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Poly-9-vinylacridine. Preparation and Some Spectral Properties

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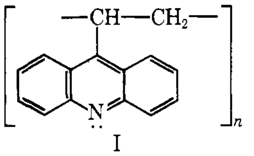
ABSTRACT: Poly-9-vinylacridine (9-PVA) was prepared by free-radical initiated polymerization of 9-vinylacridine. Using azobisisobutyronitrile as initiator a fairly high polymer ($n > 25$) was obtained, whereas benzoyl peroxide gave only low molecular weight polymer ($n \sim 5$). The spectrophotometric titration, absorption spectra, fluorescence properties and charge-transfer interactions were studied in comparison with 9-ethylacridine. The apparent pK_a of the polymer and 9-ethylacridine in 60% dioxane are 2.88 and 4.27, respectively. The absorption spectra of 9-PVA in its two ionization states showed considerable hypochromism and loss of vibrational structure. The fluorescence spectrum of the unprotonated polymer in various anhydrous solvents revealed only an excimer emission band (maximum at 520 mμ) with a relatively high quantum yield of $Q = 0.085 \pm 0.003$. In methanol, however, monomer-type fluorescence was also observed. Aqueous acid solutions of 9-PVA showed only very weak ($Q < 0.01$) monomer-type emission. The two ionization states of the polymer showed less tendency to form charge-transfer complexes than the monomer. On the basis of these observations and structural considerations it is suggested that 9-PVA has a rigid syndiotactic structure in which next nearest neighbors are stacked face to face 3.5 Å apart.

The only reports of the preparation of poly-9-vinylacridine (9-PVA) appear to be as a result of accidental polymerization of salts of 9-vinylacridine in aqueous[2a] or acetone solutions.[2b] Apart from being described as a glassy yellow solid its properties have not been recorded.



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9-PVA (I) is a potentially interesting polymer because the bulkiness of the acridine side chains probably severely limits the number of possible conformations, thus providing an excellent system for spectroscopic study of interacting chromophores. The absorption spectra should reflect the ground-state geometry of the acridine, whilst emission spectra should be sensitive to excited state conformations, notably excimer formation which has been observed in polystyrene[3-5] and polyvinylnaphthalene.[4]

The polymer can also be studied as a polycation, and the possibility exists of an intramolecular charge-transfer complex between adjacent protonated and unprotonated acridine residues, as has been observed in acridine–acridinium mixtures.[6] The properties of the array of acridine residues on the polymer may be of help in interpreting the interaction of acridines with polyelectrolytes, especially deoxyribonucleic acid.

Experimental Section

Materials. 9-Vinylacridine. Several routes to 9-vinylacridine appear in the literature.[2,7] The most convenient was found to be that of Fischer[2a] in which 9-methylacridine, prepared from acetic acid and diphenylamine, was converted by a Mannich reaction into 9-(2-dimethylaminoethyl)acridine, which after methylation and a Hofmann degradation yielded 9-vinylacridine. Purification was effected by elution from an alumina column with 1:1 benzene–petroleum ether (bp 30–60°) giving pale yellow crystals on evaporation of the solvent, mp 89° as reported.[2,7]

Poly-9-vinylacridine. Cationic, anionic and free radical initiators were tried. Boiling 9-vinylacridine with dilute hydrochloric acid did not yield any polymeric materials. Neither potassium naphthalene nor butyllithium produced any polymer from solutions of 9-vinylacridine in benzene or in the melt.

Benzoyl Peroxide Initiation. A solution of 9-vinylacridine (500 mg) and benzoyl peroxide (5 mg) in dry benzene (10 ml) with a trace of cobaltous acetate was refluxed under nitrogen for 3 days with occasional addition of more benzene to maintain the volume. The benzene was evaporated and the residue precipitated from chloroform with *n*-heptane, yield 350 mg, mp 200–220° with darkening. This proved to be low molecular weight material of $n \sim 5$ (see below).

Azobisisobutyronitrile (AIBN) Initiation. 9-Vinylacridine (760 mg) and AIBN (0.01 equiv) were polymerized in the melt *in vacuo* for 7 days at 94°. After a few days the melt solidified. Analysis on a Sephadex column (see below) showed that the product contained 20% monomer, the remainder being polymer of $n > 25$. The crude polymer

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(1) School of Chemical Sciences, University of East Anglia, Norwich, England.

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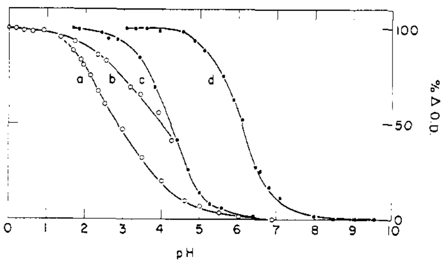

Figure 1. Spectrophotometric titration curves at 22° of (a) 9-PVA (9.10^{-5} mol residues/l.) in 60% dioxane, ionic strength 0.01 at 420 mμ; (b) 9-PVA (9.10^{-5} mol residues/l.) in 50% dioxane, ionic strength 1.0 at 420 mμ; (c) 9-ethylacridine (5×10^{-5} mol/l.) in 60% dioxane at 354 mμ; (d) 9-ethylacridine (5×10^{-5} mol/l.) in water at 353 mμ.

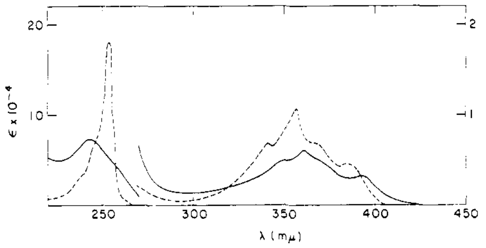

Figure 2. Absorption spectra of uncharged 9-PVA (—) and 9-ethylacridine (- - - -) in 60% dioxane, pH 9 (NaOH).

could be freed from monomer by several precipitations from chloroform with *n*-heptane and was then obtained as a yellow powder, mp 270–290° with darkening. 9-PVA is very soluble in chloroform, dioxane, and dimethylformamide, soluble in acetonitrile and dilute hydrochloric acid, sparingly soluble in alcohols, and insoluble in petroleum ether.

Molecular Weight Determination. The molecular weight of the polymer could not be estimated from sedimentation in the ultracentrifuge as the partial specific volume is not known. The most convenient method appeared to be gel filtration of an aqueous acid solution of the polymer. A 65-cm column of G-25 Sephadex (20 g) was eluted with 0.02 *M* hydrochloric acid. 9-Vinylacridine (elution volume 123 ml) and "blue" dextran (elution volume 42 ml) were used to calibrate the column. On chromatography of the AIBN-initiated polymer most of the material was eluted in a single sharp band with the void volume (42 ml) indicating a molecular weight higher than 5000. A second band (elution volume 123 ml) was the unreacted monomer and was estimated spectrophotometrically to contain 20% of the sample applied to the column. Most of the subsequent work was done on polymer purified in this way. The benzoyl peroxide initiated polymer gave a single broad band with an elution volume around 83 ml from which an average molecular weight of about 1000 was estimated.

9-Ethylacridine was prepared as reported in the literature[8] from diphenylamine and propionic acid and recrystallized twice from ethanol-water, mp 114° (lit.[8] mp 112–113°).

Solvents were of spectroscopic quality or purest available and were checked for the absence of fluorescent impurities.

Methods. Absorption spectra and optical density measurements were made on a Zeiss PMQII spectrophotometer. pH was measured on a Radiometer Model 22 pH meter. Before titrations in dioxane-water mixtures the electrodes were equilibrated in the solvent for some hours. All titrations were made from low to high pH by the addition of small quantities of 1–4 *N* sodium hydroxide solution in order to keep the ionic strength constant.

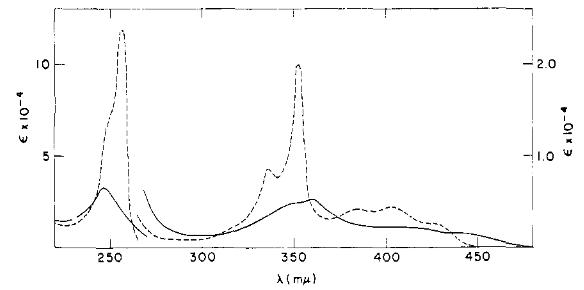

Figure 3. Absorption spectra of fully protonated 9-PVA (—) and 9-ethylacridine (- - -) in water 2×10^{-2} *M* in HCl.

Fluorescence spectra were measured on a Cary 15 spectrophotometer with Model 1512 fluorescent attachment employing front-face illumination. Fluorescence intensity measurements were made on an Aminco-Keirs spectrophotophosphorimeter by repeated comparison with a reference sample to eliminate fluctuations due to instrumental instabilities.

Quantum yields were calculated from the formula of Parker and Rees[9] by comparison with standards of known quantum yield

$$\frac{F_A}{F_B} = \frac{Q_A}{Q_B} \times \frac{OD_A}{OD_B} \qquad (1)$$

where F_A/F_B is the ratio of the areas under the fluorescence emission bands, Q_A/Q_B is the relative quantum yield and OD_A/OD_B is the ratio of optical densities at the exciting wavelengths. The standards employed were proflavin and fluorescein.

Results and Discussion

Spectrophotometric Titration. Spectrophotometric titration curves of 9-PVA and 9-ethylacridine are shown in Figure 1. Difficulty was found in finding a suitable solvent for 9-PVA. Precipitation of the free base occurred in 50% dioxane and of the protonated form in 60% dioxane. The titration curve is broad, typical of a polyelectrolyte,[10] yielding an apparent average pK_a of 2.88 in 60% dioxane ionic strength 0.01. Raising the ionic strength to 1.0 increases the basicity to an apparent average pK_a of 4.0. The titration curves of 9-ethylacridine in 60% dioxane and in water which are also shown in Figure 1 reveal the apparent pK_a in values of 4.27 and 6.10, respectively. On incorporation in the polymer the average basicity of the acridine residues decreased by about 1.4 pK units at low ionic strength, as expected for a polybase.[10]

Absorption Spectra. In Figure 2 the spectra of uncharged 9-ethylacridine and 9-PVA are compared. The long wavelength band of the polymer spectrum is red shifted by about 5 mμ and exhibits 20% hypochromism although the vibrational structure is still discernible. The large amount of hypochromism, more nearly comparable with the 30–40% of DNA[11] than that of polystyrene,[3,12] suggests that the acridine ring systems are stacked face to face in the polymer. In the short wavelength band the polymer spectrum is

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TABLE I

FLUORESCENCE CHARACTERISTICS OF 9-PVA COMPARED to 9-ETHYLACRIDINE

Compound	Solvent	Emission max, mμ	Quantum yield^{a-f} at 22°
9-PVA	10^{-2} *M* HCl	460	<0.01^{a}
9-Ethylacridine	10^{-2} *M* HCl	477	1.0^{a}
9-PVA	Dioxane	520	0.083,^{b} 0.088^{e}
	Dioxane	520	0.088,^{d} 0.086^{e}
	Acetronitrile	520	0.085^{b-e} ± 0.003
	Chloroform	520	0.085^{b-e} ± 0.01
	Methanol	520	0.04^{b-e} ± 0.01
9-PVA (low mol wt)	Dioxane	520	0.080^{b-d} ± 0.005
9-Ethylacridine	2.10^{-2} *M* NaOH	435	0.41^{f}

[a-f] Obtained by comparison with the following standards. [a] N-Methylacridinium chloride in water, $Q = 1.00$.[14] [b] Proflavine in acetate buffer, pH 4, $Q = 0.34$ [G. R. Hangen and W. H. Melhuish, *Trans. Faraday Soc.*, **60,** 386 (1964)] excited at 360 mμ. [c] As in footnote *b* but excited at 395 mμ. [d] Fluorescein in 0.1 *M* NaOH, $Q = 0.92$,[14] excited at 360 mμ. [e] As in footnote *d* but excited at 395 mμ. [f] Acridine in 2×10^{-2} *M* NaOH, $Q = 0.37$: E. J. Bowen, N. J. Holder, and G. B. Woodger, *J. Phys. Chem.*, **66,** 2491 (1962).

blue shifted and has a markedly lower extinction coefficient, but the integrated intensities of monomer and polymer are similar. Here it is difficult to assess contributions from absorption bands in the far-ultraviolet.

The absorption spectra of 9-ethylacridine and 9-PVA in acid are given in Figure 3. The two-band systems show 35% hypochromism, and the vibrational structure is almost lost. Parallel stacking of the protonated acridine residues appears unlikely. The sandwich dimers formed by 2,8-dimethylaminoacridine ("acridine orange") cations are antiparallel, with N lying approximately over C_9,[13] a conformation not possible in polyvinylacridinium ion. Some of the spectral changes may be due to perturbations in the electronic cloud of the chromophores by the high electrostatic field of the protonated polymer.

The polymer spectrum in 60% dioxane at an apparent pH equal to the average pK_a showed no anomalies in the long wavelength region where a charge-transfer band had been observed for acridine–acridinium interactions.[6] This suggests that the antiparallel face to face arrangement may also be required in the acridine–acridinium charge-transfer complex, although it is possible that the weak charge-transfer band is obscured by the red shifted polymer spectra or by hypochromism of the bound components.

Emission Properties. 9-Ethylacridine is highly fluorescent in acid solutions, the quantum yield of which is the same of that of N-methylacridinium ion ($Q = 1.0$).[14] Under the same conditions the polymer has less than 1% of the fluorescence of the monomer (see Table I). This is expected since the perturbations in the vibrational energy levels of the protonated polymer, as reflected by the lack of vibration structure in the absorption spectrum (Figure 2), may allow facile thermal deactivation of the excited state. In addition, self-quenching due to intramolecular collisions of the side chains may be very efficient.

The quantum yield of unprotonated acridine is strongly solvent dependent;[15] for acridine itself it varies between 0.37 in aqueous solution to zero in nonpolar solvents, *e.g.*, dioxane.[16] Qualitatively, the same behavior has been observed for 9-ethylacridine, which has a violet fluorescence (λ_{max} 435 mμ) in water or methanol but is nonfluorescent in dioxane. In contrast to this, 9-PVA exhibits yellow-green fluorescence (λ_{max} at 520 mμ) with the same intensity in dioxane, chloroform and acetonitrile. In methanol, however, an additional band appears in the region of the 9-ethylacridine fluorescence (see Figure 4). Deoxygenating the solutions by passage of nitrogen did not affect the intensity of any of these fluorescence bands.

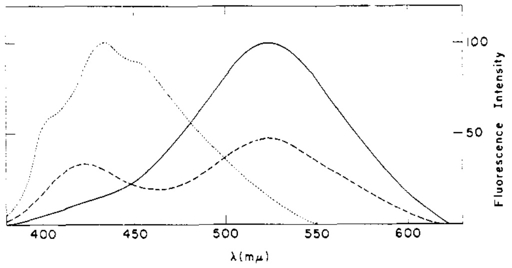

Figure 4. Fluorescence emission spectra of 9-PVA in dioxane (—) and methanol (- - -). For comparison the figure includes the emission spectrum of 9-ethylacridine in 10^{-2} *M* NaOH (· · · · ·) on a normalized scale.

The 520-mμ emission band of the polymer can be attributed to excimer emission. It is a broad structureless band, red shifted by about 5500 cm^{-1} from the 0–0 transition in the monomer band (at 405 mμ). These features are characteristic of an excimer band.[17] In addition, the excitation spectra of the polymer and monomer emission are practically identical, both having excitation maxima at approximately 360 mμ, coinciding with the maxima in the absorption spectra.

The quantum yield of the excimer fluorescence was determined by comparison with proflavin and fluorescein, which have emission maxima very close to that of the polymer (Table I). The value obtained is

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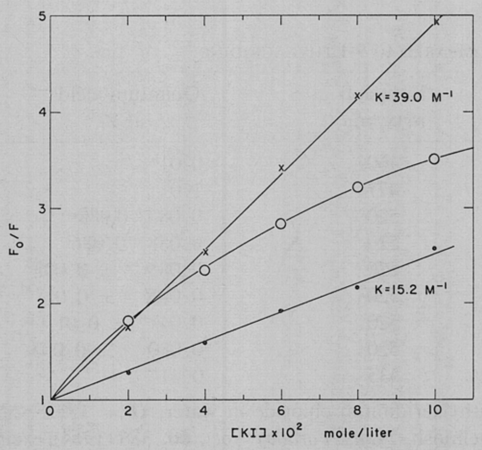

Figure 5. Quenching by potassium iodide of the fluorescence of 9-ethylacridine at 435 mμ (•—•), 9-PVA at 425 mμ (×—×) and at 520 mμ (○—○) in methanol, plotted according to the Stern–Volmer equation $F_0/F = 1 + K[Q]$. F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively, [Q] is the concentration of quencher and K is a constant.

much higher than that found for polystyrene (0.01–0.02). The low molecular weight polymer has almost the same fluorescent efficiency showing that near neighbor interactions must be responsible for the excimer formation rather than the approach of remote residues through chain coiling. The reduction of the quantum yield of the excimer band in methanol accompanied by the appearance of monomerlike fluorescence may be due to a partial disruption of the structure required for excimer formation or to a solvent dependent increase in the quantum yield of monomerlike fluorescence.

The emission of 9-PVA was found to be quenched by potassium iodide. The Stern–Volmer[18] plot of the quenching by potassium iodide in methanol is shown in Figure 5. It is somewhat difficult to explain the curvature in quenching of the excimer band. It may possibly arise because some sites are much more susceptible to quenching than others. The greater sensitivity of the monomer-type fluorescence to quenching suggests that it arises through emission from excited monomer units formed by dissociation of the excimer, rather than by direct emission.[19] We were unsuccessful in finding a suitable uncharged quencher for the fluorescence of 9-PVA. Maleic anhydride was found very effective but unreproducible, probably due to chemical reaction. Methyl iodide was found to be a rather weak quencher.

Interaction with Electron Acceptors and Electron Donors. The un-ionized form of acridine is a good π-electron donor similar to anthracene,[20] whereas its ionized form is a strong π-electron acceptor.[21] The polymer in both its protonated and unprotonated states was found to have much less tendency than 9-ethylacridine to form complexes, as judged by the appearance of a characteristic color in the presence of electron donors or acceptors. Some semiquantitative results with the electron acceptor 2,4,7-trinitro-9-fluorenone are given in Table II. The order of complexing ability is 9-ethylacridine ≫ 9-PVA (low molecular weight > 9-PVA (high molecular weight), suggesting that the terminal residues in the polymer are important complexing sites.

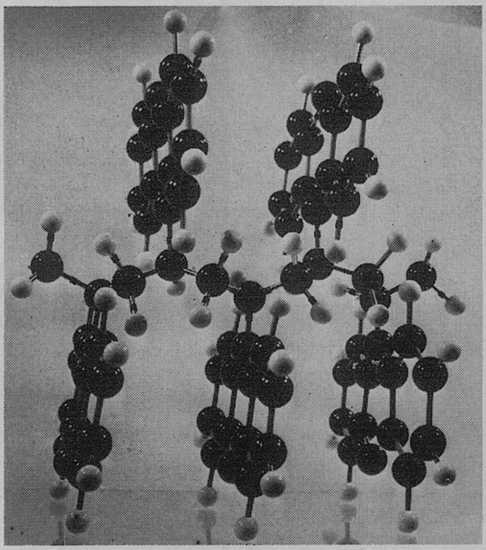

Figure 6. Suggested structure of 9-PVA.

Table II

The Intensity of the Charge-Transfer Band at 470 mμ in Dioxane Solutions of 0.1 M 2,4,7-Trinitro-9-fluorenone (TNF) in the Presence of 9-PVA or 9-Ethylacridine at a Concentration of 2.5 × 10^{-3} Mol Residues/l.

Compound	ΔOD^a
9-Ethylacridine	0.300
9-PVA (low mol wt)	0.100
9-PVA	0.079

[a] $\Delta OD = OD(\text{mixture}) - OD(\text{TNF})$.

Suggested Structure of 9-PVA. Molecular models of 9-PVA show that the acridine rings considerably restrict the number of sterically feasible conformations of the chain. A possible structure is shown in Figure 6. In this rigid syndiotactic structure the acridines are stacked with an interplanar distance between next nearest neighbors of about 3.5 Å. This conformation explains the hypochromism in the absorption spectrum and the poor π-donor properties, as only the terminal residues are readily accessible for complexing in a sandwich-type complex. This structure is also very favorable for excimer formation, which requires an interring separation in the case of benzene excimers of 3.73 Å (4,4′-paracyclophane).[4] Nearest-neighbor excimer formation, which is postulated for polystyrene,[3,19] requires a very crowded structure and is thought to be less likely. In the protonated polymer, columbic repulsions are presumably too great to allow a structure favorable for excimer formation.

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