Prototropic Tautomerism in *N,N*-Dimethyl-*N'*-(1-nitro-9-acridyl)propane-1,3-diamine and its Nitro Isomers. Application of MNDO and PPP Methods for the Examination of Structure and Electronic Absorption Spectra[1]

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N,N-Dimethyl-*N'*-(1-nitro-9-acridyl)propane-1,3-diamine (Ledakrin® or nitracrine), which exhibits distinctive anticancer activity, and its three nitro isomers can potentially exist in two or three tautomeric forms (amino, imino, *aci*). This is due to the possibility of migration of the hydrogen atom which is bound to the nitrogen atom attached to carbon C-9 to the acridine ring nitrogen, or to the nitro group. The structures of several possible tautomers were determined by geometry optimization using the MNDO method. The latter procedure enabled also the evaluation of (i) the heats of formation, (ii) the dipole moments, and (iii) the ionization potentials to be undertaken. From these structures the frequencies of electronic transitions and the oscillator strengths for individual tautomeric forms were calculated using an adaptation of the PPP method. The evaluated UV absorption spectra were compared with experimental data. It was revealed that the observed absorption in the long-wavelength region is characteristic for a given molecule. Moreover, in the case of a given isomer the absorption can be readily explained by the results of theoretical calculations presented here, assuming that the molecule may occur in various accessible tautomeric forms.

The biological activity of acridine derivatives has been known since the end of the last century.[2] Many different biological activities of acridines have been disclosed: *e.g.*, antibacterial, antivirial, antimalarial, and analeptic activities (see, *e.g.*, refs. 3–6). Special attention has been devoted to this group of compounds, however, since their anticancer activity was documented.[7–12] *N,N*-Dimethyl-*N'*-(1-nitro-9-acridyl)propane-1,3-diamine, which is known under the name nitracrine (WHO) or, in Poland, Ledakri® (*e.g.*, refs. 9–12 and references cited therein) exhibits, among other effects, such anticancer activity. The mechanism of the antitumour action of nitracrine has been the subject of extensive investigations.[9,10,13–16] In addition, other papers have been devoted to the examination of the chemical and physical properties of both nitracrine and its isomers.[11,17] These studies were undertaken in order to shed more light on the nature of the biological activity of this group of compounds, to find connections between their structure and their biological activity, and to extend our knowledge regarding the behaviour of the compounds in the liquid phase and perhaps in bodily tissues. Our two earlier reports were concerned with the latter problem.[18,19] In the present work we have turned our attention to the tautomeric phenomena in nitracrine and its three nitro isomers in solution.

The prototropic tautomerism of these derivatives is known from crystallographic investigations.[11,20–22] These studies revealed that a molecule of nitracrine in the solid phase exists in the non-planar imino tautomeric form which is characterized by the fact that the central ring system is folded at an angle along the C(9) ··· N(10) axis. Moreover, the nitro group in position 1 is tilted, with a torsion angle of *ca.* 60° to atoms in the acridine ring.[20] On the other hand, the 2-nitro isomer exists as a planar amino tautomer, with coplanarity between the acridine nucleus and the nitro group.[21] These distinctive structural differences between nitroacridin-9-amines have been considered to be the main factor determining the unique anticancer activity of nitracrine.[8,20–22] It is also worth mentioning that differences in the behaviour of nitracrine and its isomers upon hydrolysis have been attributed to the existence of tautomeric equilibria in aqueous solution.[23] The main aim of the present study is to reveal which structures can be expected for nitracrine and its three nitro isomers in different accessible tautomeric forms, in gaseous and inert liquid phases and, further, how these structures influence physicochemical properties of individual tautomers and their electronic absorption spectra. Lastly, we wanted to know which forms are probable in liquid solutions and how features of the medium may influence existing equilibria. The results of this study may shed more light on the features of specific biological activity of the drug and on the structure–activity relationships.

To achieve these goals we combined UV absorption measurements with theoretical calculations using semiempirical MNDO and PPP methods. Application of the MNDO method resulted from the fact that it correctly reproduced tautomeric equilibria in several other systems.[24,25]

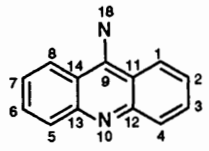
Materials and Methods

Chemicals.—*N,N*-Dimethyl-*N'*-(1-nitro-9-acridyl)propane-1,3-diamine and its isomers were synthesized as dihydrochlorides and purified by the method reported in the literature.[26,27] The compounds were kindly supplied by Professor J. Konopa and his research group 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 toluene. The extracts were subsequently dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The purity of both hydrochlorides and free bases was examined by TLC.[28]

Solvents, *i.e.* hexane, methanol, and toluene, all from Mallinckrodt, and ethanol (P.O.Ch., Poland) of spectroscopic grade were used as received.

Apparatus.—Spectral measurements in the UV and visible regions were carried out on SPECORD M40 and VSU-2P (Carl Zeiss, Jena) spectrophotometers.

Calculations.—The geometry optimizations were performed by the standard MNDO method with the DFP optimization procedure.[29] For these optimizations it is necessary to know the starting geometry of the compounds studied. Such geometry can be chosen from the crystallographic data. Unfortunately, so far only crystal structures for the 1-nitro isomer (which crystallizes in the imino tautomeric form) and the 2-nitro isomer (which crystallizes in the amino form) are known.[20,21] Using these data we chose starting structures of amino forms of all four isomers by keeping the solid-state geometry of the acridine nucleus of the 2-nitro isomer and changing the position of the nitro group. Analogously, the starting structures of all imino tautomers were chosen by adopting the crystallographic data for the 1-nitro isomer. Because of the lack of crystallographic data for *aci* tautomeric forms we assumed starting structures of appropriate 2-nitro and 4-nitro isomers by chemical intuition. As seen in substructure (**1**) the acridine skeleton in the *aci* form is similar to that for the amino form except for the fragment C(14)–C(9)–C(11)–N(18). The latter is quite characteristic of the imino form. Therefore, the starting structure of the acridine nucleus in the *aci* form was assumed to be a combination of the structural elements of both amino and imino forms. This skeleton was combined with structural elements of the *aci*-nitro group which were obtained from the optimization of nitromethane in the *aci* form by use of the MNDO method.

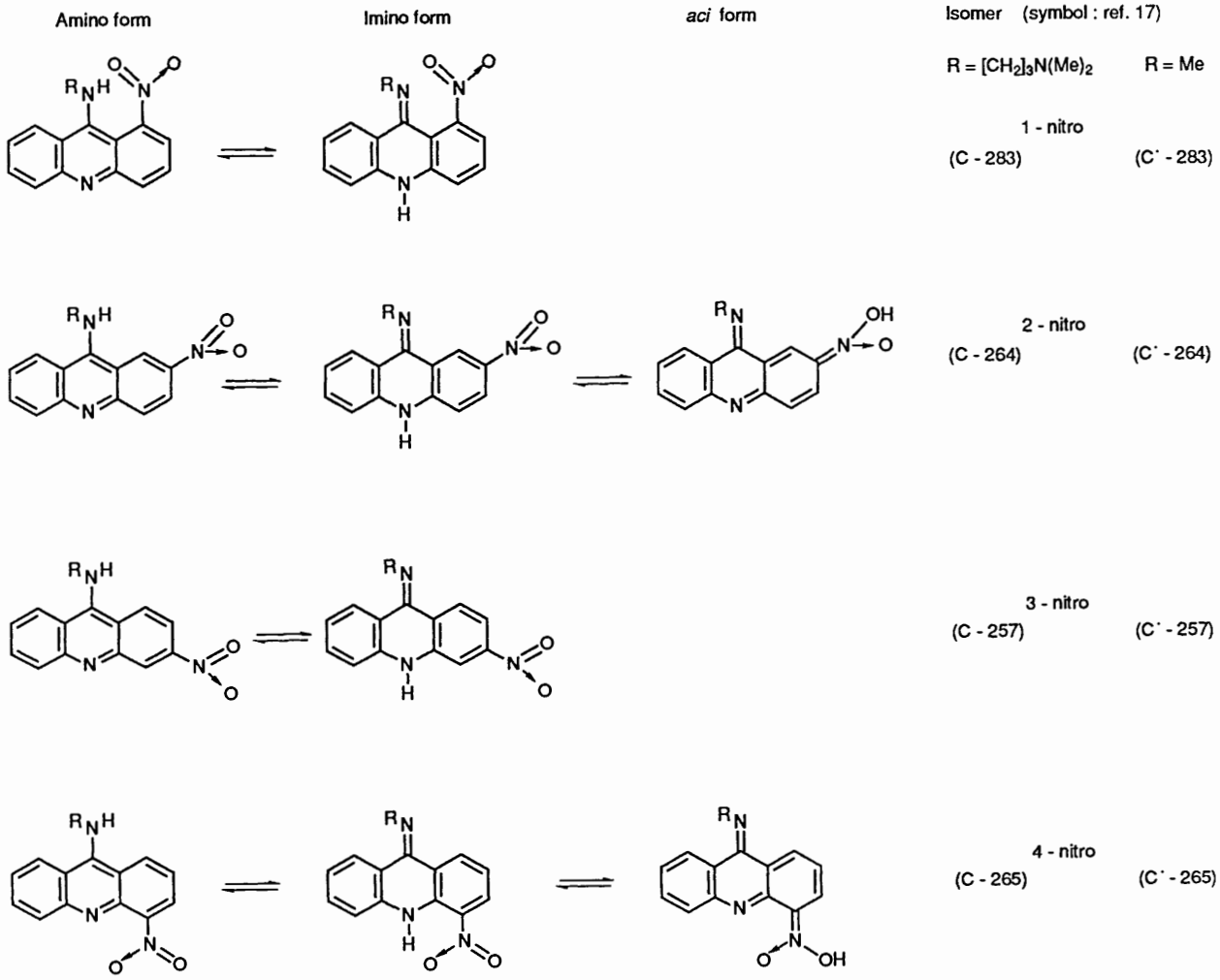


(**1**)

Numeration of atoms in acridin-9-amines.[17]

Because of the limited capability of our computer (all calculations were performed on an IBM PC/AT), we kept unchanged during the optimizations the internal co-ordinates of all the hydrogen atoms except those in the close neighbourhood of the polar groups. Further, in amino and *aci* tautomeric forms we assumed the geometry of the acridine nucleus to be planar. Lastly, we substituted a methyl group for R (see Scheme). The above mentioned simplifications undoubtedly influenced somewhat the physicochemical characteristics obtained on the basis of the MNDO method (Table 1) but should not affect the subsequently derived spectral characteristics. For the structures obtained by optimization with the MNDO method several characteristics were also computed on applying the INDO procedure.[30] These characteristics are shown in Table 1.

Primarily we used the CNDO/S CI method[31] to predict the UV spectra of the compounds studied. Unfortunately, the derived characteristics (*i.e.*, positions of electronic transitions and oscillator strengths) corresponded very poorly to the experimental absorption spectra. In particular, the method did not predict any bands in the long-wavelength region, *i.e.*



Scheme. Tautomeric equilibria in nitro isomers of *N*,*N*-dimethyl-*N'*-(nitro-9-acridyl)propane-1,3-diamines and *N*-(nitro-9-acridyl)methanamines.

Table 1. Physicochemical and spectral characteristics for isomers of *N*-(nitro-9-acridyl)methanamine.

Compound		Physicochemical characteristics						Spectral characteristics for the long-wavelength region	
		MNDO				INDO			
Symbol (see Scheme 1)	Tautomer	ΔH_f/ kcal mol^{-1} [a]	Dipole moment μ/D[b]	Energy of HOMO/eV	LUMO/eV	E_{tot}/eV	μ/D[b]	λ/nm	Oscillator strength
C′-283	amino	104.0	3.45	−8.796	−1.661	−4 708.471	2.97	485.8	0.225
								342.3	0.142
								324.5	0.189
	imino	91.1	6.48	−8.723	−0.882	−4 709.302	7.61	433.5	0.633
								427.5	0.169
								296.4	0.133
C′-264	amino	93.0	6.20	−8.742	−1.700	−4 708.871	5.33	478.7	0.189
								399.9	0.097
								345.8	0.256
	imino	88.1	8.32	−8.929	−1.142	−4 709.530	8.46	466.7	0.731
								407.0	0.210
								309.6	0.117
	aci	112.9	1.36	−8.249	−1.851	−4 705.916	3.29	412.3	1.186
								309.9	0.167
								311.4	0.259
	aci-anion							541.6	1.154
								416.3	0.260
								320.0	0.088
C′-257	amino	93.1	7.67	−8.929	−1.142	−4 708.929	7.34	498.5	0.141
								450.8	0.123
								319.6	0.146
	imino	89.8	6.60	−8.804	−1.282	−4 709.428	6.35	495.6	0.141
								439.2	0.504
								386.2	0.159
C′-265	amino	108.6	8.29	−8.644	−1.640	−4 708.241	8.51	461.8	0.350
								370.5	0.100
								316.9	0.339
	imino	94.4	4.54	−8.747	−1.203	−4 709.488	3.76	516.7	0.555
								403.0	0.260
								306.2	0.046
	aci	112.7	1.65	−8.120	−1.661	−4 694.984	2.67	483.4	0.528
								377.4	0.330
								322.1	0.055
	aci-anion							585.5	0.589
								390.6	0.323
								361.4	0.062

[a] 1 cal = 4.184 J. [b] 1 D = 3.345 64 C m.

Table 2. PPP Parameters for $-NO_2H$ and $-NO_2^-$ groups.

Group	Atom	Valance state ionization potential	One-centre integrals	Resonance integrals
	C	−11.57	11.33	β_{C-N} 2.55
	N	−22.27	15.72	β_{N-O} 2.58
	O←	−21.24	15.35	β_{N-O} 2.11
	O−	−28.54	17.80	
	C	−12.14	11.61	β_{C-N} 2.14
	N	−22.31	15.74	β_{N-O} 2.70
	O	−19.58	14.74	

$\lambda > 400$ nm. Therefore, we made an attempt to employ the PPP method for spectral evaluations.[32] Another reason for using the PPP method was that predictions regarding the energy of electronic transitions by this method are usually better than in the case of the CNDO/S method.[33] The parametrizations for carbon atoms were typical.[34] The PPP parameters for the nitro group were assigned following Gordon and Neumer.[35] For nitrogen atoms in an acridine ring and in the amino group the values of the PPP parameters were estimated using the results of MNDO optimizations and employing a procedure described elsewhere.[36,37] For these evaluations the core charges obtained from the MNDO calculations were used to derive the valence-state ionization energies, and these values were then employed to evaluate the one-centre electron-repulsion integrals on the basis of the method described by Dewar and Morita.[38] The values of resonance integrals ($\beta_{i,j}$) were estimated following the empirical expression of Kulkarni *et al.*,[37] which relates $\beta_{i,j}$ values with changes in the valence-state ionization potential of the *i*-th atomic centre. Similarly the values of the PPP parameters for the nitro group in either neutral *aci* or appropriate anionic forms were derived. For this purpose we used values of core charges derived by the MNDO method for both the neutral *aci* form and the appropriate anionic form of nitromethane. To our knowledge PPP parameters for the nitro group in the *aci* form have not, so far, been reported. Thus they are shown in Table 2.

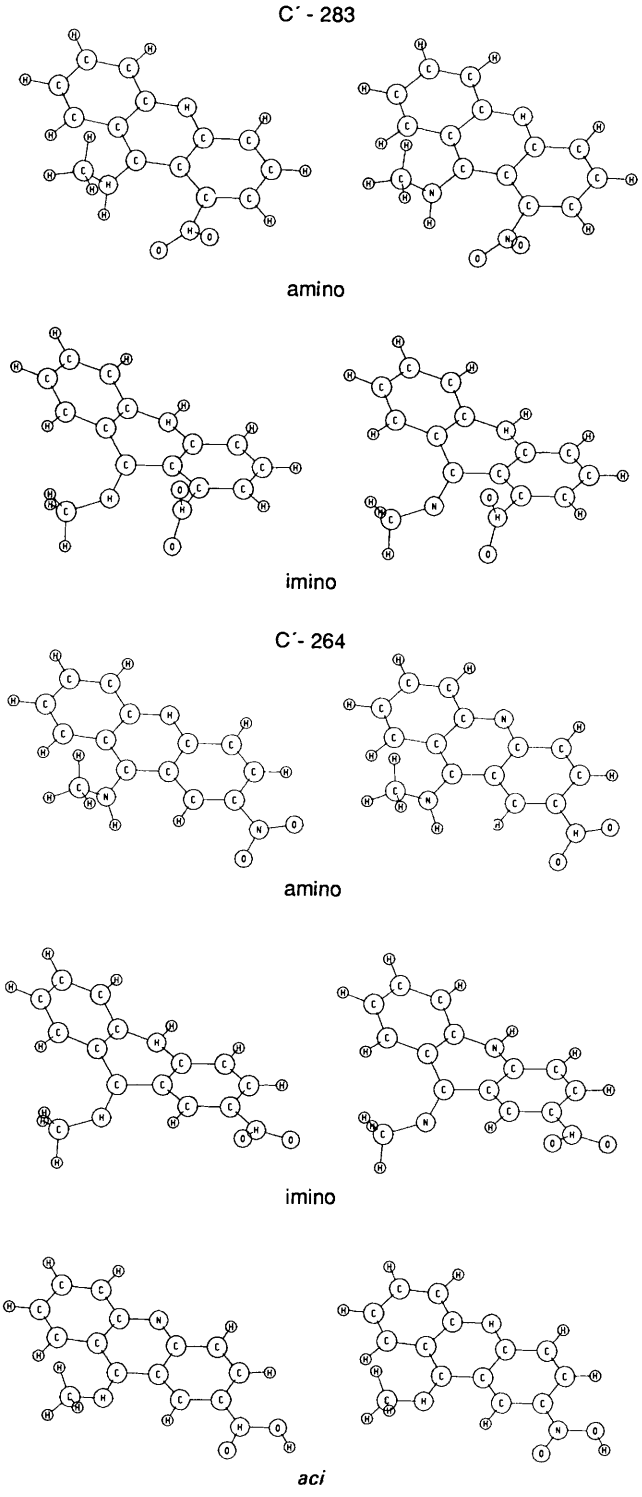


Figure 1. The stereoviews of C′-283 and C′-264 molecules in their accessible tautomeric forms.

To estimate the changes in values of resonance integrals with distance, Kon's formula was applied.[39] The changes in $\beta_{i,j}$ integrals caused by nonplanarity of the central ring in the imino form or by the twist of nitro or amino groups against the acridine nucleus were accounted for by making them dependent on the appropriate torsion angle.[40] The two-centre repulsion integrals were evaluated using the Mataga–Nishimoto formula.[41]

Results and Discussion

Structural Features of N-(*Nitro-9-acridyl*)*methanamines.*—The views of the C′-283 and C′-264 molecules in their accessible tautomeric forms are presented in Figure 1. Examination of all four isomers in all their possible tautomeric forms revealed that the structural features of both amino and imino forms of the 1-nitro isomer differ from those characteristic of other isomers. This particularly concerns the arrangement of the nitro group. The torsion angle of the $-NO_2$ group against the closest aromatic ring is 40 and 87° for the amino and imino forms of C′-283, respectively. In the amino and imino tautomers of the other three isomers the nitro group is much less tilted from planarity with the benzene ring to which it is attached. The optimization procedure also predicted that the $-NO_2H$ fragment of the *aci* nitro group in C′-264 and C′-265 is almost planar with the acridine ring. In the latter compound the OH fragment of the *aci* nitro group is directed outwards N(10). Because of the steric hindrances caused by the R substituent at N(18) the amino group is always twisted against the acridine ring at angles of 69, 57, 63, and 59° for C′-283, C′-264, C′-257, and C′-265, respectively. In the imino and *aci* tautomers the arrangement of the imino group is governed by the double-bond character of the C(9)–N(18) bond. Further information provided values of some dihedral angles, showing the nonplanarity of the central acridine ring in the imino form. So, for example, the C(14)–C(9)–C(11)–C(12) angle is 25, 27, 24, and 21° for the C′-283, C′-264, C′-257, and C′-265 isomers, respectively. The above discussion, as well as further examination of structures, reveals that the 1-nitro isomer occupies a distinctive position among the compounds studied. This results from the presence of two relatively large groups close together in the C′-283 molecule, namely the nitro group and the substituted amino group.

Physicochemical Characteristics and Relative Stability of Tautomers.—Some physicochemical characteristics obtained after optimization by the MNDO method, as well as those obtained using the INDO method, enable a comparison of the expected stability and behaviour of various isomers and their tautomeric forms to be undertaken. Both the MNDO heats of formation and the total energies (E_{tot}) from the INDO method seem to indicate that imino tautomers should be thermodynamically more stable than the corresponding amino ones. On the other hand, the *aci* forms in structures C′-264 and C′-265 are characterized by higher values of ΔH_f (from MNDO) and E_{tot} (from INDO) which means that the existence of the compounds in these structures is not very likely, either in the gaseous phase or in solution. It may, however, be noted that the INDO total energy for the *aci*-C′-264 tautomer is lower in comparison with that for the *aci*-C′-265 one. The values of the dipole moment (μ) evaluated by both the MNDO and INDO methods are comparable. Generally, the lowest μ values characterize the *aci* tautomeric forms. The examination of the data in Table 1 reveals that for 1-nitro and 2-nitro isomers μ values for imino tautomers are higher than those for the appropriate amino tautomers. For C′-257 and C-265 isomers the tendency is reversed. Additional information provided values of energies of HOMO and LUMO orbitals. Among others they may be related to the redox properties of the compounds studied.

UV Spectra of N,N′-*Dimethyl*-N′-(*nitro-9-acridyl*)*propane*-1,3-*diamine Isomers.*—The experimental absorption spectra of the four *N,N*-dimethyl-*N′*-(nitro-9-acridyl)propane-1,3-diamine isomers studied are shown in the upper graphs in Figures 2–5. These spectra reveal the existence of essentially two bands in the long-wavelength region. The lowest energy band, with a maximum at *ca.* 450 nm, extends up to 600 nm. The

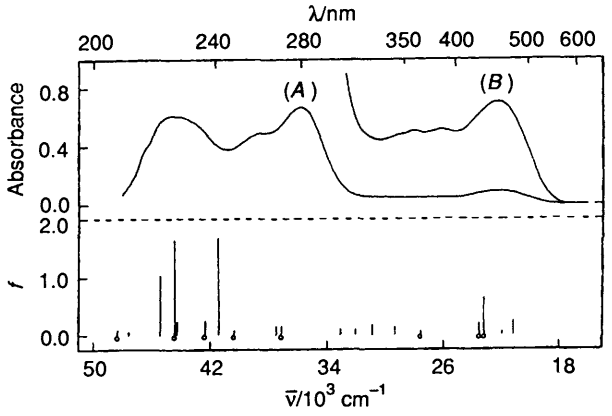


Figure 2. The experimental (*A*, *c* 4.6 × 10^{-5} mol dm^{-3} in hexane; *B*, spectrum amplified 8 times) absorption spectra of *N*,*N*-dimethyl-*N'*-(1-nitro-9-acridyl)propane-1,3-diamine [absorbance $\log_{10}(I_0/I)$] together with the calculated transition frequencies for amino (|) and imino (⫰) tautomeric forms.

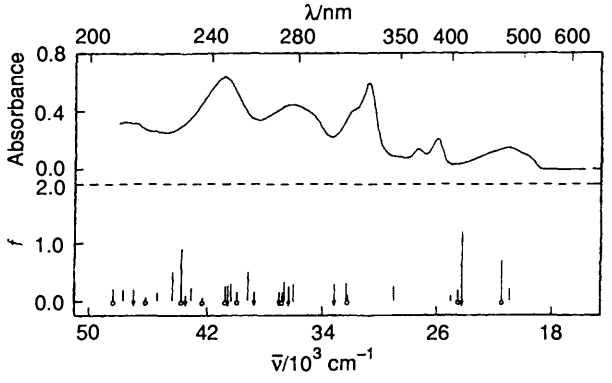


Figure 3. The experimental absorption spectrum (*c* 9.3 × 10^{-5} mol dm^{-3} in hexane) of *N*,*N*-dimethyl-*N'*-(2-nitro-9-acridyl)propane-1,3-diamine together with the calculated transition frequencies for amino (|), imino (⫰), and *aci* (↓) tautomers.

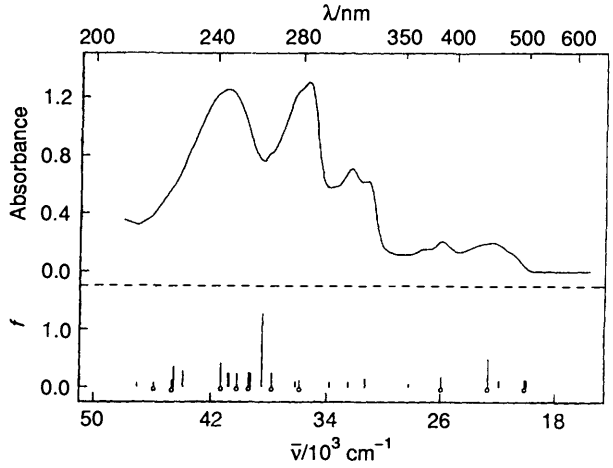


Figure 4. The experimental (*c* 4.6 × 10^{-5} mol dm^{-3} in hexane) absorption spectrum of *N*,*N*-dimethyl-*N'*-(3-nitro-9-acridyl)propane-1,3-diamine together with the calculated transition frequencies for amino (|) and imino (⫰) tautomers.

position of this band is affected only slightly by the properties of the medium in the case of C-264,[18] C-257,[18] and C-265[1] isomers. However, the long-wavelength band of the 1-nitro isomer is observed only in non-polar and aprotic solvents (Figure 2).[18]

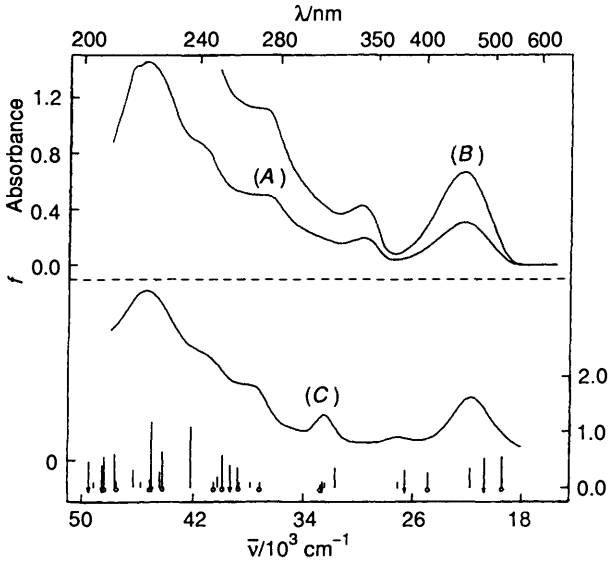


Figure 5. The experimental (*A*, *c* 4.6 × 10^{-5} mol dm^{-3}; and *B*, *c* 9.3 × 10^{-5} mol dm^{-3}; both in hexane) and predicted (*C*) absorption spectra of *N*,*N*-dimethyl-*N'*-(4-nitro-9-acridyl)propane-1,3-diamine together with the calculated transition frequencies for amino (|), imino (⫰), and *aci* (↓) forms.

On moving towards the higher energy region, a second band system occurs between 300 and 400 nm. Contrary to the long-wavelength absorption presented above the latter one, *i.e.* between 300–400 nm, is somehow characteristic of a given isomer. Moreover, it is influenced noticeably by the properties of the solvent.[1,18] In the case of the C-283 isomer, the long-wavelength band (at 450 nm) declines in polar and protic solvents, whereas the band between 300–400 nm becomes stronger.[18] All the above facts would suggest that both band systems discussed, *i.e.* the one at *ca.* 450 nm and that occurring between 300–400 nm, might arise from two different forms of the compounds, co-existing in the liquid phase.

Experimental versus *Theoretical Electronic Absorption Spectra*.—The above hypothesis necessitates further confirmation, which is provided by the results of theoretical evaluations. They are presented on the lower graphs in Figures 2–5, in which calculated energy transitions are drawn as lines. The height of each line is proportional to the evaluated oscillator strength (*f*) for a given electronic transition. To facilitate the comparison of experimental absorption characteristics with theoretical ones let us primarily assume that the spectra result from the existence of the compounds studied in accessible tautomeric forms. As demonstrated in Figures 2–5, in the region discussed above, the PPP method predicts electronic transitions for all possible tautomers. Since the thermochemical data in Table 1 tend to exclude the possibility of the occurrence of isomers C-264 and C-265 in *aci* tautomeric forms it may be assumed that only electronic transitions for amino and imino forms actually determine the absorption spectra. It is somewhat difficult to imagine the shape of the analysed spectra on the basis of the predicted electronic transitions. Thus, for the C-265 isomer we made an effort to evaluate the spectrum by assuming that each transition can be approximated by a Gaussian curve. Further, we chose band widths and a ratio between both tautomers such as to obtain the best fit to the experimental absorption spectrum. As seen in Figure 5 the shape of the predicted spectrum approximates well to the experimental curve at a ratio of [amino]:[imino] 0.65:0.35. For other isomers such a procedure did not lead to such a good reproduction of the shape of the experimental absorption

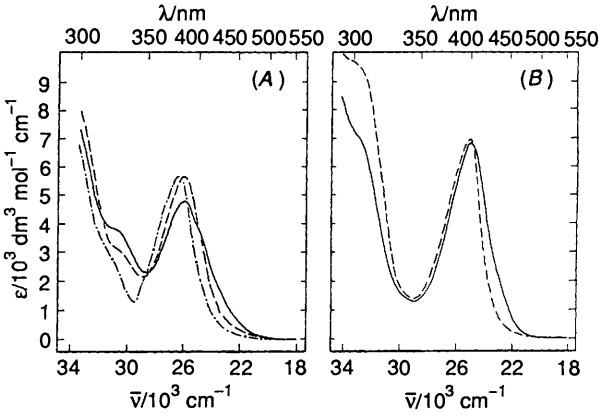


Figure 6. The absorption spectra of compounds (I) (*a*) and (II) (*b*), with known imino structure, in hexane (— · —), methanol (——), and toluene (— —).

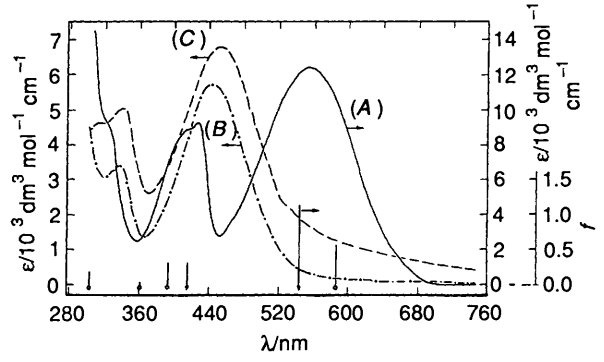
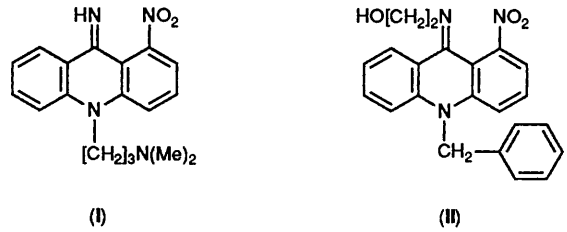


Figure 7. The absorption spectra of *N,N*-dimethyl-*N'*-(2-nitro-9-acridyl)propane-1,3-diamine in ethanolic sodium ethanolate (*A*), and *N,N*-dimethyl-*N'*-(4-nitro-9-acridyl)propane-1,3-diamine in ethanol (*B*) and ethanolic sodium ethanolate (*C*), together with the calculated transition frequencies for *aci*-anionic forms of C'-264 (↓) and C'-265 (↓).

spectra as shown for C-265. Data in Figures 2–4 demonstrate that, for C-283, C-264, and C-257 isomers, the first long-wavelength transition corresponds to amino forms, whereas for the C-265 isomer it corresponds to the imino form. The separation of the predicted transitions corresponding to the amino and imino forms is not, generally, large enough to enable one to ascribe the observed long-wavelength bands in the experimental spectra to certain tautomeric forms of a given isomer.

Owing to the lack of symmetry elements in the compounds studied it is rather difficult to describe these electronic transitions on the basis of group theory, as has been done for related symmetric compounds.[42] Analysis of the direction of the transition moment indicates, however, that electronic excitation corresponding to the first long-wavelength transition is always accompanied by a shift of electronic density towards the nitro group.

To gather more information regarding the above analysis of the long-wavelength absorption region we have presented in Figure 6 the spectra of two available compounds in which the imino structure was blocked. It can be seen that compounds (I) and (II) are structural analogues of the 1-nitro isomer in the imino form. Compound (I) was synthesized by Horowska *et al.*[43] The second, compound (II), was prepared by Wysocka-Skrzela, and its structure was confirmed by *X*-ray analysis.[44] The substituents at N(10) of these derivatives are such that they should not influence the long-wavelength electronic transitions. Indeed, comparison of the spectra shown in Figure 5 with those



(I) (II)

The compounds with blocked imino structure.

reported earlier in ref. 18 (Figure 2) clearly indicate that the band system occurring between 350–400 nm corresponds to the imino tautomer of *N,N*-dimethyl-*N'*-(1-nitro-9-acridyl)-propane-1,3-diamine.

The Existence of aci *Tautomers.*—As has been noted above the *aci* tautomers of isomers C-264 and C-265 are thermodynamically much less stable than the other two forms. This led to the conclusion that, essentially, both the above compounds cannot exist in the *aci* form. Nevertheless, it would be interesting to show that the *aci* structure can exist. In the case of the equilibria studied, such structures would be constrained by the imposition of certain conditions. Some published reports suggest that the tautomeric conversion of the nitro group into the *aci* structure takes place in an EtOH–EtONa medium.[45,46] The absorption spectra of the 2- and 4-nitro isomers in an EtOH solution of sodium ethanolate are presented in Figure 7. The spectrum of the 2-nitro isomer exhibits a new, strong absorption with a maximum at *ca.* 550 nm. The band ascribed to the amino form, which generally occurs between 450 and 500 nm [Figure 2(*B*), ref. 18], completely disappears. In moving towards higher energy transitions a second band, with two maxima between 400 and 420 nm, is seen. This remarkable change in the absorption spectrum of the 2-nitro isomer may, of course, be observed visually. Solutions of *N,N*-dimethyl-*N'*-(2-nitro-9-acridyl)propane-1,3-diamine in ethanol (or neutral media) are red–yellow, whereas the compound dissolved in ethanolic sodium ethanolate is blue–violet. The 4-nitro isomer dissolved either in ethanol or in EtOH–EtONa does not change its colour noticeably. The absorption spectra of C-265 in both media are very similar (Figure 7), although the spectrum in EtOH–EtONa exhibits a characteristic tail. It is worth mentioning that the absorption of 2-nitro and 4-nitro isomers observed in the EtOH–EtONa medium, described above, disappears in neutral and acidic media and reoccurs in EtONa solution. Isomers C-283 and C-257, naturally, cannot exhibit the absorption, which is characteristic of *aci* structures, although in the EtOH–EtONa medium the intensity of the bands resulting from amino and imino forms changes in comparison with that observed in neutral media. The latter phenomenon is presumably due to the changes in the polarity of the medium which is used. It is worth mentioning also that the existence of an *aci* form of 2-nitroacridin-9-amines has also been put forward on the basis of polarographic studies.[47,48] In strongly alkaline media the structure which can actually exist is an anionic form of the appropriate *aci* tautomer. For such structures, obtained by the removal of the proton from the $-NO_2H$ group, the electronic transitions were evaluated by the PPP method. The predicted long-wavelength transitions for the *aci* anionic structure of C-264 correspond well to the experimental absorption bands. For the C-265 *aci* anionic form the PPP method also predicts transitions in the long-wavelength region. The corresponding bands are not, however, observed in the experimental absorption spectrum. It is difficult to explain why the C-264 isomer can be forced into an *aci* anionic structure and C-265 can not. The explanation might be that the total energy value, from INDO, is

much higher for the *aci*-C-265 form than for the *aci*-C-264 tautomer. If the transition state for the formation of anionic forms were the appropriate *aci* forms it would mean that the conditions chosen were not sufficient to force a C-265 molecule, of relatively lower thermal stability compared with that of *aci*-C-264, into an anionic form.

Influence of Solvent on the Electronic Absorption Spectra and Tautomeric Equilibria.—The results of theoretical calculations regarding structural examinations, as well as the physicochemical characteristics and spectral properties of the nitro isomers of *N,N*-dimethyl-*N'*-(nitro-9-acridyl)propane-1,3-diamine, imply that the two long-wavelength bands probably arise from the two tautomeric forms of the compounds existing in the liquid phase. The imino structure of the 1-nitro isomer, in which the compound exists in the solid phase, is also retained in polar or protic solvents.[18] However, the equilibrium moves towards the amino tautomeric form in non-polar and aprotic solvents.[18] This phenomenon can partially be accounted for by the difference in polarity between different forms. The imino form, which is characterized by a relatively high μ, is stabilized in polar media. On the other hand, the amino form, much less polar, should predominate in an inert media. This latter behaviour does not correlate with the relative energies of both tautomeric forms. As shown in Table 1, the amino forms are always less thermodynamically stable than the corresponding imino tautomers. The influence of polarity of the medium on the tautomeric equilibria can be considered analogously in the case of the remaining compounds studied. Both the amino and imino tautomers of C-264 and C-257 are characterized by having comparable μ values. Thus, both forms should behave similarly in polar and inert media, as was demonstrated by the absorption spectra.[18] The difference in polarity again shows C-265 to have an imino form which is much less polar than its amino form. Examination of the long-wavelength absorption of the compound in various solvents indicated that the bands between 350 and 400 nm disappear and at the same time there is a simultaneous increase of the lowest energy band.[1] This means that the equilibrium in the case of the 4-nitro isomer would move towards the amino tautomer in the liquid phase.

The tautomeric equilibrium state should be influenced also by the possibility of interaction with the solvent through hydrogen bonding. The effect can be predominant in protic solvents. The results of our present calculations do not, however, enable us to draw any further conclusions due to the complexity of this type of interaction.

The above discussion clearly demonstrates that in the liquid phase the tautomeric equilibrium state depends on the properties of the medium. Examination of this problem was not the primary aim of the present work. Nevertheless, to have some idea of the order of the value of the equilibrium constant in inert solvents we estimated the c_{am}/c_i ratio for C-283 dissolved in hexane. For this purpose it was assumed that the absorption coefficient at λ_{max} is proportional to the concentration of a given species. Furthermore, we assumed the absorption coefficient of the imino tautomer of C-283 to be identical with that characteristic of compound (**II**) (Figure 6). Since the electronic absorption is characterized by many bands overlapping each other we deconvoluted the spectra of C-283 (Figure 2) and the reference substance (Figure 6) by assuming Gaussian profiles of individual bands and by using the Marquardt optimization method.[49] This procedure gives a value for the equilibrium constant of *ca.* 0.17. This roughly estimated value indicates that in the inert liquid phase the equilibrium moves greatly towards the amino structure. This structure is not represented in the crystal phase of the compound.

Conclusions.—The MNDO method applied in this work enabled evaluation of the structures of several tautomeric forms of *N*-(nitro-9-acridyl)methanamines. Moreover, the theoretical calculations performed provided some information, not previously available, regarding the physicochemical characteristics of these derivatives. The important conclusion of the present study is that nitracrine and its nitro isomers in solution do not exclusively maintain the structure characteristic of the crystal phase. Furthermore, the structure in the liquid phase depends heavily on the properties of the medium. It may thus be expected that under physiological conditions the structure can also change. Consequently, this feature of the compounds examined can affect transport phenomena, as well as the mechanism of their biological action. For further insight into this problem it would seem to be important to investigate the influence of the properties of the medium and temperature on the tautomeric equilibria. Another interesting subject is the tautomerism of protonated forms of the compounds. This is of special interest since the dihydrochlorides of these derivatives are soluble in water, and thus are suitable for medical applications. We are currently working on these problems.

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