromic shift of $\Delta\lambda$ 15 to 40 nm in the electronic spectra.

The effect of ethanol on the position of the absorption maxima of the various protolytic species is rather small. In this respect only substance *IV* was studied in detail. The change is most pronounced in the case of the anionic L^- species, exhibiting a hypsochromic shift of the maximum of 22 nm with decreasing permittivity of medium. The neutral HL species shows a shift in the same sense, but much less pronounced, the protonized H_2L^+ species displays an opposite bathochromic shift of as little as 6 nm (Table II).

To a greater extent is the nonaqueous medium manifested in the dissociation constant values. The decisive factors are here the relative permittivity and the charge of the conjugate pair species. In the case of acids in which the charge is lowered on the dissociation, the acidity constant increases (pK_a decreases) with decreasing permittivity of medium; and, conversely, if the conjugate base acquires a higher charge than the acidic species, the constant decreases (pK_a increases)[26,27]. This is borne out by the constants of some common weak acids, including catinoid ones, in aqueous–ethanolic solutions[27].

In accordance with this are also the dissociation constants of gallocyanine methyl ester (*IV*) in solutions with various proportions of ethanol (Table I), for both protolytic equilibria, $H_2L^+ \rightleftharpoons HL$ (K_{a1}) and $HL \rightleftharpoons L^-$ (K_{a2}).

The way of measuring the pH is of significance in the determination of the dissociation constants in mixed solvents. The electromotive voltage of the cell measured is well reproducible, but – in contrast to purely aqueous solutions – a high contribution of the liquid potential is involved. The pH value in mixed solvents for a cell calibrated by means of standard aqueous buffers is[28]

$$pH = pa_H + \delta ,$$

where the correction factor δ relates the conventional pH value with the activity pH scale for the mixed solvent in question.

The dissociation constant calculated from the pa_H value for a certain content of ethanol differs from the mixed constant by a value increasing with the decreasing relative permittivity of medium (Table I). This difference is in accordance with the theoretical δ value for aqueous–ethanolic solutions[28].

Theoretical considerations as well as the experimental results of study of the acid–base equilibria for the dyes *I – VIII* indicated that not all of them are equally well suited as neutralization or complexometric indicators; the differences in their properties are due to the substituents on the 7-dimethylamino-3-phenoxazone skeleton. As the most suitable emerge the derivatives involving an OH group in position 4, and of these, the compound *IV* (Prune) seems to be the most promising; this substance has been employed with success as an acid–base indicator[6] as well as a complexing agent[7–10]. Notably good results in neutralization titrations can be achieved by using this substance as a blended indicator in mixture with methyl orange[13], similarly as in the case with substance *V* (ref.[6]). The colour changes of the protolytic species of the remaining dyes are less pronounced as compared with *IV*, and consequently the titration errors are considerably higher; therefore, these dyes cannot be recommended for analytical use in acid–base reactions.

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