Acid-base Equilibria of Dipyridylethylenes
Studied by Absorption and Fluorescence Spectrometry

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In connection with photochemical and fluorimetric studies and with previous pK measurements on styrylpyridines, the acid-base equilibria of the *trans*-1,2-dipyridylethylenes have been studied by absorption and fluorescence spectrometry. The results obtained by the two methods are compared and the pK's of first and second protonation discussed in dependence with the nitrogen positional isomerism.

The measurements of the dissociation constant of the *trans*-3-styrylpyridinium ion is also reported as a particularly convenient application of fluorescence spectrometry.

Our interest in the acid-base equilibria of dipyridylethylenes (DPE's) is twofold. Firstly, it is possible to correlate the protonation state of these compounds with their photochemical behaviour at different pH's, as in the case of styrylpyridines (StP's) which have been extensively investigated in this laboratory (3,4). Secondly, their conjugated dications are dibasic acids and the acid-base equilibria of this class of acids have also been studied at different times in this laboratory (5).

Absorption spectrophotometry in the visible and/or ultraviolet regions is one of the more extensively applied and useful methods for the determination of the protonation equilibria of both monobasic and dibasic acids.

Few examples are found in the literature where emission spectrometry, namely fluorescence, was used for this purpose. Curves of fluorescence intensity versus pH allowed, in some favourable cases, the determination of the ionization constants of acids or bases in their first excited singlet states (6) when the rate of equilibrium attainment of the electronically excited molecule with the proton is comparable to or faster than its deactivation. Only a limited number of compounds studied so far satisfy this requirement; most of them fluoresce from a non protolytically equilibrated excited state (see below for 3-StP).

This is apparently the case with our compounds, which, based on preliminary experiments, seem to be characterized by very short pre-radiative lifetimes (7). The fluorescence intensity-pH curves show deflection points similar to those of the absorbance-pH curves; hence, they can be used for the measurement of protolytic equilibria of the

ground state molecules. However, the method is more delicate than the usual absorption spectrophotometry because of the implication of auto-absorbance and collisional quenching phenomena and of the necessity of calibration curves of fluorescence intensity versus concentration. This method could be used, however, when the spectral differences between the basic and acidic forms are greater in the fluorescence than in the absorption spectra.

This paper reports the results obtained from the fluorimetric and UV spectrophotometric determination of the ionization constants for *trans*-3-StP and the six isomeric *trans*-DPE's, comparing briefly the two methods employed and discussing the effect of the positional isomerism of the ring-nitrogen on the acid-base properties.

The DPE's examined were 1,2-di(2-pyridyl)ethylene (2,2 DPE); 1-(2-pyridyl), 2-(3-pyridyl)ethylene (2,3 DPE); 1-(2-pyridyl), 2-(4-pyridyl)ethylene (2,4-DPE); 1,2-di(3-pyridyl)ethylene (3,3 DPE); 1-(3-pyridyl), 2-(4-pyridyl) ethylene (3,4 DPE); and 1,2-di-(4-pyridyl)ethylene (4,4 DPE), all in their *trans* forms.

Results and Discussion.

3-Styrylpyridine.

Unlike the 2 and 4 isomers, *trans*-3-StP displays only slight modifications in the absorption spectrum due to protonation, making the pK determination more difficult (4,8). Since the difference between the fluorescence spectrum of 3-StP and its conjugated acid (Fig. 1a) is somewhat large, the ionization constant was measured by fluorimetry.
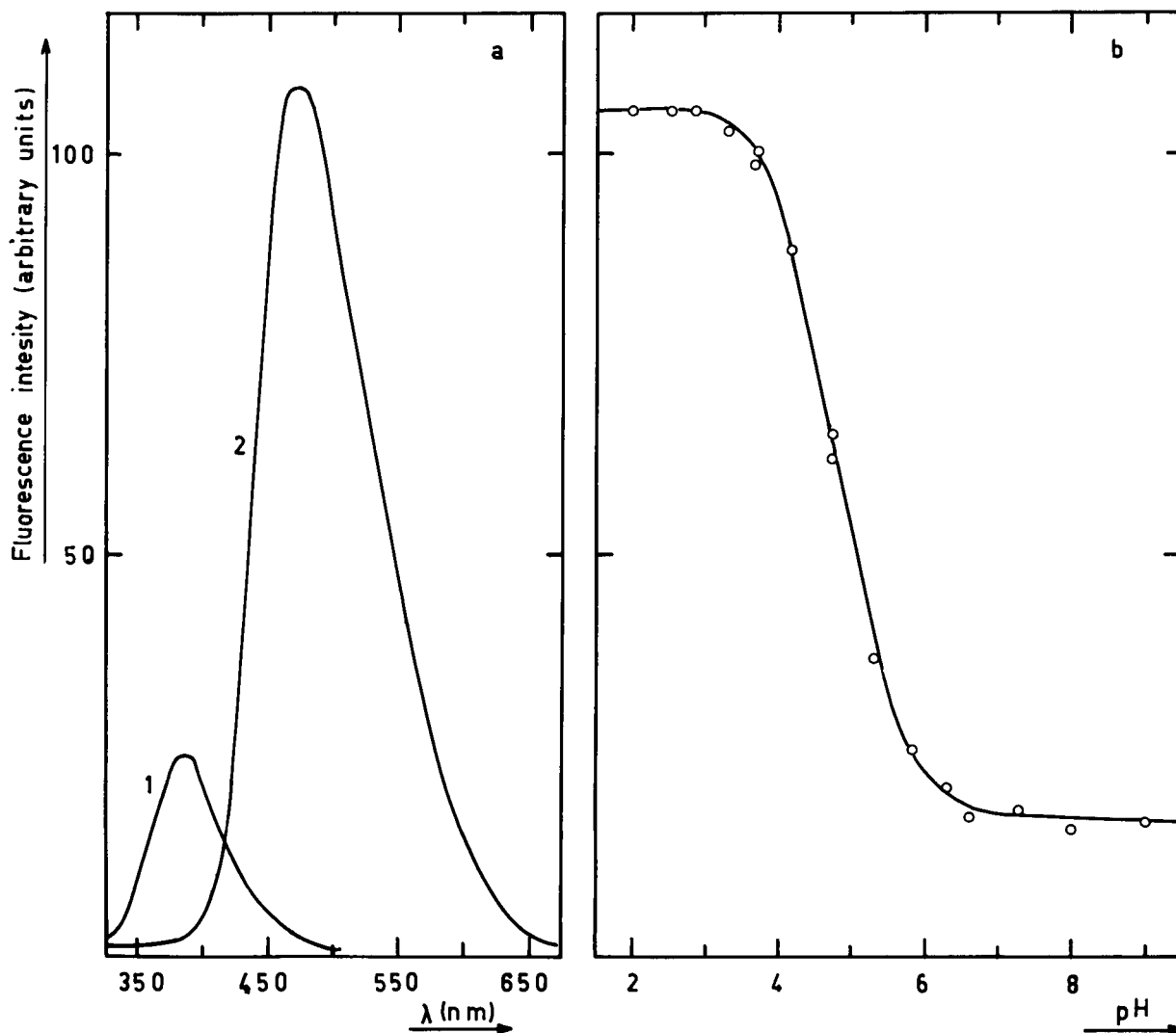
Figure 1. (a) Fluorescence spectra of trans-3-styrylpyridine (1) and 3-styrylpiridinium cation (2); (b) Fluorescence-pH curve for the determination of pK_a.

As a matter of fact, 3-StP exhibits emission characteristics of the protonated form (see Table I) up to $pH > 12$, even if $pK < 5$. The intensity ratio of the StPH$^+$ band at 470 nm in acidic ($pH \sim 2$) and basic solution (from pH 6 to ~ 11) is about 3:1, indicating a partial equilibrium of the excited molecule with the proton. This behaviour, which indicates a greater basicity of 3-StP in the first excited singlet state, is now under study and will be reported in detail in a separate paper.

Due to the large difference between pK_a in the ground and excited state, the measurement of the ground state pK_a by fluorescence should be reliable. A regular

$I_{fluor.}/pH$ curve was in fact obtained, as shown in Fig. 1b, from which $pK_a = 4.75 \pm 0.02$ was calculated, after correction for the ionic strength (K_a is the dissociation constant of the styrylpyridinium ion).

In this particularly favourable case, fluorimetry appears to be a more convenient method than absorption spectrophotometry, the value obtained being in good agreement with that previously reported (4.73 ± 0.07) (4) and rather improved in precision.

Dipyridylethylenes.

Table I shows the results obtained for DPE's, both by absorption and fluorescence measurements.

TABLE I

Ionization Constants of trans-1,2-Dipyridylethylenes at 25° by Absorption and Fluorescence Measurements. (K's are the dissociation constants of mono-protonated and diprotonated cations of DPE's).

DPE's	pK_1			pK_2		
	Fluor.	Abs.	Aver.	Fluor.	Abs.	Aver.
2,2	4.8	4.8	4.8	2.2_5	2.6	2.4
2,3	5.0_5	4.9_5	5.0	2.8_5	3.1	3.0
2,4	5.5_5	5.4_5	5.5	2.8_5	2.9_5	2.9
3,3	5.0		5.0	2.6_5		2.6_5
3,4	5.6	5.5_5	5.6	3.2	3.5	3.3_5
4,4		5.6	5.6	3.6	(3.7)	3.6_5

Singular behaviour by 4,4 DPE was observed. Both absorbance-pH and fluorescence intensity-pH curves display one inflection even though in different pH regions; out of these regions the spectra are apparently unaffected by change of pH. The question was whether two acid-base forms have the same absorption and fluorescence spectra or whether one of the protonation steps occurs at a pH not reached in the explored pH interval (0-10). More careful investigation revealed very slight modifications of the absorption spectrum (hypsochromic shift of the maximum and bathochromic shift of the isosbestic point, both of the order of the reproductibility error) around pH = 3.7, where the change of fluorescence occurs. The first explanation, based on the similarity of spectra, seems the more reliable one, by comparison with the acid-base behaviour of the other isomers and from other considerations, i.e. unlikelihood of intervention of protolytic equilibrium in the excited state in our measurements and comparison with the pK's of dipyridyls (9). Based on these considerations, it seemed reasonable that the addition of the first proton could be followed by absorption and the second protolytic equilibrium by fluorescence spectra (see tentative attribution in the table).

For the 3,3 DPE, the spectrophotometric determination of pK₁ and pK₂ was practically impossible owing to the close similarity of its absorption spectra at various pH's. Fluorescence spectra, however, are strongly affected by changes of pH and permitted the determination of both pK's with a good precision.

Comparison of the data in Table I for the other isomers shows good agreement between the absorption and fluorescence measurements. Obviously, the uncertainty (± 0.06, as a mean value) depends case by case on the differences displayed by the absorption and fluorescence spectra in the successive steps of protonation.

In a discussion of the data in Table I, the heteroatom and the protonated heteroatom can be considered as substituents and the effect of N or NH^+, respectively, on the acid-base properties of the other basic center can be compared.

The protolytic equilibria of diazaheterocycles with the nuclear nitrogen atoms in the same ring have been previously considered (10). The effects of the introduction of the second nitrogen and that of the nitrogen positional isomerism on the ionization constants have been found to be very strong. The present work evaluates the transmission of these effects between two rings linked by an ethylenic group.

The problem is which of the nitrogen atoms is to be the first protonated in the unsymmetric 2,3, 2,4 and 3,4 DPE's. In the case of dipyridyls, the pK's relative to the first and the second proton additions have been reported (9) but which ring-nitrogen is involved in the first protonation was not discussed. The behaviour of the DPE's should not be much different from dipyridyls in spite of the fact that slight additional mesomeric effects of the ethylenic bond are probably operative. A comparison between the two classes of compounds showed in fact that both first and second pK's display a very similar trend.

The insertion of a second nitrogen atom in the π-deficient N-heteroaromatics is base-weakening, the competition of the two basic centers towards the mobile electrons decreasing their charge excess. Naturally, less base-weakening occurs here, as the two heteroatoms are in different rings separated by the ethylenic bridge.

Since a ring-nitrogen atom has electronic effects (-I and -M) similar to those of a nitro-group, the first proton addition of DPE's should occur at slightly lower pH than for styrylpyridines (4,8). Table I shows that this is in fact the case, at least for the III-derivatives (for 4-StP, pK = 5.7 (4)). The order of pK for the DPE isomers is the same found for the stability constants of charge transfer complexes with iodine (11), indicating that the electronic effects dominate over steric effects in the formation of the complex.

The second proton addition occurs naturally at much lower pH since the basic strength had been weakened by the strong -I effect of the protonated NH^+ group as well as by the repulsive coulombic effect (12).
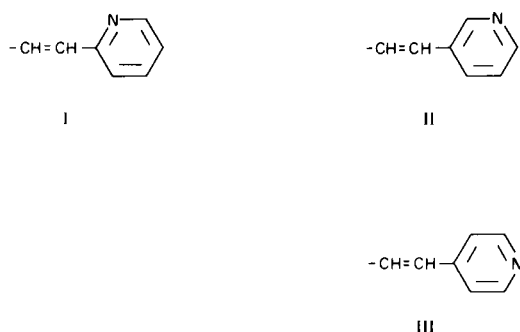
The assignment of the two macroscopic dissociation constants to the individual nitrogens is not without question, taking also into account that the strength of the three isomeric pyridine nuclei is of the same order of magnitude.

It should be noted, however, that the experimental values show a satisfactory precision (see the agreement between the two experimental methods); this, coupled with the comparison with the acid-base behaviour of the

StP's, would seem to make the following discussion reasonable.

Series 2,4- 3,4- 4,4.

The p-(C$_5$H$_4$N–CH=CH–) group, like the styryl group, can be expected to behave as an electron donor due to a combination of -I and +M effects with a prevalence of the mesomeric one. The pK$_1$'s are in fact higher than for pyridine since the cations are stabilized through quinonoid forms. The noticeable red shift of the spectrum supports the argument for this stabilization. Taking 5.06 the pK$_a$ of pyridine (4), and ρ = 5.71 (13), a σ_p = -0.07 was obtained, approximately constant for the three isomeric positions of the heteroatom:

-CH=CH— [structure I, pyridyl ring with N at top left]

-CH=CH— [structure II, pyridyl ring with N at top right]

I

II

-CH=CH— [structure III, pyridyl ring with N at bottom right]

III

This σ_p value is lower than that obtained for the styryl group (σ_p = -0.12 (4)) because of an enhanced -I effect.

A study of the two basic centers shows that the charge density (14) on the para nitrogen is higher for the 3,4 and a little smaller for the 2,4 isomer, but steric considerations led to the reasonable hypothesis that the first protonation occurs at the (4-pyridyl)-group for all three isomers. It is to be noted in this respect that while the net charge can be a good indication of the basicity of an ionizing group, no direct relationship exists between it and the K$_a$ of the heteroaromatics (12).

The second protonation of 1-(4-pyridinium), 2-pyridyl-ethylenes occurs at the three isomeric positions of the second ring in the order para (III) > meta (II) > ortho (I). Addition of the second proton to the resonance stabilized 2,4 and 4,4 cations should be more difficult than to the unconjugated meta nitrogen of the 3,4 DPE, but a combination of base-weakening effects leads to the experimental order. The 2,4 isomer has in fact the lower pK value owing to both inductive and steric effects.

Since in the second protonation a greater influence of the positional isomerism is operative, due to a stronger -I effect of the NH$^+$ group and to the reduced resonance stabilization in DPEH$_2^{++}$ (the spectrum is in fact shifted towards the blue with respect to DPEH$^+$), the pK increases with the distance of the basic center from the C$_5$H$_4$NH$^+$–CH=CH– substituent. This fact leads to a

small ΔpK between first and second protonation of 4,4 DPE, as in the case of dipyridyls (9).

Series 2,2- 2,3- 2,4.

With the o-(C$_5$H$_4$N–CH=CH–) pyridyls, it was found that the substituents I and II are base-weakening compared to III probably because of the predominant -I effect. The ortho nitrogen should be the second to be protonated in the 2,4 compound, while the situation is more uncertain in the 2,3 isomer as the reduced mesomeric effect from the II-group could increase the basic strength of the ortho nitrogen; this is also expected from charge density calculations (14). For the first protonation, the order of the basic strength is para (2,4) > ortho or meta (2,3) > ortho (2,2). The structural effect is here more noticeable than in the first protonation of the preceeding series as the pK is more sensitive to the nitrogen positional isomerism when the basic center changes its position in the ring in which the protonation occurs (see the second protonation of the precedent series). As to the second proton addition in this series, the pK changes very little, with a small loss of basic strength of the 2,2 isomer for steric reasons.

Series 2,3- 3,3- 3,4.

The first and third isomers have already been considered in the two preceeding series. The absence of +M effect in the 3,3 isomer would indicate a low pK$_1$, which was found to be the case experimentally. It follows from the reported assumptions and the charge density data (14), that the second proton addition should occur on the meta nitrogen for the three isomers. If this is the case, the experimental order of basicity, 3,4 > 2,3 > 3,3 would be expected since the 3,3 isomer displays smaller base-weakening effects, both steric and inductive, and the absence of base strengthening mesomeric effect.

EXPERIMENTAL

Materials.

3,3 DPE was prepared by a standard method (15). The other DPE's were Fluka commercial products purified by recrystallization before use. Trans-3-StP had been prepared in previous research by a classical route (15).

Fluorescence Measurements.

The spectra were recorded by a CGA DC 3000/1 spectrophotofluorimeter with an Osram XBO 150W high pressure xenon arc as exciting source. An alcoholic solution of anthracene was used for calibrating the instrument. More detailed information on the fluorimetric measurements of this class of compounds is reported elsewhere (16).

On irradiation by the exciting source the fluorescence spectra underwent fast modifications due to trans → cis photoisomerization and prevalent photodegradation. Because of the low photochemical stability of DPE solutions, the automatic recording of the spectra was impossible in most cases and a manual procedure was necessary, renewing the samples at every reading. Absence of

emission from the solvent was checked in preliminary experiments.

Because of its noticeable quenching effect on the fluorescence, the ionic strength was kept constant in the selected pH range by potassium chloride additions (μ = 0.1 was chosen to allow a comparison between the buffered solutions and the acidic ones). Only in this way did the $I_{fluor.}/pH$ curves reach a regular trend.

In every set of pK measurements the exciting wavelength chosen was that near to the isosbestic points between the acidic and basic forms. The precision of the fluorescence readings was not too high, depending mainly on the photostability of the solutions; for the less weak intensities, the reproducibility was within 2-3%.

Absorption Measurements.

An Optica CF4 DR double beam and an Optica CF4 single beam spectrophotometer were used. The pK's were obtained by absorption measurements made under the same conditions described for fluorescence.

pK Measurements.

The ionization constants were determined at room temperature ($\sim 25°$) in a series of Britton buffer solutions (from pH 8 to pH 2) and in hydrochloric acid solutions for lower pH's; the aqueous solutions contained 10% ethanol by volume for solubility requirements (15% for 3-styrylpyridine, for comparison with absorption measurements previously reported) (4). Solute concentration for both absorption and fluorescence measurements was of the order 2.10^{-5} M, at which value the emission intensity is proportional to concentration. The alcohol content of the solutions was low enough to make it possible to use the pH-readings, obtained after calibration of the pH-meter by aqueous standard buffer solutions, without introducing any serious error (17). The pH-meter used was a Radiometer PHM 22r type with a glass electrode.

The overlapping ionization constants of the dibasic acids were calculated from the experimental absorbance-pH curves using the method previously described (5a, 18). The shape of the curves at the analytical wavelengths exhibited a minimum or a maximum depending upon whether the intermediate species (DPEH$^+$) displayed lower or higher extinction coefficients. This same method was applied to the fluorescence intensity versus pH curves, which also gave the requisite maximum or minimum.

Thermodynamic values in the table were derived from experi-

mental data corrected for the ionic strength, using the approximate Debye-Hückel formula for the activity coefficients, log γ_i = $-0.57z_i^2$ $\sqrt{\mu}/(1 + \sqrt{\mu})$, which accounts for the ethanol percentage.

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