

Synthesis, Characterisation & Dissociation Constants of 1-(3-Aryl-5-hydroxy-4-isoxazolylazo)-4-sulphonic Acid & Its Derivatives

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1-(3-Aryl-5-hydroxy-4-isoxazolylazo)-4-sulphonic acid (PHI-4S) and its derivatives (methyl, methoxy and bromo) have been prepared and characterised on the basis of elemental analysis, IR and NMR spectroscopic studies. Dissociation constants of the ligands have been found to decrease with increase in temperature and ionic strength of the solution. Ion-size parameter (a_0) is evaluated from modified Debye-Huckel equation and found to be 5.10 and 4.90 Å in 30% aqueous methanol and aqueous media respectively. A linear relationship of pK_a versus $1/D$ and/or mol fraction (n_2) has been recorded while the plots of pK_a versus volume (%), weight (%) are non-linear. The order of dissociation constants is BrPHI-4S < PHI-4S < MePHI-4S < MeOPHI-4S. Isoequilibrium temperature (β) value is far below the experimental temperature (290-315 K) indicating the importance of entropy factors in controlling the dissociation equilibria.

A number of isoxazole derivatives have been found to be potential antibacterial, antitubercular, antifungal and/or antiviral reagents. Isoxazoles, having the arylazo group at position-4, have been reported to possess antidiabetic and fungicidal activity. The biological activity of such ligands, is known to be enhanced on complex formation with metals¹⁻⁶. The azo dye derivatives of sulphonic acids containing pyridine moiety have gained much prominence as analytical reagents in complexometric titrations⁷⁻¹⁰. A perusal of literature reveals that synthesis, characterisation and dissociation constant studies pertaining to 1-(3-aryl-5-hydroxy-4-isoxazolylazo)-4-sulphonic acids (PHI-4S) are scanty. This prompted us to synthesise a variety of PHI-4S compounds and study their dissociation constants by potentiometric and spectrophotometric methods.

Materials and Methods

All the chemicals used were BDH or E Merck reagents. Methanol was further purified according to literature procedures¹¹. The pH corrections for aquo-organic solvents were applied by the method of Van Uitert and Haas¹².

Synthesis of PHI-4S compounds

The ligands under investigation are azodye derivatives of isoxazolones. These were synthesized by diazotising sulphanilic acid^{4,13-15} and subsequently coupling the diazotised product with 3-aryl-5-isoxazolone. 3-aryl-5-isoxazolone exists in ketoenol equilibrium and acts as a phenol type compound readily undergoing electrophilic substitution reaction with diazonium ion at 4-position. 3-aryl-5-isoxazolone (0.05 mol) was dissolved in 20%KOH solution

and the solution added to a suspension of diazotised sulphanilic acid with vigorous stirring at 5°C. The mixture was allowed to stand for 10 min. A yellow coloured dye stuff separated as a crystalline paste. The product was filtered and repeatedly recrystallized from methanol to get an analytically pure sample.

Determination of dissociation constants

Potentiometric and spectrophotometric methods were used to evaluate the dissociation constants of ligands^{16,17}. The potentiometric method used was Calvin-Bjerrum technique as adopted by Irving and Rossotti¹⁷. The ligands were titrated potentiometrically against standard alkali in an inert (nitrogen) atmosphere. From the titration curves the average number of protons associated with the ligand (\bar{n}_H) was calculated. From the $\bar{n}_H - pH$ relationship, dissociation constants were evaluated at $\bar{n}_H = 0.5$. Concordant results were obtained by point-wise calculation and linear plots. These values were further confirmed by Albert's spectrophotometric method¹⁶ according to the relationship,

$$pK_a = pH + \log(d_1 - d)/(d - d_M)$$

where d_1 , d_M and d indicate the optical densities of ionised, molecular and observed optical density at analytical wavelength (405 nm) respectively.

These studies were carried out as a function of ionic strength (μ), temperature and dielectric constant (D). The pK_a values are reproducible within the limits of ± 0.05 units.

Results and Discussion

The PHI-4S compounds have been characterised

Table 1—Analytical Data of the Azo Dye Derivatives of Isoxazolones

Compound (Mol. formula)	Yield (%) (Colour)	Found (Calc.) %			PMR (DMSO- d_6) δ (PPM)
		C	H	N	
PHI-4S ($C_{15}H_{11}N_3O_5S$)	72 (Pale yellow)	51.41 (52.17)	3.82 (3.21)	12.48 (17.17)	12.19 (–OH, in heterocyclic ring); 8.00-6.80 (two sets of Ar-H in ring A); 8.04-7.88, 6.93, 6.33 (2Ar-H <i>ortho</i> to –SO ₃ Na, <i>d-ortho</i> to –N=N– in ring B).
MePHI-4S ($C_{16}H_{13}N_3O_5S$)	60 (yellow)	52.82 (53.48)	3.71 (3.65)	11.75 (11.69)	12.19, C–OH, in heterocyclic ring); 8.04-6.80, 3.30(s) (two sets of Ar-H, R-H in ring A), 8.04-7.88, 6.93, 6.33 (2Ar-H <i>ortho</i> to –SO ₃ Na, <i>d-ortho</i> to –N=N in ring B)
MeOPHI-4S ($C_{16}H_{13}N_3O_6S$)	65 (yellow)	50.96 (51.20)	3.42 (3.49)	11.44 (11.20)	12.19 (–OH in heterocyclic ring); 8.00-6.80, 3.08 (two sets of Ar-H, R-H in ring A), 7.28-7.18, 6.93, 6.33 (2Ar-H <i>ortho</i> to –SO ₃ Na, <i>d-ortho</i> to –N=N– in ring B).
BrPHI-4S ($C_{15}H_{10}N_3O_5SBr$)	54 (yellow)	41.68 (42.47)	2.32 (2.38)	10.08 (9.91)	12.19 (w, –OH, in heterocyclic ring); 6.97-6.88 (two sets of Ar-H in ring A); 8.04-7.88, 6.93, 6.33 (2Ar-H <i>ortho</i> to –SO ₃ Na, <i>d-ortho</i> to –N=N– in ring B).

by elemental analysis (Table 1), IR and NMR spectroscopic studies. Melting points of all the compounds have been determined in open capillaries and are uncorrected. These compounds have been observed to be high melting ($> 300^\circ\text{C}$). Purity of the compounds has been checked by TLC.

Infrared spectra of the compounds have been recorded on a "Shimadzu" spectrophotometer using KBr pellets. The IR spectra of PHI-4S show two peaks at 3400 and 1720 cm^{-1} assignable to νOH and $\nu\text{C}=\text{O}$ frequencies. The existence of $\nu\text{O}-\text{H}$ band at such low frequency and its broad nature can be attributed to the strong intramolecular hydrogen bonding^{18,19}. Observation of both $\nu\text{C}=\text{O}$ and νOH modes indicates that keto-enol tautomerism exists in these compounds. Similar observations have been made in other isoxazolone derivatives²⁰. The $\nu\text{N}=\text{N}$ vibration of azo compounds is IR-forbidden but $\nu_{\text{as}}\text{N}=\text{N}$ occurs near 1429 cm^{-1} . Isoxazolone shows the corresponding band at 1420 cm^{-1} which is weak due to non-polar nature of the bond.

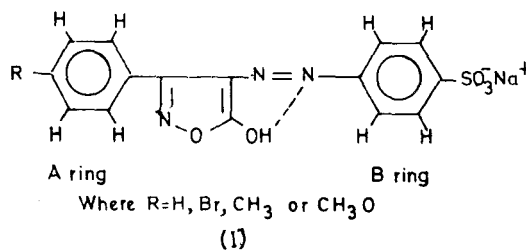
The four peaks observed around 1600, 1580, 1500 and 1450 cm^{-1} may be assigned to aromatic $\text{C}=\text{N}$ and $\text{C}=\text{C}$ stretching modes. The bands near 745 and 860 cm^{-1} may be associated with heterocyclic "ring breathing" modes.

NMR spectra of sodium salts of PHI-4S were recorded on FT NMR model-FX-90 Q using DMSO- d_6 as the solvent and TMS as the internal standard. The data are incorporated in Table 1. A doublet around 6.33 and 6.93 δ may be due to protons of the aromatic ring (B), situated at *ortho* positions to

azo group ($\text{N}=\text{N}$). The other two protons of ring B, which are *ortho* to sulphonic group (SO_3Na^+) appear downfield at 7.18, 7.28 δ (2H) due to the effect of increased electronegativity of sulphonic group. In the case of methyl derivative, the two protons, adjacent to sulphonic group, have been observed at 7.88 and 8.04 δ . Peaks observed around 6.8 to 8.0 δ may be assigned to aromatic protons of ring A.

A singlet peak appearing around 12.19 δ accounts for a proton corresponding to OH group, though it is generally expected at 10.0 δ . Such a downfield shift may probably indicate that the proton is involved in intramolecular hydrogen bonding. Similar absorptions have been found for other compounds except the bromo derivative. The spectrum of the bromo derivative shows weak signals which may be due to presence of two electronegative groups attached to the two phenyl rings (A and B).

In the ligand (PHI-4S), no aliphatic proton has been observed. However, in the case of methyl and methoxy ($-\text{OCH}_3$) derivatives a singlet was observed at 3.30 and 3.08 δ respectively. On the basis of foregoing discussion the structure of the compounds could be given as shown in (I).



Dissociation constants (K_a) of PHI-4S compounds

The ligands are monobasic acids. The ligand titration curve falls below the acid titration curve indicating the liberation of a proton. From the acid and ligand titration curves, \bar{n}_H values have been calculated which are in the range of 0.30 to 0.96 for PHI-4S, 0.20 to 0.97 for MePHI-4S 0.28 to 0.96 for MePHI-4S and 0.20 to 0.92 for BrPHI-4S. From the proton-ligand formation curves (\bar{n}_H versus pH), dissociation constants were evaluated at $\bar{n}_H = 0.5$ (Table 2). These were found to be in concordance with the values obtained from linear plots ($\log [\bar{n}_H/(1 - \bar{n}_H)]$) as a function of pH , pointwise calculations and Albert's spectrophotometric method¹⁶. The degree of formation for unprotonated (α_L) and protonated (α_{HL}) species of the ligand has been calculated¹⁷ and the two species are found to be present in equal amounts at a pH equal to pK_a .

Effect of ionic strength (μ) on pK_a

The pK_a values of PHI-4S ligands are found to decrease on increasing ionic strength (μ) of the system (Table 3). The data obtained are cast into the modified Debye-Huckel relationship²¹⁻²³,

$$pK_a = pK_a^0 - (\Delta\Delta Z^2 \mu^{1/2} / 1 + Ba_0 \mu^{1/2})$$

where Z is the valency of the species. For uni-univalent electrolytes, a_0 is assumed as 4 Å. The Debye-Huckel constants A and B have been calculated according to the expressions cited in literature²⁴. The values for A and B are found to be 0.625 and 3.44×10^7 respectively for 30% aqueous methanol. For aqueous system, the values of A and B are reported as 1.846 and 5.05×10^7 . The plots of pK_a versus $\mu^{1/2}/(1 + Ba_0\mu^{1/2})$ are in accordance with the above equation. But the slope values ($m = \Delta\Delta Z^2$) do

Table 2—Computation of Dissociation Constants by Various Methods

{[Acid] = 2.50×10^{-3} mol dm⁻³; [T_L] = 2.50×10^{-3} mol dm⁻³; $\mu = 0.05$ mol dm⁻³; Temp. = 293 K}

Ligand	Point-wise calculation	Half integral	Linear plots	Albert's spectrophotometric
(A) Aqueous system				
MeOPHI-4S	7.68	7.70	7.69	7.67
MePHI-4S	7.35	7.36	7.36	7.35
PHI-4S	7.08	7.08	7.08	7.06
BrPHI-4S	6.55	6.56	6.56	6.54
(B) 40% Aqueous methanol system				
MeOPHI-4S	8.16	8.19	8.18	8.16
MePHI-4S	7.85	7.87	7.86	7.85
PHI-4S	7.58	7.59	7.58	7.56
BrPHI-4S	7.16	7.16	7.16	7.15

Table 3—Calculated and Observed Data of Ionic Strength (μ) Effect on pK_a of MeOPHI-4S

{[T_L] = 2.50×10^{-3} mol dm⁻³; Temp. = 293 K}

Property	Aqueous methanol system		Aqueous system	
	Calc. ($a_0 = 5.10$ Å)	Obs. ($a_0 = 4.00$ Å)	Calc. ($a_0 = 4.90$ Å)	Obs. ($a_0 = 4.00$ Å)
Slope ($\Delta\Delta Z^2$)	0.650	0.590	1.850	1.620
Intercept (pK_a^0)	8.085	8.160	7.950	7.930
ΔZ^2	1.000	0.943	1.000	0.876

not seem to agree with the expected values. The expected ΔZ^2 values are +1.0 and -1.0 for monobasic acids and monoacidic bases respectively. The deviation in the observed $\Delta\Delta Z^2$ values from theoretical values may be attributed to the discrepancy in the value of theoretical a_0 . In order to gain the correct a_0 value, the plots of pK_a as a function of $\mu^{1/2}/(1 + Ba_0\mu^{1/2})$ have been tested by hit and trial method, using the value of a_0 in the range 3.0 to 6.0 Å. The effect has been found to be fruitful when $a_0 = 5.10$ Å and 4.90 Å in 30% aqueous methanol and aqueous system respectively for all the ligands studied (Table 3). Accordingly, the observed values of most accurate slopes ($\Delta\Delta Z^2$), a_0 values and intercepts have been compiled in Table 3 for MeOPHI-4S as a typical case.

Effect of solvent on the dissociation of PHI-4S compounds

The pK_a values are found to increase in solvents of low dielectric constant (D) (Table 2). The values of D for various proportions of aqueous methanol have been obtained from the data compiled by Akkerlof²⁵. Though the plots of pK_a versus volume (%) and weight (%) of methanol are nonlinear, it is interesting to note that the plots of pK_a against mol fraction (n_x) and $1/D$ are linear with regression coefficient (γ) 0.985. These are consistent with the relationship proposed by Irving and Rossotti²⁶, with the intercept equal to $pK_a(W)$ (dissociation constant in aqueous medium). Further, the plot of ΔpK_a versus $(1/D_s - 1/D_w)$ has been found to be linear according to Born's relationship.

$$\Delta pK_a = \frac{e^2}{2.303 r_0 kT} (1/D_s - 1/D_w)$$

where ΔpK_a = difference in the pK_a values in aqueous methanol and water, e = charge on the electron, k = Boltzmann constant, T = temperature, r_0 = mean radius of anion, D_s = dielectric constant of aqueous

Table 4—Dissociation Constants and Thermodynamic Parameters of Substituted PHI-4S Ligands (RPHI-4S)

{[T_L] = 2.50 × 10⁻³ mol dm⁻³; μ = 0.05 mol dm⁻³}

Compound	<i>pK_a</i>		-Δ <i>H</i>	-Δ <i>G</i>	Δ <i>S</i> JK ⁻¹ mol ⁻¹
	293 K	313 K			
(A) Aqueous medium					
MeOPHI-4S	7.69	7.27	36.9	43.1	-21.2
Me.PHI-4S	7.36	7.06	26.4	41.3	-50.9
PHI-4S	7.08	6.53	48.2	39.8	28.7
Br.PHI-4S	6.56	6.12	38.7	36.8	6.5
(B) 40% Aqueous methanol medium					
Me.OPHI-4S	8.18	7.68	43.9	45.9	-6.83
Me.PHI-4S	7.86	7.41	39.6	44.1	-15.4
PHI-4S	7.58	6.96	54.5	42.6	40.6
Br.PHI-4S	7.16	6.23	81.6	40.2	141.3

*Error limits for (i) Δ*H* & Δ*G* = ± 2.0 kJ mol⁻¹

(ii) Δ*S* = ± 5 JK⁻¹ mol⁻¹

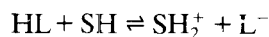
(iii) *pK_a* = ± 0.05 units.

methanol, *D_w* = dielectric constant of water. From the slopes of these plots, values of mean radius (*r*₀) of the ion has been calculated (7.67 Å for PHI-4S, MePHI-4S and MeOPHI-4S and 8.87 Å for BrPHI-4S respectively).

Effect of substituents and temperature on the dissociation of PHI-4S compounds

In the present investigation, the *pK_a* values of the ligands have been found to be in the order: MeOPHI-4S > MePHI > PHI-4S > BrPHI-4S at all temperatures and in all the dielectric media indicating that the dissociation is more favourable with the electron releasing substituents (Table 2). This also indicates the importance of high electron density in facilitating the dissociation of PHI-4S compounds.

The *pK_a* values are found to decrease with an increase in temperature. The thermodynamic parameters (Table 4) calculated for dissociation of PHI-4S ligands reveal that the entropy factor is either positive or negative. For electron releasing groups it has been found to be negative. However, Δ*H* is positive in all the cases. This trend indicates that the equilibrium is more endothermic in aqueous methanol medium. The negative entropy of methoxy and methyl substituted compounds can be explained in terms of the following equilibria



$$\Delta S = (\Delta S_{\text{SH}_2} + \Delta S_{\text{L}^-}) - (\Delta S_{\text{HL}} + \Delta S_{\text{SH}}) + \Delta S(\text{Sol-Sol-int})$$

$$\Delta S = (\Delta S_{\text{SH}_2} - \Delta S_{\text{SH}}) + (\Delta S_{\text{L}} - \Delta S_{\text{HL}}) + \Delta S(\text{Sol-Sol-int})$$

$$\Delta S = x + y + \Delta S(\text{Sol-Sol-int})$$

In the above equation the first term is negative since SH₂⁺ ion is highly solvated resulting in the decrease in entropy with respect to SH. Similarly, the second term is also negative leading to overall negative values. Δ*S* (Sol-Sol-int) will depend on the mol fraction of the organic component and its ability to stabilise or destabilise the structure of water. Similar observations were made earlier on the dissociation constant studies of acetic acid, 1-hydroxy-2-nitroso-4-sulphonic acid²⁸, 2-hydroxy-1-naphthoic acid and *o*-pyrocatechuic acid²⁹.

In order to know to nature of equilibrium³⁰, the thermodynamic data and dissociation constant data have been cast into Leffler's³¹ and Exner's plots³². A linear plot of Δ*H* as a function of Δ*S* (Leffler's plot) indicates the iso-equilibrium temperature (*β*) to be 260 K. By and large a similar value (253 K) has been obtained by the Linear plot of *pK_a* at 293 K versus *pK_a* at 313 K. Though the *β* values differ by 10 K from one method to the other, both the values fall far below the experimental temperature range (293–313 K) indicating that the equilibrium is controlled by entropy factors.

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