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Absorption and Luminescence Spectroscopic Analysis of Tautomeric Forms of Protonated *N,N*-Dimethyl-*N'*-(1-nitro-9-acridinyl)-1,3-propanediamine (Nitracrine) and Its Nitro Isomers in Poly(vinyl alcohol) Films

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The electronic absorption, fluorescence, and phosphorescence excitation spectra, as well as the fluorescence and phosphorescence spectra, at either room or liquid nitrogen temperatures, were measured for *N,N*-dimethyl-*N'*-(1-nitro-9-acridinyl)-1,3-propanediamine and its three nitro isomers in acidified poly(vinyl alcohol) (PVA) film. The spectral characteristics obtained reveal the existence of the compounds studied in at least two structural forms. The results are interpreted in terms of the tautomeric phenomena which originate due to the migration of the hydrogen atom, which is bound to the nitrogen atom attached to the carbon atom (9), to the acridine ring nitrogen.

KEY WORDS: Nitracrine; absorption; fluorescence; phosphorescence; tautomers; PVA films.

INTRODUCTION

Numerous acridine derivatives exhibit a broad spectrum of selective biological activity which enables their application is chemotherapy (e.g., Refs. (1–3). Particular attention has, however, been devoted to those acridine derivatives the anticarcenogenic activity of which has already been reported [3–7]. Such activity is also exibited by *N,N*-dimethyl-*N'*-(1-nitro-9-acridinyl)-1,3-propanediamine, known as nitracrine (the WHO term) or, in Poland, as Ledakrin (e.g., Refs. 8–10 and references cited therein) (Scheme I). The admission of this compound for therapeutic applications has been followed by extensive studies the aim of which was to get an insight into the mechanism of its antineoplastic activity [9–20], as well as to reveal its physical and chemical features [8,21–24]. These investigations have also been undertaken to answer the questions why nitracrine, being one of numerous synthesized acridine derivatives, exhibits selective biological activity and which structural features may affect this activity. The present work is concerned primarily with the latter problem.

The crystallographic investigations [23,25–27], as well as our recent theoretical and spectral examinations [24,28], revealed the occurrence of prototropic tautomerism in nitracrine and its nitro isomers (the tautomeric forms of nitracrine are presented in Scheme II). This phenomenon, which plays an important role in chemistry and biology, is accompanied by the migration of the hydrogen atom bound to N(18) (amino form) to the acridine ring nitrogen, N(10) (imino form), or, in the case of 2-nitro and 4-nitro isomers, to the nitro-group oxygen (aci form) [23–28]. Recent MNDO [24] and complete AM1 [28] calculations regarding the geometry and energetics of the appropriate forms revealed that all four isomers could actually exist in amino and imino tautomeric forms, since aci tautomers of both C-264 and C-

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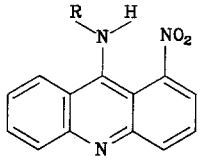
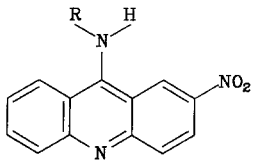
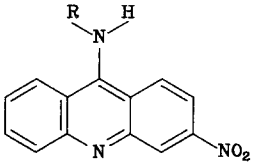
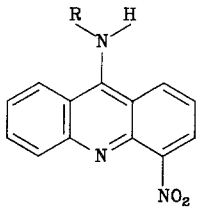

nitracrine
1–nitro isomer
(C–283)

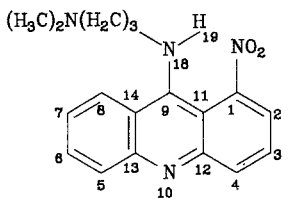

2–nitro isomer
(C–264)

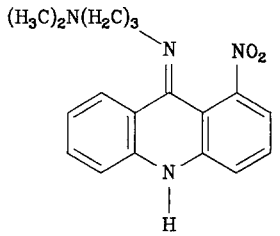

3–nitro isomer
(C–257)


4–nitro isomer
(C–265)

$R=(CH_2)_3N(CH_3)_2$

Scheme I. The compounds studied—nitracrine and its nitro isomers. Symbols for the compounds are given in parentheses.


amino form


imino form

Scheme II. Tautomeric forms of nitracrine.

265 were markedly less stable energetically. In solution, an equlibrium between the amino and the imino tautomers can be accomplished, this being roughly confirmed by the examination of theoretical and experimental electronic absorption spectra in various solvents [21,24,28]. The problem could not, however, be resolved definitely due to the complexity of the electronic transitions, the uncertainty in the theoretical predictions, and the fact that the absorption bands of both forms usually overlap. The luminecsence spectroscopy seems to be the promising method for further examination of the tautomeric phenomena in nitracrine and its nitro isomers. The usefulness of this technique in the studies of various features of acridine derivatives has already been widely evidenced [29–41]. The existence of the compounds in various structural forms should be reflected by the differences between the absorption and the excitation characteristics, as well as by the temperature-dependent changes in the fluorescence characteristics. This work has been devoted to the investigation of the absorption and luminescence spectra, some of them as a function of temperature, of protonated nitracrine and its nitro isomers in poly (vinyl alcohol) films. The study undertaken, except purely scientific aspects, may also shed some more light on the nature of the biological activity of the drug, the structure–activity relationships, transport phenomena, etc.

MATERIALS AND METHODS

Chemicals. *N,N*-dimethyl-*N'*-(1-nitro-9-acridinyl)-1,3-propanediamine and its isomers were synthesized as dihydrochlorides and purified by the method reported in the literature [42,43]. The compounds were kindly supplied by Prof. J. Konopa and his co-workers from the Technical University of Gdańsk. The free bases were obtained by the alkalization of aqueous solutions of original compounds with K_2CO_3 and extraction with benzene. The extracts were subsequently dried over anhydrous $MgSO_4$; filtered, and evaporated under reduced pressure. Some of them were additionally subjected to lyophilization. The purity of both hydrochlorides and free bases was checked by thin-layer chromatography (TLC) [44].

Poly(vinyl alcohol) was purchased from Aldrich. Methanol (from Mallinckrodt), of spectroscopic grade, was used as received.

PVA Film Preparation. Poly(vinyl alcohol) films containing nitracrine and its nitro isomers were prepared similarly as described by Tanizaki *et al.* [45]. According to this method, methanolic solutions of C-283, C-264, C-257, and C-265, acidified with hydrochloric acid, were introduced into a 12% aqueous solution of poly(vinyl alcohol). Subsequently, the mixture was allowed to dry, usually for about 2 days. The initial concentrations of the compounds studied were such as to obtain films with an optical density (OD) adequate for the UV-VIS absorption and emission measurements (OD of films never exceeded 0.3). On the other hand, the amount of HCl in the film was chosen so that similar absorption spectra of a chromophore were obtained in the film and in a strongly acidified methanolic medium (1 *M* HCl solution). Since the poly(vinyl alcohol) film itself creates a slightly acidic medium [46], the procedure described above ensured a certain degree of protonation of the compounds studied.

Apparatus. The absorption in the UV and visible region was measured using Specord M40 and VSU-2P (Carl Zeiss, Jena) spectrophotometers. The original absorption spectra of the compounds in PVA were corrected for the absorption of a neat film. Spectral measurements of the films were carried out only at wavelengths exceeding 340 nm due to the lack of transmittance of a neat PVA polymer at shorter wavelengths [46].

The fluorescence, phosphorescence, and luminescence excitation spectra were measured by means of an apparatus described elsewhere [47]. The excitation source was a 250-W xenon lamp. The required wavelengths were selected using a prismatic monochromator (Carl Zeiss, Jena). A similar monochromator was used for the observation of the luminescence characteristics. Two synchronized choppers powered with a generator working at a frequency of 80 Hz, placed in the excitation and detection beams, enabled the time separation of fluorescence and phosphorescence signals. Such experimental arrangement permitted also selective and phase-sensitive amplification of the photomultiplier signal.

Luminescence characteristics were measured at ambient and/or liquid nitrogen temperatures. For this purpose, a special optical cryostat was used. The emission parameters of the systems studied, as well as the experimental method applied, enabled fluorescence excitation spectra to be observed only in the region corresponding to the long-wavelength fragment of the absorption spectra. The emission spectra were corrected for the sensitivity of an apparatus by the use of quinine sulfate, anthracene, and DPO as standards [48,49]. The luminescence excitation spectra were corrected relative to the emission characteristic of the light source.

The measured absorption and emission spectra (with resolution better than 5 nm) were analyzed numerically with the approximation by splain functions [50]. Furthermore, all spectra were normalized to the maximal readings, for which a value equal to 1 was ascribed. The latter operation permitted easier comparison of the absorption and fluorescence excitation spectra. This approach enables more distinct demonstration of the differences in the shape of various spectra, as well as the shifts of the emission bands upon excitation at various wavelengths.

RESULTS

Form of the Compounds in the PVA Foil

At the beginning, the foils were prepared by introducing nitracrine and its nitro isomers, dissolved in methanol, into PVA. The absorption spectra of the films thus prepared were, however, different from those characterizing the compounds in ethanolic solutions [21,22]. This fact might suggest that the form of the compounds in the PVA polymer varies from that in the ethanolic liquid phase. This effect might be accounted for by the possibility of the protonation of nitracrine and its nitro isomers during the film formation. Such a possibility results from the strong basic character of the compounds studied, owing to the presence of three basic nitrogen atoms in their molecules, and the slightly acidic character of the PVA foil [46]. Next, the question arises whether the protic equilibria in PVA films are moved completely toward certain protonated forms of the molecules. The comparison of the electronic absorption spectra of nitracrine and its isomers in nonacidified PVA foils with those measured in various solvents [21,22,24] and in a 1 *M* solution of HCl in methanol (Fig. 1) does not confirm this. Therefore, all luminescence studies were carried out for acidified PVA foils. As revealed by the comparison of the absorption spectra in such a medium (Figs. 2, 4, 7, and 10) with those in acidified liquid phase (Fig. 1), we are dealing in both cases with the same protonated forms of the compounds studied.

Features of the Absorption Spectra

The long-wavelength absorption spectra of protonated nitracrine and its isomers in both PVA (Figs. 2, 4, 7, and 10) and methanolic solutions (Fig. 1) exhibit a rather complex nature. This, of course, may be due to the specific features of these derivatives but can also be interpreted as the effect of the existence of these mole-

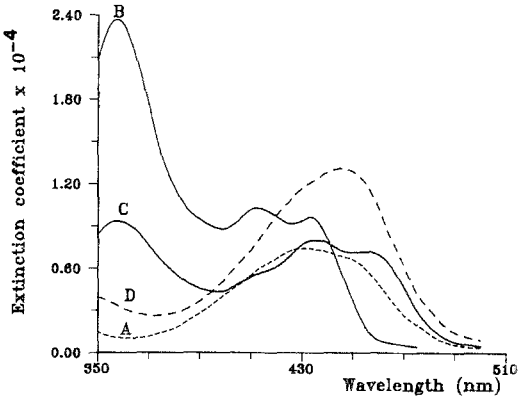


Fig. 1. Absorption spectra of C-283 (A), C-264 (B), C-257 (C), and C-265 (D) isomers in a 1 *M* methanolic solution of HCl. $l = 1$ cm; $c \simeq 5 \times 10^{-5}$ mol dm^{-3}.

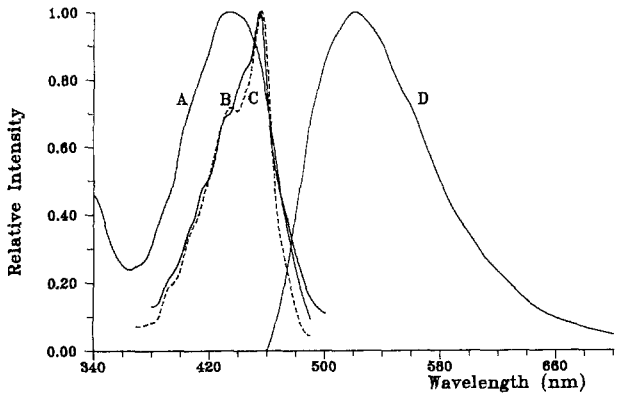


Fig. 2. Absorption spectrum (A), fluorescence excitation spectra, λ_{obs} = 530 nm (B, C), and fluorescence spectrum, λ_{ex} = 420 nm (D), of C-283 in acidified PVA film. (A, B, D) Measured at room temperature; (C) measured at liquid nitrogen temperature.

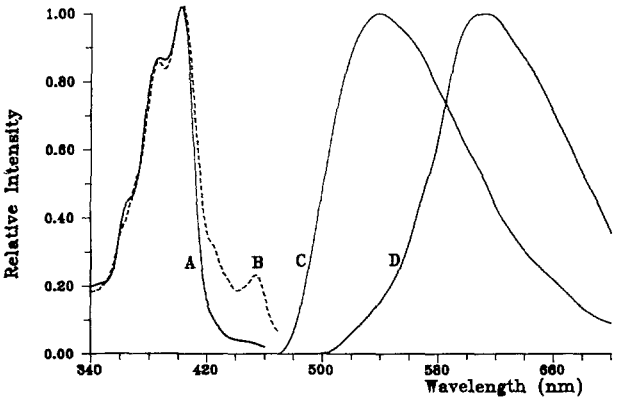


Fig. 3. Phosphorescence excitation spectra, λ_{obs} = 550 nm (A) and 630 nm (B), together with phosphorescence spectra, λ_{ex} = 400 nm (C) and 440 nm (D), of C-283 in acidified PVA. All measured at liquid nitrogen temperature.

cules in various structural forms. We discuss this subject in subsequent sections.

Features of the Emission Characteristics

Nitracrine (C-283). The fluorescence and phosphorescence excitation spectra together with fluorescence and phosphorescence spectra of the compound are shown in Figs. 2 and 3. The position and shape of the fluroescence emission of C-283 is not influenced by the excitation wavelength and temperature (identical spectra correspond to room and liquid nitrogen temperatures). Therefore, only one of the spectra is shown in Fig. 2D. The fluorescence excitation spectra, measured at the emission maximum (530 nm; Figs. 2B, C), vary markedly from the absorption characteristic (Fig. 2A). The position on the wavelength scale of both spectra is the same; the excitation spectra, however, exhibit two maxima, at 435 and 460 nm, whereas the absorption spectrum has only one maximum, at 435 nm. These features of the fluorescence excitation characteristics suggest the existence of at least two species in the PVA polymer. This is not revealed, however, either in absorption or in fluorescence spectra. The fact that only a single emission band is observed is particularly interesting. Such behavior of the system can be explained by the similarities in the emission characteristics of various forms, by significant differences in the efficiency of their emission, so that actually only one form emits radiation, and also by the possibility of fast rearrangement of the excited molecules toward only one form capable of emitting light. The amounts of two forms are slightly affected by temperature, this being evidenced by the comparison of the width and relative intensities of bands in the fluorescence excitation spectra measured at room and liquid nitrogen temperatures (Figs. 2B, C). The latter effects can be explained by taking into account the possibility of the temperature changes in the relative efficiencies of emission of different forms and the influence of temperature on the equilibrium state of the system.

The phosphorescence characteristics of protonated C-283 are shown in Fig. 3. Distinct differences in the excitation spectra, observed at 550 and 630 nm, as well as in the emission spectra measured at two excitation wavelengths corresponding to the maxima in the excitation spectrum, i.e., 400 and 440 nm, can be noticed. The phosphorescence, similarly to the fluorescence characteristics, can be explained assuming the existence of at least two forms of the compound in the PVA polymer. This is particularly pronounced in the excitation characteristics. Upon observation at 630 nm, a peak appears at 460 nm which is not revealed in the excitation spectrum corresponding to the observation at 550 nm.

New features of the system are revealed when comparing the fluorescence and phosphorescence characteristics. The strong band in the phosphorescence excitation spectrum (at 400 nm) is blue-shifted relative to the one in the fluorescence excitation spectrum. Only the position of the peak at 460 nm in the phosphorescence excitation spectrum (Fig. 3B) corresponds well to that of the strongest peak in the fluorescence excitation spectrum (Figs. 2B, C). It would mean that the excitation of phosphorescence of at least one form of the molecule, presumably responsible for the peak at 400 nm in the phosphorescence excitation spectrum, is accompanied by the substantial vibrational excitation of the molecule.

The maxima of both phosphorescence spectra are red-shifted relative to the maxima in the fluorescence spectrum. The difference of ca. 10 nm between the maximum in the fluorescence spectrum (Fig. 2D) and that in the short-wavelength phosphorescence spectrum (Fig. 3C) is too small for both emissions to be ascribed the same structural form of the compound. More probably, the long-wavelength phosphorescence (Fig. 3D) and fluorescence (Fig. 2D) spectra arise from one form responsible for peaks at 460 nm in the fluorescence and phosphorescence excitation spectra. Phosphorescence emitted in the high-energy region (Fig. 3C) seems to correspond to the form with short-wavelength peaks in the fluorescence and phosphorescence excitation spectra. The presence of the latter form, however, does not seem to be manifested in the fluorescence emission.

2-Nitro Isomer (C-264). The fluorescence excitation spectra of the protonated C-264 molecule measured, at room temperature, in the PVA polymer exhibit maxima at roughly the same wavelengths as the absorption spectrum, except for the fact that the relative intensities of the relevant bands are reversed (Fig. 4). The fluorescence excitation sprectra corresponding to the ambient temperature (Figs. 4B, C) show some differences in the shape and relative intensities of the bands at ca. 415 and 440 nm, when measured at two different observation wavelengths. At liquid nitrogen temperature, the fluorescence excitation spectra (Fig. 5) are quite similar to those measured at room temperature, although the peaks at 415 and 440 nm are somewhat better separated. The fluorescence spectra, at both room and liquid nitrogen temperatures, always exhibit, irrespective of the excitation wavelength, the same shape. Therefore, only the

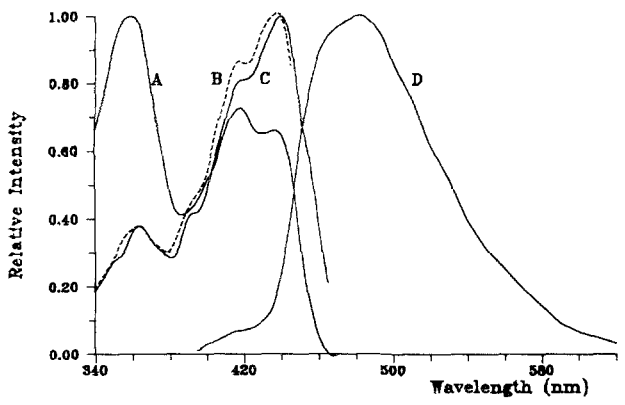


Fig. 4. Absorption spectrum (A), fluorescence excitation spectra, λ_{obs} = 470 nm (B) and 495 nm (C), and fluorescence spectrum, λ_{ex} = 360 nm (D), of C-264 in acidified PVA film. All measured at room temperature.

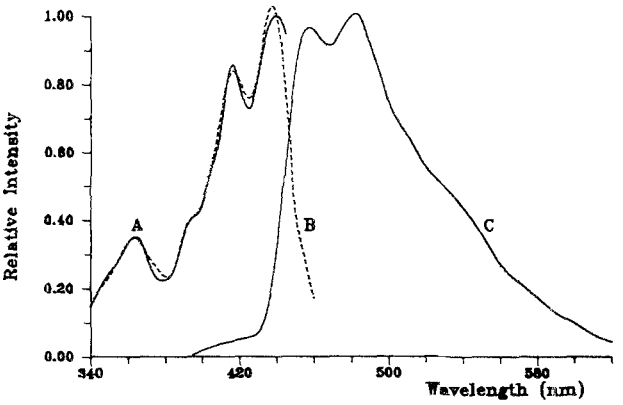


Fig. 5. Fluorescence excitation spectra, λ_{obs} = 460 nm (A) and 485 nm (B), together with fluorescence spectra, λ_{ex} = 380 nm (C), of C-264 in acidified PVA. All measured at liquid nitrogen temperature.

examples of these spectra are given in Figs. 4D and 5C. However, the room-temperature fluorescence spectrum appears as one broad band with a maximum at 485 nm, whereas the one measured at liquid nitrogen temperature exhibits two maxima, at 460 and 485 nm. Such an effect could be due to the substructure of the transition but may also be accounted for by the existence of two emitting states of molecules the relative amount of which is affected by temperature. The existence of two forms, at least in the ground state, seems to be indicated by the comparison of the fluorescence excitation and absorption spectra.

The phosphorescence spectrum corresponding to the excitation at 360 nm exhibits a weak band with a maximum at 500 nm followed by a system of bands, with the strongest one at 545 nm (Fig. 6C). The latter emission is also characteristic of the excitation at 430 nm (Fig. 6D). The phosphorescence excitation spectra for two observation wavelengths, i.e., 500 and 540 nm, differ markedly as regards their shape and positions of the peak maxima. Moreover, the spectrum corresponding to the short-wavelength observation (Fig. 6A) is blue-shifted relative to the spectrum characteristic of the long-wavelength observation (Fig. 6B). The above-presented and other features of phosphorescence emission further confirm the complex nature of the protonated C-264 molecule.

The comparison of the fluroescence (Figs. 4D and 5C) and phosphorescence (Fig. 6D) emissions of protonated C-264 suggests that the long-wavelength peaks arise most probably from the same form of the molecule. The phosporescence excitation spectrum corresponding to this form (Fig. 6B) is correlated well with the fluo-

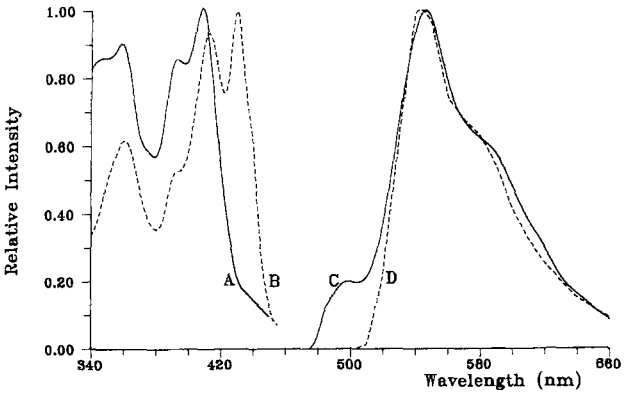


Fig. 6. Phosphorescence excitation spectra, λ_{obs} = 500 nm (A) and 540 nm (B), together with phosphorescence spectra, λ_{ex} = 360 nm (C) and 430 nm (D), of C-264 in acidified PVA. All measured at liquid nitrogen temperature.

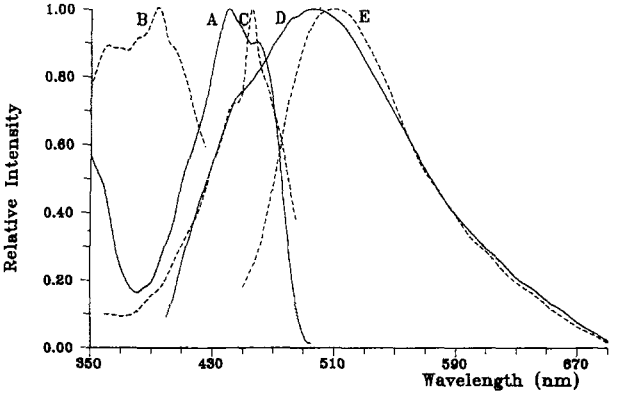


Fig. 7. Absorption spectrum (A), fluorescence excitation spectra λ_{obs} = 440 nm (B) and 520 nm (C), and fluorescence spectra, λ_{ex} = 380 nm (D) and 420 nm (E), of C-257 in acidified PVA film. All measured at room temperature.

rescence excitation spectra (Figs. 4B and C and 5A and B), which implies that both emissions require primarily the excitation to the same electronic singlet state. The second form, responsible for the short-wavelength spectral characteristics (Fig. 6), is revealed upon excitation in the higher-energy region. The fluorescence excitation spectrum of this form presumably overlaps with the spectrum characteristic of the other form. Its phosphorescence excitation spectrum (Fig. 6A) is, however, quite different from that attributed to the other form (Fig. 6B). Since that former spectrum is blue-shifted relative to the fluroescence spectra, it means that intersystem crossing is, in this case, accompanied by vibrational excitation.

3-Nitro Isomer (C-257). The long-wavelength fluorescence spectrum of C-257 measured at room temperature (Fig. 7E) exhibits a smooth shape characteristic of the emission from well-defined species. This shape of the spectrum is preserved at liquid nitrogen temperature, although the band is slightly blue-shifted (Fig. 8D). The fluorescence spectra resulting from the excitation at shorter wavelengths seem to be composed of two overlapping emission transitions (Figs. 7D and 8C). As compared to the spectra resulting from the long-wavelength excitation, they exhibit an additional emission band around 430 nm. The relative intensities of both bands, in the latter spectra, are somewhat affected by temperature. The change in the observation wavelength from 440 to 520 (or 540) nm leads to quite different excitation spectra (Figs. 7B and C and 8A and B). The short-wavelength fluroescence excitation spectra (Figs. 7B and 8A) do not show any correlation with the absorption spectrum (Fig. 7A), their shape and spectral range being influenced only slightly by temperature. On the other

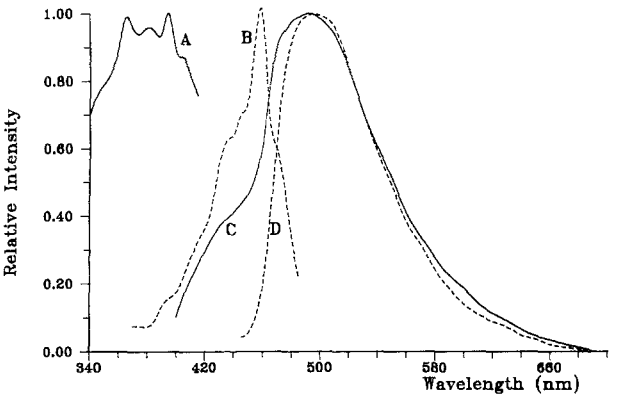


Fig. 8. Fluorescence excitation spectra, λ_{obs} = 440 nm (A) and 540 nm (B), together with fluorescence spectra, λ_{ex} = 380 nm (C) and 420 nm (D), of C-257 in acidified PVA film. All measured at liquid nitrogen temperature.

hand, the long-wavelength fluorescence excitation spectra (Figs. 7C and 8B) occur in the region of the long-wavelength absorption of the protonated C-257 molecule (Fig. 7A). Both the emission and the absorption spectra are band systems with two maxima. The relative intensities of peaks in the latter excitation spectra are, however, reversed in comparison with those in the absorption spectrum. The intensities of peaks in the long-wavelength fluorescence excitation spectra are also slightly affected by temperature. All facts presented above clearly demonstrate that, similarly as in the case of compounds discussed earlier, the protonated C-257 molecule exists in the PVA foil in at least two forms having varied spectral features.

The above conclusion resulting from the examination of the fluroescence spectral characteristics applies fully to phosphorescence. As demonstrated in Fig. 9, two non overlapping phosphorescence bands were obtained at two chosen excitation wavelengths (Figs. 9C, D). The phosphorescence excitation spectra measured at two various observation wavelengths occur in a similar wavelength region, although the shape of the spectra (Fig. 9A, B) indicates that these characteristics correspond to two different species.

If all long-wavelength emission characteristics, i.e., fluorescence and phosphorescence spectra, as well as fluorescence and phosphorescence excitation spectra, are ascribed to one form of the protonated C-257 molecule, then the short-wavelength spectra would arise from the other form of the compound. Such an alternation of spectra correlates well with a general knowledge of the emission phenomena. It is, however, interesting that only long-wavelength fluorescence excitation spectra occur in the region of a long-wavelength absorption, and short-wavelength spectra are not at all correlated with absorption. The explanation might be that the form responsible for the short-wavelength fluorescence is already created in the excited state of the molecule. Since the short-wavelength fluorescence excitation spectra fall down into the region where no absorption band is observed, the probable rearrangement of a molecule in the excited state requires substantial vibrational excitation. The short-wavelength phosphorescence excitation spectrum (Fig. 9A) and the relevant fluorescence spectrum (Fig. 8A) occur in roughly the same spectral region, which suggests that fluorescence and phosphorescence originate from the same excited state. The long-wavelength phosphorescence excitation spectrum (Fig. 9B) is somewhat blue-shifted relative to the appropriate fluorescence spectra (Figs. 7C and 8B). If in the latter case the same assignment as above is made, it would mean that the intersystem crossing is accompanied by the vibrational excitation of the electronically excited molecule.

4-Nitro Isomer (C-265). The long-wavelength fluorescence excitation spectrum of protonated C-265 (Fig. 10C) occurs essentially in the region of the long-wavelength absorption of the compound (Fig. 10A). However, the correlation between two spectra is rather poor. The maximum of the long-wavelength excitation spectrum is 20 nm red-shifted relative to the maximum of the absorption band. Nevertheless, it may be presumed that the emission in the long-wavelength region is accomplished from the excited state of the form already existing in the ground state. The smooth shape of the fluorescence spectra corresponding to the excitation in this region (Figs. 10E and 11D) suggests that the emission takes place from a single form of the compound, this also being indicated by the shape of the relevant phosphorescence spectrum (Fig. 12D). Further confirmation is provided by the thermal behavior of the system, namely, that neither fluorescence excitation (Figs. 10C and 11B) nor fluorescence (Figs. 10E and 11D) spectra are influenced by temperature.

The short-wavelength fluorescence excitation spectrum Figs. 10B and 11A) lies in the high-energy region, which is far beyond the range of the long-wavelength absorption band. Moreover, the shape of the spectrum indicates its complex origin, which is particularly pronounced upon changing the temperature. The possible reasons for such untypical behavior are discussed sub-

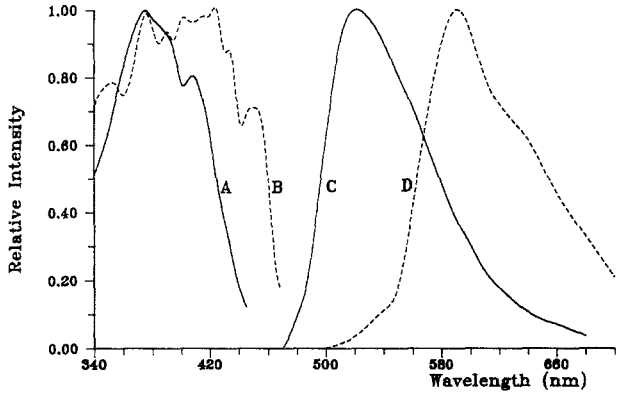


Fig. 9. Phosphorescence excitation spectra, λ_{obs} = 540 nm (A) and 600 nm (B), together with phosphorescence spectra, λ_{ex} = 380 nm (C) and 460 nm (D), of C-257 in acidified PVA. All measured at liquid nitrogen temperature.

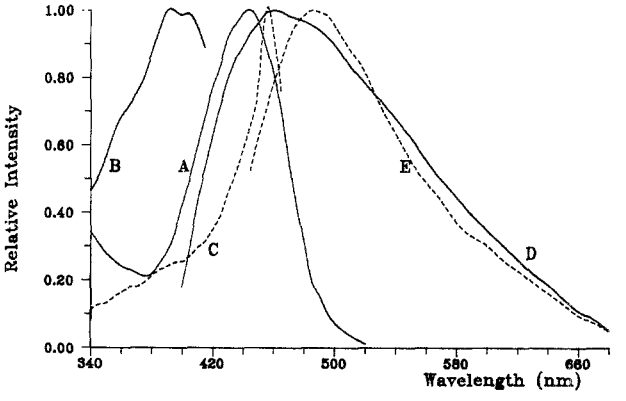


Fig. 10. Absorption spectrum (A), fluorescence excitation spectra, λ_{obs} = 450 nm (B) and 520 nm (C), and fluorescence spectra, λ_{ex} = 380 nm (D) and 420 nm (E), of C-265 in acidified PVA. All measured at room temperature.

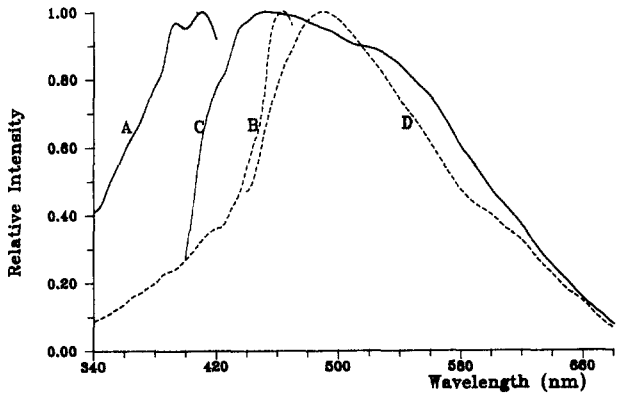


Fig. 11. Fluorescence excitation spectra, λ_{obs} = 450 nm (A) and 520 nm (B), together with fluorescence spectra, λ_{ex} = 380 nm (C) and 420 nm (D), of C-265 in acidified PVA. All measured at liquid nitrogen temperature.

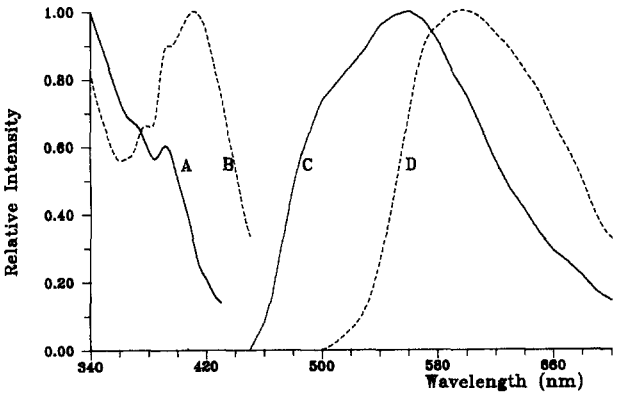


Fig. 12. Phosphorescence excitation spectra, λ_{obs} = 510 nm (A) and 600 nm (B), together with phosphorescence spectra, λ_{ex} 380 nm (C) and 450 nm (D), of C-265 in acidified PVA. All measured at liquid nitrogen temperature.

sequently. Further information is provided by both the fluorescence and the phosphorescence spectra. The short-wavelength fluorescence spectra (Figs. 10D and 11C) constitute broad bands the shapes of which indicate that they result from overlap of more than two emission bands. The complex nature of these bands can be seen better on changing from room to liquid nitrogen temperature. The corresponding short-wavelength phosphorescence emission (Fig. 12C) also demonstrates the overlapping of the two bands. All short-wavelength luminescence characteristics seem to indicate that the emission in this region arises from at least two independent structural forms of the protonated C-265 molecule.

The sequence of the appearance of emission characteristics appropriate for certain forms of the protonated C-265 molecule follows generally recognized rules. The position of luminescence excitation characteristics relative to each other and to the absorption band requires, however, some comment to be made. As presented above, the long-wavelength fluorescence excitation arises most probably from the form existing in the ground state. On the other hand, the short-wavelength fluorescence excitation spectra (Figs 10B and 11A) fall down in the region where no absorption transition is observed. Such a relation between the fluorescence excitation and the absorption spectra may imply that the emitting states are created upon the rearrangement of the primarily excited form of the molecule existing also in the ground state. According to the spectral characteristics obtained, such a process will require additional vibrational excitation.

The short-wavelength phosphorescence excitation spectrum (Fig. 12A) occurs essentially in the same region as appropriate fluorescence spectra. This fact may suggest the closeness of the singlet and triplet states involved in the S → T transition. On the other hand, the lack of any correlation in the shape of the two abovementioned spectra may additionally support the idea that emission, due to the excitation in this region, arises from at least two independent forms of the molecule.

DISCUSSION

Possible Forms of Protonated Molecules in the PVA Foil

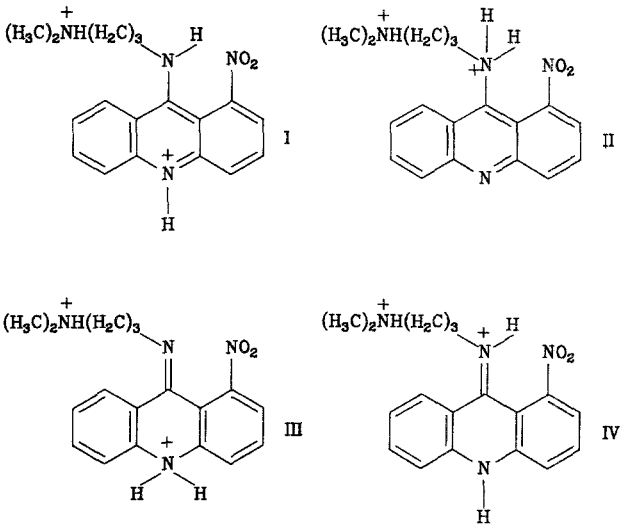
As demonstrated by the discussion in the previous section, all emission characteristics reveal the appearance of protonated nitracrine and its nitro isomers in at least two forms. In most cases, these forms should already exist in the ground state of molecules; in others, i.e., in the case of 3-nitro or 4-nitro isomers, they are presumably created also in the excited state. One possible explanation of the nature of this phenomenon would be that the compounds studied undergo aggregation in the PVA polymer. The formation of dimers of protonated acridine derivatives in solution [33,51–53] and even in polymeric films [54] has been considered in the past. In our case, however, it does not seem likely since we are dealing with a rather low concentration of the compounds and a rigid dense phase. Moreover, it would be difficult to account for the substantial discrepancies between absorption and fluorescence excitation spectra, the differences between fluorescence and phosphorescence excitation spectra at various observation wavelengths, as well as the differences in the emission characteristics

measured at various excitation wavelengths, and the effect of temperature on the emission characteristics, in the category of monomer–dimer equilibria. The spectral features of the systems studied can, however, be readily accounted for by assuming the possibility of the hydrogen atom migration within the molecule. Such a phenomenon has been known as a prototropic tautomerism.

Prototropic Tautomerism in the Ground State of Molecules

The prototropic tautomerism in neutral [55–59] and protonated [29,56,59–63] 9-acridinamine derivatives has long been known. In nonprotonated nitracrine and its 2-nitro isomer, the occurrence of this phenomenon has been evidenced based on X-ray investigations [23,25–27]. It was further confirmed by quantum chemistry calculations and theoretical and experimental examinations [24,28] of the electronic absorption [24–28].

The possible tautomeric forms of neutral nitracrine are shown in Scheme II. In acidic media we are dealing with the charged forms of the molecules. The compounds studied contain three basic sites localized at nitrogen atoms. Therefore, they are capable of participating in three protolytic equilibria. Based on the current knowledge of this problem, one can believe that only two protons are actually bound to the compounds [8]. It can be presumed that the most basic character is exhibited by nitrogen at the end of the alkylamino side chain and that this atom first undergoes protonation. The second proton can be localized either on the heterocyclic acridine ring nitrogen or on the exocyclic nitrogen of the aromatic amino group. Since the molecules of the compounds studied can occur in two, amino and imino, tautomeric forms (see, e.g., Scheme II) [24], one can predict four diprotonated tautomeric forms for nitracrine and each of its nitro isomers (Scheme III). Two or more such structures are adopted by the compounds studied in the PVA polymer. It is rather difficult to predict a priori the relative stabilities of these forms. The answer to this question could be provided by quantum chemistry calculations. Unfortunately, such information is not yet available, although we are currently working on this problem. Most authors considering the protonation of 9-acridinamine [60–62] and its derivatives [35,37,39–41] and the problem of prototropic tautomerism in these compounds [60–62] believe that, in the amino tautomeric form, the acridine ring nitrogen exhibits more a basic character than the exocyclic amine one, whereas in the imino tautomeric form, the basicity of N(18) is higher than that of N(10). It is, perhaps, worth mentioning that some studies seem to indicate that, in monocation of 9-acridinamine, the imino tautomeric structure is more stable in the ground state [60,61,64].



Scheme III. Diprotonated forms of nitracrine tautomers.

Possiblity of Proton Transfer in the Excited States

Upon examination of the behavior of neutral nitracrine and its nitro isomers in the liquid phase, it has been found that the change in the solvent brings about remarkable changes in the shape and relative intensities of the long-wavelength absorption bands [21,22,24]. These findings have been attributed to the existence of equilibria betwen amino and imino tautomeric forms of these derivatives (Scheme II). Since the relative abundances of these two forms change in various solvents, the free energies of the amino and imino forms are comparable and the configurational reorganization, accompanied by the hydrogen transfer, does not require a substantial activation barrier to be overcome.

There exist serious premises indicating that tautomeric phenomena appear also in the protonated forms of the compounds studied and their excited states [29,60–62,64,65]. It is, however, much more difficult to predict the relative stabilities of various forms, particularly in the excited states. The long-wavelength luminescence characteristics of all compounds studied and short-wavelength characteristics in the case of C-283 and C-264 seem to indicate that the emission observed occurs from the tautomeric forms, which exist primarily in the ground state. Untypical short-wavelength fluorescence and phosphorescence excitation spectra, in the case of C-257 and C-265, may be accounted for by considering that

after electronic excitation, accompanied by excitation to higher vibrational states, tautomeric rearrangement of molecules takes place. Such a process, which can also occur in excited C-283 and C-264 molecules, may be accomplished as well with the participation of H^+ from the environment due to the fact that, under the experimental conditions, a large excess of these ions is dealt with. The possibility of the configurational rearrangement of molecules in the excited state can readily explain the complex nature of the short-wavelength fluorescence and phosphorescence spectra of C-265. The luninescence spectra in the latter case are untypical and indicate the coexistence of at least three emitting states. It seems worth mentioning that the possibility of a similar rearrangement of molecules in the excited states has been considered in other systems (e.g., Refs. 29, 61, 66, and 67).

Relations Between the Energy Levels of Electronically Excited States of Molecules

The fluorescence excitation spectra, except for the short-wavelength ones in the case of C-257 and C-265, roughly fall in the region of the occurrence of the absorption spectra. This implies that the energeticity of the $S_0 \rightarrow S_1$ transitions is reflected well by both spectral characteristics. This, however, concerns only transitions in those tautomeric forms of the molecules which exist in the ground state. The energy of forms not appearing in the ground state can be expected to be higher in the excited states. However, presumably only in the case of C-257 and C-265 can tautomeric structures which do not exist in the ground state be formed upon excitation by the relatively low-energy radiation used in our experiments. Moreover, as indicated by the short-wavelength fluorescence excitation spectra of the latter molecules, the $S_0 \rightarrow S_1$ absorption followed by tautomeric rearrangement in the excited state would require a substantial excess of energy to be provided.

The intersystem crossing in the acridine molecule is accomplished between S_1 and T_2 or T_3 states [30,34,36,68]. One can believe that such an energetical sequence of states of different multiplicity is also characteristic of the compounds studied. The fact, however, that the phosphorescence excitation spectra are in general shifted toward the higher-energy region relative to the relevant fluorescence spectra implies that the triplet state participating in the transition is situated somewhat above the first singlet state of molecules.

An Attempt to Ascribe Spectral Characteristics to Certain Tautomeric Forms of Molecules

Characteristic of the amino tautomeric structures shown in Scheme III is the fact that the conjugation extends over the aromatic system embracing 16 bonds. Moreover, in the amino structure with the protonated acridine ring nitrogen (**I**), there exists the possibility of conjugation of the free electron pair at exocyclic N(18) with the whole aromatic system. One may expect that such a form is of a higher thermodynamical stability compared to structure **II**. In both imino tautomers of the compounds studied (Scheme III) the conjugation covers a 15 aromatic-double-bond system. The form denoted **IV** is also characterized by the fact that the free electron pair at the cyclic nitrogen atom can participate in the conjugation. This form should, therefore, be more stable than **III**. By the above qualitative approach, it is not possible to differentiate between the amino and the imino forms. The question, therefore, remains which of the tautomeric structures, **I** or **IV**, may be expected to be more stable. Several authors studying spectral features of monocationic forms of 9-acridinamine [60,64] and its derivatives [61,64,65] believe that in both the ground and the electornic excited states the imino tautomeric structure is more stable. If such a sequence of stability of tautomeric forms is retained in the case of the compounds studied, the long-wavelength absorption and fluorescence characteristics can be attributed to imino structures shown in Scheme II as **IV**.

CONCLUDING REMARKS

The results of the present work reveal the existence of protonated nitracrine and its nitro isomers in poly(vinyl alcohol) film in two or more structural forms resulting from the prototropic tautomerism phenomena. The possibility of the appearance of the compounds in various forms, which may be different in the ground and excited states, accounts well for the observed discrepancies in the absorption and luminescence excitation spectra, as well as, in some cases, for the complex nature of the luminescence characteristics.

Better insight into this subject can be gained by the static emission studies in solution and by the examination of the time-resolved luminescence characteristics. We believe that also quantum chemistry methods should provide information not yet available regarding the structure of tautomers, their relative stability, energies of

electronic transitions, and their other features. We are currently working on these problems.

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