Monatshefte für Chemie 135, 261–267 (2004)
DOI 10.1007/s00706-003-0116-z

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

The Dissociation Constants of 1,1′-Bis (pyridinium-4-aldoxime)trimethylene dibromide

Blaženka Foretić[1] and **Nicoletta Burger**[1,*]

[1] Department of Chemistry and Biochemistry, Faculty of Medicine, University of Zagreb, 10000 Zagreb, Croatia

Received July 7, 2003; accepted September 18, 2003
Published online December 30, 2003

Summary. The dissociation constants of the two oxime groups of 1,1′-bis(pyridinium-4-aldoxime)-trimethylene dibromide (*TMB-4*) were determined using spectrophotometric data. Two numerical methods were applied to treat the overlapping equilibria. The results obtained by both agreed with each other and their mean values at 25°C corrected for the ionic strength of 0.05 mol dm^{-3} are $pK_{a1} = 7.49 \pm 0.11$ and $pK_{a2} = 8.96 \pm 0.09$. These values were discussed in terms of the pK_as of 1,1′-bis(pyridinium-4-aldoxime)oxydimethylene dichloride (*Toxogonin*), a similar dioxime, which were derived by extrapolation of literature data.

Keywords. 1,1′-Bis(pyridinium-4-aldoxime)trimethylene dibromide; Dissociation constants; Spectrophotometry.

Introduction

Oximes having different chemical structures are known to form coloured complexes with a great number of metal ions as well as with the aquapentacyanoferrate(II) ion [1, 2]. The latter reactions have been the subject of our investigations for several years. Many oximes, especially those of the bispyridinium type, are potent reactivators of the acetylcholinesterase inhibited by organophosphorus poisons (insecticides, nerve gases) and seem to have further multiple pharmacological effects [3]. One of the most powerful among them is 1,1′-bis(pyridinium-4-aldoxime)trimethylene dibromide (*TMB-4*). It was found to react with the aquapentacyanoferrate(II) ion, $[Fe(CN)_5H_2O]^{3-}$, by forming a blue coloured oximatopentacyanoferrate(II) complex whose composition, spectral characteristics, and stability constant were described earlier [4].

Pentacyanoferrate(II) complexes are often used as models in fundamental and applied studies. Trying to give more insight into the correlation between the chemical

* Corresponding author. E-mail: nicoletta.burger@mef.hr

structure and the biological function of oximes, we undertook a kinetic study of the reactions of the aquapentacyanoferrate(II) ion with 1,1′-bis(pyridinium-4-aldoxime)trimethylene dibromide and another dioxime, 1,1′-bis(pyridinium-4-aldoxime)oxydimethylene dichloride (also known as *Toxogonin*, *Obidoxime*, or *LüH*-6). During these investigations we encountered the necessity of determining the dissociation constants of the oxime groups at defined reaction conditions. This was necessary, because of the highly different reactivity of the particular ionic species present in solutions of these compounds toward the aquapentacyanoferrate(II) ion. The pK_a values of the two oxime groups of *Toxogonin* found in the literature [5, 6] were interpolated to give values appropriate to our kinetic experiments. It was not possible to get such values for *TMB-4*, because of the scarce literature information related to its oximes' pK_as [7, 8]. Thus, this investigation was carried out.

Results and Discussions

The UV/VIS absorption spectrum of *TMB-4* was recorded in water solutions of different acidity (Fig. 1). It exhibits two intensive *pH* dependent bands as a result of $\pi \rightarrow \pi^*$ transitions within the pyridiniumaldoxime aromatic system [9]. The maximum at $\lambda = 282$ nm is characteristic for the protonated pyridiniumaldoxime group while the maximum at $\lambda = 345$ nm is due to the absorption of the deprotonated pyridiniumaldoxime group. Both maxima are in accordance with the ^1L_b absorption band of the substituted pyridine ring. The less intensive absorption at $\lambda = 245$ nm, whose position shifts hypsochromically to $\lambda = 242$ nm upon raising the *pH* of the medium, is also attributed to the deprotonated pyridiniumaldoxime group and it is in accordance with the so called second band (*2nd band*) of the pyridine aromatic system.

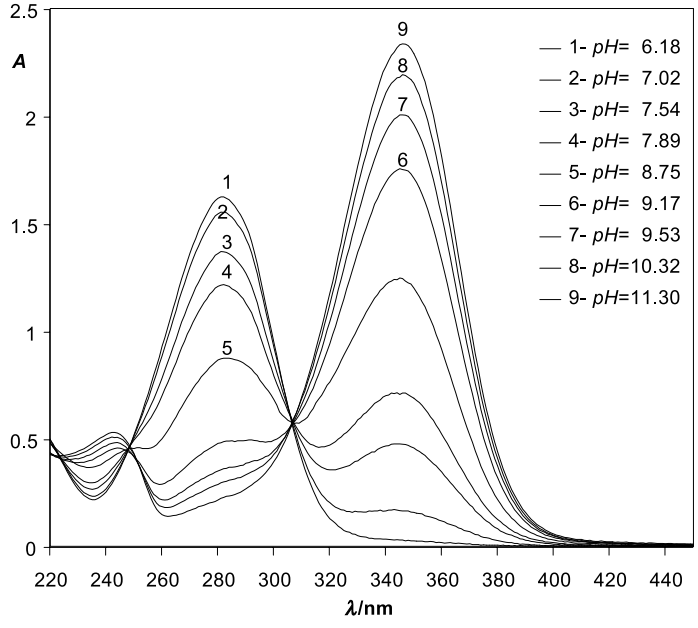
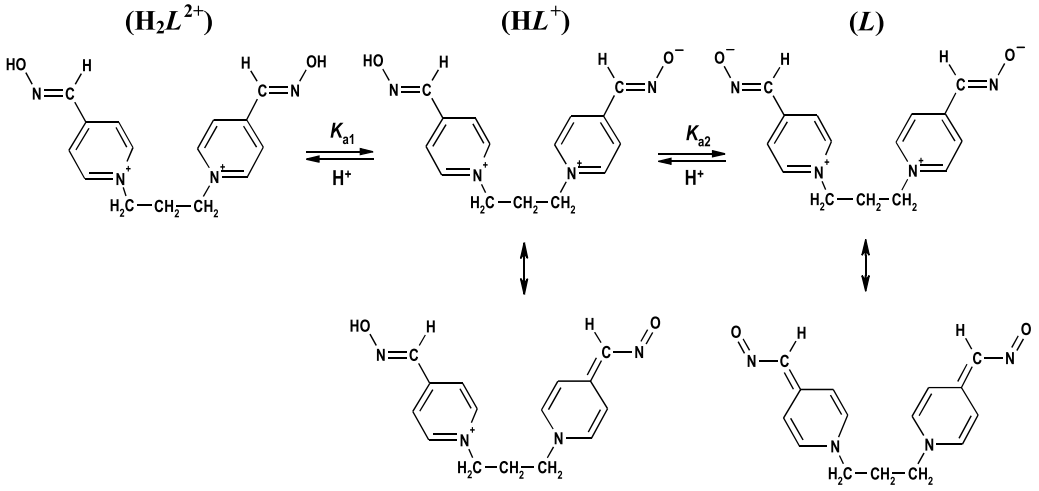


Fig. 1. Absorption spectra of *TMB-4* solutions ($c = 4 \cdot 10^{-5}$ mol dm^{-3}, $t = 25$°C) at different acidities



Scheme 1

The appearance of two sharply defined isobestic points refers to the acid-base equilibria (Scheme 1) or shortly to Eqs. (1) and (2), in which L stands for the neutral species.

$$H_2L^{2+} \rightleftharpoons H^+ + HL^+ \quad K_{a1} \tag{1}$$

$$HL^+ \rightleftharpoons H^+ + L \quad K_{a2} \tag{2}$$

The UV/VIS absorption spectra and the later derived absorbance versus acidity curves (Fig. 2) clearly show that the quoted acid-base equilibria and thus the corresponding dissociation constants of the two oxime groups overlap. Such effects are observed when the pK values of compounds with two ionizing groups are separated by only $3\,pK$ units. The situation is complex because not only the dissociation constants but also the absorbance of the monoprotonated species are unknown and cannot be determined independently. To overcome these difficulties two numerical methods for calculation were used.

The observed absorbance at an analytical wavelength for a given pH value, where all three species are present is given in Eq. (3) where A_0, A_1, and A_2 are the absorbances of the pure diprotonated, monoprotonated, and nonprotonated species respectively, while x_0, x_1, and x_2 represent their mole fractions in the solution.

$$A = A_0 \cdot x_0 + A_1 \cdot x_1 + A_2 \cdot x_2 \tag{3}$$

Since the total concentration of the ligand in solution is given in Eq. (4) the mole fractions of the diprotonated, monoprotonated, and nonprotonated species are defined by Eqs. (5)–(7).

$$c = [H_2L^{2+}] + [HL^+] + [L] \tag{4}$$

$$x_0 = \frac{[H_2L^{2+}]}{c} = \frac{[H^+]^2}{[H^+]^2 + K_{a1} \cdot [H^+] + K_{a1} \cdot K_{a2}} \tag{5}$$

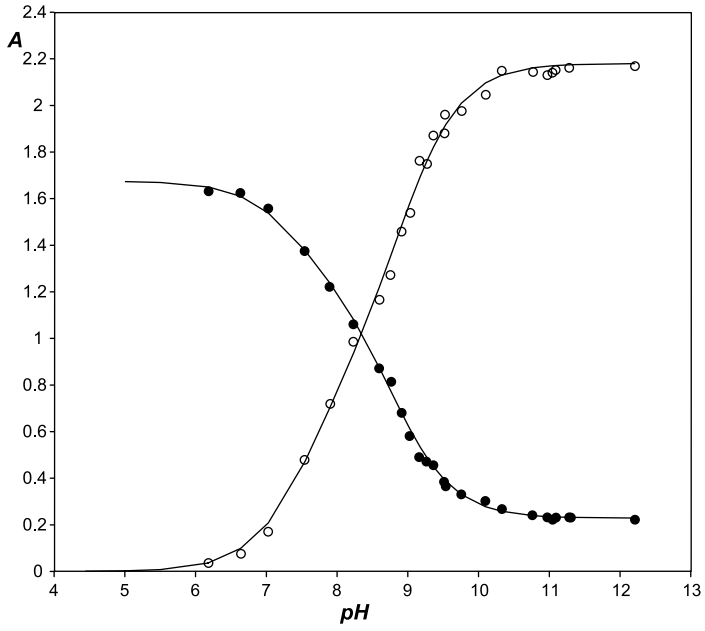


Fig. 2. Absorbance dependence of *TMB-4* solutions ($c = 4 \cdot 10^{-5}$ mol dm^{-3}) on *pH*; exp. data ○ $\lambda_m = 345$ nm and ● $\lambda_m = 282$ nm, solid line: non-linear regression

$$x_1 = \frac{[HL^+]}{c} = \frac{K_{a1} \cdot [H^+]}{[H^+]^2 + K_{a1} \cdot [H^+] + K_{a1} \cdot K_{a2}} \tag{6}$$

$$x_2 = \frac{[L]}{c} = \frac{K_{a1} \cdot K_{a2}}{[H^+]^2 + K_{a1} \cdot [H^+] + K_{a1} \cdot K_{a2}} \tag{7}$$

$[H_2L^{2+}]$, $[HL^+]$, and $[L]$ were obtained by application of the mass action law to Eqs. (1) and (2). The substitution of these equations into Eq. (3) leads to Eq. (8).

$$A = \frac{A_0 \cdot [H^+]^2 + A_1 \cdot K_{a1} \cdot [H^+] + A_2 \cdot K_{a1} \cdot K_{a2}}{[H^+]^2 + K_{a1} \cdot [H^+] + K_{a1} \cdot K_{a2}} \tag{8}$$

A non-linear least-square fit of the absorbance versus *pH* data by Eq. (8) led to the curves presented in Fig. 2 and the two dissociation constants of *TMB-4*. The calculations were performed at $\lambda_m = \lambda$(maximum) of the nonprotonated and protonated ionic forms and resulted in $pK'_{a1} = 7.56$ and 7.43 and $pK'_{a2} = 8.93$ and 8.81, respectively.

Another numerical method, supported by a computer program, which includes activity corrections ($t = 25°C$) and thus the evaluation of thermodynamic values of K_{a1} and K_{a2}, was used according to *Albert* and *Serjant* [10]. The calculations begin with successive approximations. Initially, the two particular dissociations are treated as if they were independent. Thus, the experimental data are divided into two segments; those which cover the *pH* range where the first dissociation step occurs (I) and those which concern with the second dissociation step (II). The unknown

molar absorption of the monoprotonated species is estimated by solving the two Eqs. (9) and (10) derived from Eq. (8).

$$\left(1+\frac{K_{a2}}{[\mathrm{H}^+]}\right)\cdot(A_I - A_2\cdot x_2) = A_1 + \frac{[\mathrm{H}^+]}{K_{\mathrm{a1}}}\cdot(A_0 - A_I + A_2\cdot x_2) \tag{9}$$

$$\left(1+\frac{[\mathrm{H}^+]}{K_{a1}}\right)\cdot(A_{II} - A_0\cdot x_0) = A_1 + \frac{K_{\mathrm{a2}}}{[\mathrm{H}^+]}\cdot(A_2 - A_{II} + A_0\cdot x_0) \tag{10}$$

It is assumed that the terms $(1+K_{\mathrm{a2}}/[\mathrm{H}^+])$ and $(1+[\mathrm{H}^+]/K_{\mathrm{a1}})$ equal to unity while $A_2\cdot x_2$ and $A_0\cdot x_0$ equal to zero. The mean value of A_1 from the separate least squares solutions of Eqs. (9) and (10) is substituted in Eq. (8) which is then solved for K_{a1} and K_{a2} utilizing all the experimental data uniformly. These initial estimates of K_{a1} and K_{a2} are used to calculate the terms $(1+K_{\mathrm{a2}}/[\mathrm{H}^+])$ and $A_2\cdot x_2$ in Eq. (9) and $(1+[\mathrm{H}^+]/K_{\mathrm{a1}})$ and $A_0\cdot x_0$ in Eq. (10). The mean value of the newly calculated A_1 is then substituted in Eq. (8). This process is continued iteratively until there is no significant difference between the successive values of K_{a1} and K_{a2}. Corrections for the ionic strength of $0.05\,\mathrm{mol\,dm^{-3}}$ at $t=25^\circ\mathrm{C}$ were based on the general Eq. (11) in which I is the ionic strength while Z_{i} are the charges of the diprotonated, monoprotonated, and nonprotonated species of the ligand. pK_{a} and pK'_{a} are the thermodynamic and the nonthermodynamic (uncorrected) pK_{a}s, respectively.

$$pK_a = pK'_a \pm \frac{0.512\cdot A(Z_i^2)\cdot\sqrt{I}}{1+1.5\cdot\sqrt{I}} \tag{11}$$

Since the charge of the ionic species of *TMB-4* is rather disperse, in spite of its net charge, the ligand was treated as an ampholyte. Such a treatment showed the best agreement between the results obtained by those two numerical methods (Table 1).

The concentration profile shown in Fig. 3 was constructed using the mean values of the thermodynamic dissociation constants obtained for both characteristic absorption maxima.

The dissociation constants of the two oxime groups of the structurally similar *Toxogonin* determined at $t=25^\circ\mathrm{C}$ by potentiometric titration [5, 6] are $pK'_{\mathrm{a1}}=7.59$,

Table 1. Dissociation constants of *TMB-4* and spectral characteristics of its ionic forms in solution ($c=4\cdot10^{-5}\,\mathrm{mol\,dm^{-3}}$, $t=25^\circ\mathrm{C}$)

λ/nm		pK_{a1}	pK_{a2}	$\varepsilon(\mathrm{H_2}L^{2+})/\mathrm{dm^3\,mol^{-1}\,cm^{-1}}$	$\varepsilon(\mathrm{H}L^{+})/\mathrm{dm^3\,mol^{-1}\,cm^{-1}}$	$\varepsilon(L)/\mathrm{dm^3\,mol^{-1}\,cm^{-1}}$
345	A	7.474	9.016	0	22251	54387
	B	7.555	9.053	0	23061	53875
282	A	7.347	8.896	41757	30057	5600
	B	7.597	8.880	40625	29729	5625
Mean values:		7.49 ± 0.11	8.96 ± 0.09	0	22656	54131
				41191	29893	5612

A: values obtained by non-linear least square fit corrected by Eq. (11)

B: values obtained by numerical method according to Ref. [10]

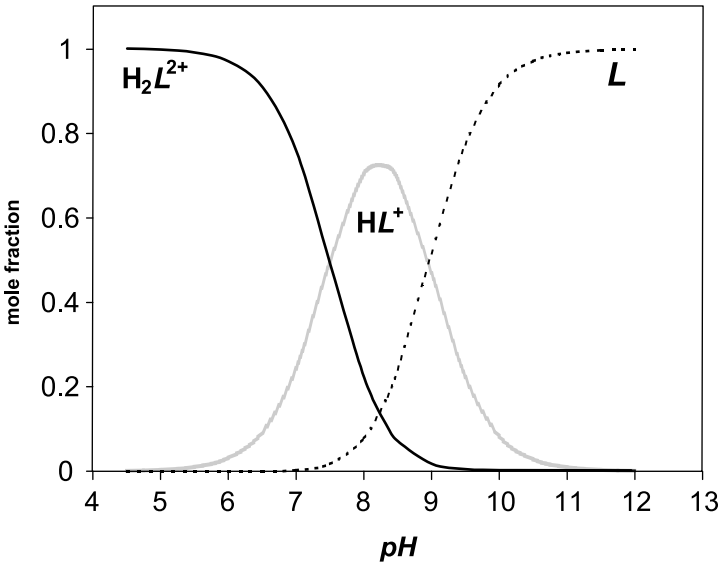


Fig. 3. *pH* Profile of *TMB-4* species in solution

$pK'_{a2} = 8.32$ at $I = 0.1\,\mathrm{mol\,dm^{-3}}$ and $pK'_{a1} = 7.61$, $pK'_{a2} = 8.25$ at $I = 1.0\,\mathrm{mol\,dm^{-3}}$. The simultaneous moderate increase of the pK'_{a1} value and decrease of the pK'_{a2} value with rising ionic strength, which is a characteristic of ampholytic molecules, suggests that *Toxogonin* has a similar charge distribution as *TMB-4*. The cited literature data extrapolated and corrected by Eq. (11) to $I = 0.05\,\mathrm{mol\,dm^{-3}}$ were $pK_{a1} = 7.50$ and $pK_{a2} = 8.42$.

The first dissociation constants of both dioximes are almost identical while the greater value of pK_{a2} obtained for *TMB-4* indicates additional effects of the first oxime group on the dissociation of the second one. It was shown earlier [11, 12] that the quotient of the dissociation constants of pyridinium dioximes increases with the decrease of their symmetry. The thus calculated K_{a1}/K_{a2} ratios amount to 29.5 and 8.3 for *TMB-4* and *Toxogonin*, respectively. This can refer to the lower symmetry of the *TMB-4* molecule. Dioximes like *TMB-4* and *Toxogonin* can assume the form of three different configuration isomers: the symmetric (*E*,*E*) and (*Z*,*Z*) and the nonsymmetric (*E*,*Z*). The symmetric (*E*,*E*) configuration was found to predominate in the solid state and in solutions of *Toxogonin* [13]. A different configuration of the oxime groups of *TMB-4* could influence the second dissociation step and increase its pK_{a2} value. On the other hand the lower pK_{a2} value of *Toxogonin* and thus the smaller K_{a1}/K_{a2} ratio is in accordance with the well known fact that *Toxogonin* is a more effective acetycholinesterase reactivator than *TMB-4*. More *Toxogonin* anions are expected to be available at physiological *pH* values to reactivate the inhibited enzyme.

Experimental

All the chemicals used were of analytical-reagent grade. 1,1′-Bis(pyridinium-4-aldoxime)trimethylene dibromide (*TMB-4*) was prepared and recrystallized by a known procedure [14]. It is a yellow-white powder (mp 220–222°C, dec.), soluble in water, and its aqueous solutions remain stable for days. The

characteristic absorptions of its IR spectrum are: $\nu(C{=}N) = 1640$, $\delta(OH)_{oxime} = 1300$, $\nu(NO) = 1015\,cm^{-1}$ as well as bands around $\bar{\nu} = 3430$ and $3125\,cm^{-1}$ due to $\nu(OH)$ stretching frequencies of the free and the associated oxime group. The constant ionic strength of $0.05\,mol\,dm^{-3}$ was maintained with sodium chloride. *Britton* and *Robinson* buffers were prepared by mixing $100\,cm^3$ of a mixture of phosphoric, boric and acetic acids (all $0.04\,mol\,dm^{-3}$) with appropriate volumes of $0.2\,mol\,dm^{-3}$ sodium hydroxide. Each solution of *TMB-4* contained a *Britton* and *Robinson* buffer and sodium chloride to a total ionic strength of $0.05\,mol\,dm^{-3}$. Redistilled water was used throughout.

Absorption measurements were performed against appropriate blanks on a UNICAM UV/VIS spectrophotometer UV4-100 at 25°C and with 1-cm silica-glass cells. A *pH*-meter with a saturated calomel-glass electrode system was used for *pH* measurements accurate to ± 0.05 *pH* units. The *pH*-meter was calibrated at 25°C by the two-point calibration method using commercially available Titrival® standard buffer solutions with an uncertainty of ± 0.05 *pH* units. The pK_a values of the two oxime groups were determined from spectrophotometric data.

References

[1] Chakravorty A (1974) Coord Chem Rev **13**: 1
[2] Burger N, Hankonyi V, Smerić Z (1989) Inorg Chim Acta **165**: 83
[3] van Helden HPM, Busker RW, Melchers BPC, Bruijnzeel PLB (1996) Arch Toxicol **70**: 779
[4] Burger N, Hankonyi V (1986) Polyhedron **5**: 663
[5] Christenson I (1968) Acta Pharm Suec **5**: 23
[6] Christenson I (1972) Acta Pharm Suec **9**: 309
[7] Burrows GE, Feitknecht UF, Miranda PM, Gibbon SL, Way JL (1972) J Chromatogr **66**: 156
[8] Bieger D, Wasserman OJ (1967) Pharm Pharmacol **19**: 844
[9] Stern ES, Timmons CJ (1970) Gillam and Stern's Introduction to Electronic Absorption Spectroscopy in Organic Chemistry. Edward Arnold, London, p 149
[10] Albert A, Serjeant EP (1971) The Determination of Ionization Constants. Chapman and Hall, London, p 44
[11] Schoene K, Starke EM (1971) Biochem Pharmacol **20**: 1041
[12] Gray AP (1984) Drug Metab Rev **15**: 557
[13] Spöhrer U, Eyer P (1995) J Chromatogr A **693**: 51
[14] Hagedorn I, Stark I, Schoene K, Schenkel H (1978) Arzneim-Forsch/Drug Res **28**: 2055