Characterization of the Ultraviolet Absorption Spectra of Para-Substituted Derivatives of Benzamidine[1]

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Abstract: Attempts are made to establish correlations between the absorption bands of benzene and those of substituted benzamidines. The molar extinction coefficients of the following para-substituted benzamidinium ions were determined: $-NH_2$, $-OH$, $-OCH_3$, $-CH_3$, $-H$, $-F$, $-Cl$, $-Br$, $-CO_2Et$, and $-NO_2$. The spectra were obtained in acid, neutral, and alkaline aqueous media and in 1-butanol. The observed bathochromic shifts caused by the substituents agree well with the values calculated by MO theory using the perturbational method. There exist strong correlations between $-\sigma_R$ and the shifts caused by the introduction of substituents, by the change in pH, and by the change in solvent polarity. The shifts caused by the substituents also correlate well with σ_p, but with two intercepting regression lines. The shifts are interpreted in the language of the valence bond theory. All the compounds exhibited a $^1A-^1L_a$ band, while the $^1A-^1L_b$ band only appeared in the spectra of the benzamidinium and *p*-carbethoxybenzamidinium ions. This fact permits the inference that the single band which appears in the spectra of the remaining compounds is a composite of the $^1A-^1L_a$ and $^1A-^1L_b$ absorptions.

Benzamidine and its ring-substituted derivatives constitute a group of compounds which are potent reversible inhibitors of the serine proteases trypsin,[2a-d] human urinary kallikrein,[2e] and rat urinary kallikrein.[3] References to benzamidine derivatives in the literature are quite scarce, and the articles which have been published refer to either their synthesis[4,5] or their behavior as enzyme inhibitors.[6] Very few studies have been made of their ultraviolet, infrared, or nuclear magnetic resonance spectra, their acid-base behavior, or their quantum mechanical characteristics. In this series of articles, we present a systematic study of the π-electronic structure of a series of para-substituted benzamidines to better understand their physical-chemical properties and their interactions with the active center of trypsin. In the present study, we have attempted to establish a correlation between the absorption bands of benzene and the benzamidine derivatives, as well as to interpret, in terms of resonance structures, the effect on the ultraviolet spectra caused by a change in pH, variation of solvent, and ring substitution.

Results

Effects of Para Substituents, pH, and Solvent. As can be seen in Figure 1, A and B, the spectra are quite symmetrical about the absorption maxima except for the *p*-hydroxybenzamidinium ion, whose band asymmetry suggests that it is a composite band. The $^1A-^1L_b$ band is only visible in the spectra of the benzamidinium, *p*-carbethoxybenzamidinium, and *p*-carboxybenzamidinium ions. An interesting observation is the fact that the $^1A-^1L_b$ band, with some exceptions, only appears in the spectra of para-substituted derivatives in which the two substituent groups have approximately the same overall electron-withdrawing or -donating power (qualitative). Table I lists the experimental λ_{max}, molar extinction coefficient, and the spectral band width for each of the benzamidine derivatives. The data in Table II show that the substituent effect on the bathochromic shift of the $^1A-^1L_a$ band at pH 8.0 is $O^- > NH_2 > OCH_3 > NO_2 > Br > CH_3 > Cl \simeq CO_2R \simeq COO^- > F > H$. Deprotonation of the para-substituted benzamidines causes a hypsochromic shift of the $^1A-^1L_a$ band in the following order: $CH_3O > CH_3 > Br > F > Cl > COOH \simeq H \simeq COOR$. A bathochromic shift occurred with three other substituents in the order $NH_3^+ > OH > NO_2$. Also, varying the solvent from water to 1-butanol causes a bathochromic shift of the $^1A-^1L_a$ band for these derivatives in the order: $NH_2 > OH > CH_3O > Br > H > Cl \simeq COO^- > COOR \simeq F \simeq CH_3$. A hypsochromic shift was exhibited by two substituents in the order $O^- > NO_2$.

Discussion

The Stevenson[7] modification (eq 1) of the Petruska[8] equation for substituted benzenes was used for the calculation of the $^1A-^1L_a$ absorption maxima for the para-substituted benzamidines, ν_x and ν_b being the ν_{max} for para-substituted benzamidinium ions and benzene, respectively.

$$\nu_b - \nu_x = K(l_0 + l_3) + (x_0 - x_3)^2 \tag{1}$$

The parameter l refers to the shift in ν_{max} of benzene caused by the substituent. The value of K depends on the number of substituents on the benzene ring. The values of x_0 and x_3 depend on the electron-withdrawing or -donating ability of the substituents in the para position. The values of the parameters of eq 1 are shown in Table III. The parameters l_0 and x_0 for the amidine and amidinium groups were not determined; for the purpose of making semiquantitative predictions, these were assumed to be equal to those of the carboxylate and carboxyl groups, respectively. This assumption is supported by the following observations. (1) There is a structural similarity between the amidinium and carboxyl groups on one hand and between amidine and carboxylate on the other, each pair having the same geometric form and the members of each pair being approximate isosteres. (2) The $^1A-^1L_b$ band appears in the spectrum of the *p*-carboxybenzamidinium ion, suggesting that these substituents are approximately equivalent in their withdrawing or donating ability as was noted above for homo-substituted benzenes.[9] (3) There is a strong correlation between the bathochromic shift caused by substituents in para-substituted benzoic acid derivatives and para-substituted derivatives of benzamidine (Figure 2).

Effect of Substituents on the Spectra. Table III shows that there is very good agreement between the bathochromic shifts (relative to benzene) observed with substituents and the shifts calculated by eq 1. This fact suggests that the intense band ($\epsilon_M \simeq 10^4$) in the spectra of the para-substituted benzamidinium ions originates from the $^1A-^1L_a$ transition. On the other hand, the fact that the $^1A-^1L_b$ band only appears in the spectra of the benzamidinium, *p*-carbethoxybenzamidinium, and *p*-carboxybenzamidinium ions suggests that, in the case of the other derivatives, the in-

Table I. Absorption Spectral Data of Para-Substituted Benzamidinium Ions

Substituent X	λ_{max}, nm	Molar extinction Coeff, $M \times 10^4$ cm^{-1}	Bandwidth, nm
OH	303.0	1.878 ± 0.026	0.45
NH_2	292.0	1.528 ± 0.046	1.04
OCH_3	258.0	1.386 ± 0.023	0.26
Br	244.0	1.997 ± 0.029	0.70
Cl	240.0	1.467 ± 0.029	0.70
F	231.0	1.361 ± 0.021	0.66
CH_3	241.0	1.023 ± 0.022	0.61
COO^-	234.0	1.019 ± 0.012	0.72
COOR	238.5	1.736 ± 0.032	1.00
NO_2	256.0	1.377 ± 0.031	0.24
H	225.0	0.939 ± 0.079	1.10

tense absorption ($\epsilon_M \simeq 10^4$) is a composite band resulting from the superposition of the $^1A-^1L_b$ and $^1A-^1L_a$ bands.[7] The superposition may result from the fact that the $^1A-^1L_a$ π-electron transition is more sensitive to perturbation by substituents than the $^1A-^1L_b$ transition.[9] In general, the intensities and frequencies of the $^1A-^1L_b$ absorption cannot be determined because of this superposition. The intensities of the absorptions of the $^1A-^1L_a$ bands also do not serve for comparison since they are not electronically pure, being strongly influenced by atomic vibrations in the molecule. Stevenson[7] divided the substituents into two groups: electron acceptors A and electron donors D. The bathochromic shifts[10] of the $^1A-^1L_a$ bands in the spectra of monosubstituted benzenes are, for electron donators (D), $O^- > NH_2 > OCH_3 > OH > Br > Cl > CH_3$ and, for electron acceptors (A), $NO_2 > CHO > COCH_3 > COOH > CN > SO_2NH_2 > NH_3^+$. For para-disubstituted benzenes, if both the substituents are donors (DD) or acceptors (AA) of electrons, the bathochromic shifts are similar to those of the monosubstituted benzenes. However, when the two substituents are complementary (AD or DA), the shift is very large. The para-substituted derivatives of benzamidine of the complementary type follow the order of bathochromic shift given above, except for the *p*-methylbenzamidium ion. It is possible that the anomalous effect shown by this derivative is due to a solvation energy which is different from that of the other derivatives. The noncomplementary para-substituted benzamidines follow the order shown above ($NO_2 > CO_2R$).

Simpson and Looney[11] emphasized the importance of intramolecular charge transfer in the excited state of the $^1A-^1L_a$ transition. Thus, the electron-donating groups O^-, NH_2, OH, and OCH_3, acting by a σ-inductive mechanism (I^-), would destabilize the excited state and provoke a hypsochromic shift, which is contrary to the experimental observations. If the substituents were to act through resonance (M^+), they should stabilize the excited state (II in eq 2), causing a bathochromic shift in the order $O^- > NH_2 > OH > OCH_3$. The experimental results follow this order except that $OCH_3 \simeq OH$. In fact, there is a good correlation ($r = 0.9472$) between $\Delta\nu_{max}$ and the resonance parameters ($-\sigma_R$) except for F, CO_2R, and NO_2. Also, Figure 3 shows that there is a good correlation between $\Delta\nu_{max}$ and the normal σ para values of Hammett ($\sigma_p = \sigma_I + \sigma_R$) for electron donors, thus confirming that the resonance stabilization effect is important in this group.

On the other hand, these electron donors could act via a π-inductive effect which would also stabilize the excited state, leading to a bathochromic shift. The stabilization by resonance may be more efficient, however. The order of the experimental bathochromic effect for the para-substituted benzamidines (Br > Cl > F) is the inverse of the order of the resonance parameters. If the halogens acted by an in-

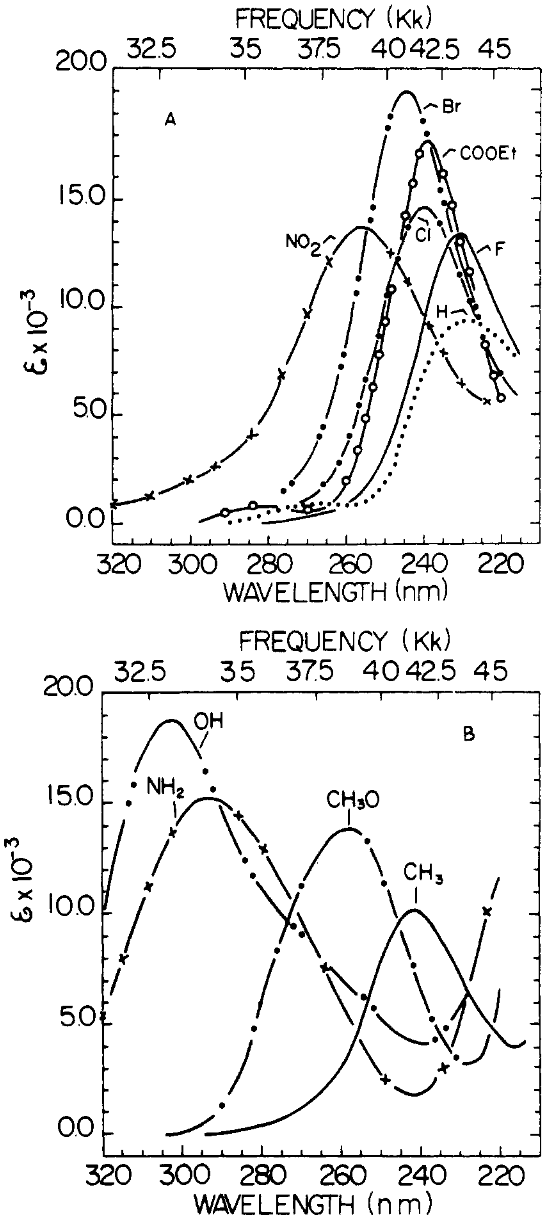
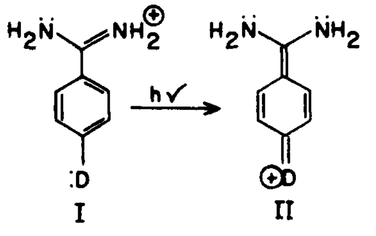


Figure 1. The absorption spectra of para-substituted benzamidine derivatives in Tris 0.10 *M*, pH 8.0, at 25°. (A) derivatives with $\sigma_p > 0$: (••••••) BzA^+; (—) *p*-$FBzA^+$; (— • — • —) *p*-$ClBzA^+$; (— • • —) *p*-$BrBzA^+$; (—o—) *p*-EtO_2CBzA^+; (—×—) *p*-NO_2BzA^+. (B) Derivatives with $\sigma_p < 0$: (—) *p*-CH_3BzA^+; (— • —) *p*-CH_3OBzA^+; (—×—) *p*-NH_2BzA^+; (— • • —) *p*-$HOBzA^+$.



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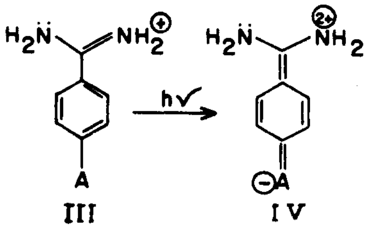


III IV

(3)

Table II. Effect of the Variation of pH and Solvent Polarity on the Absorption Maxima of Para-Substituted Benzamidinium Ions

Substituent X	ν_{max} (pH 1), kK	ν_{max} (pH 8), kK	ν_{max} (pH 13), kK	ν_{max} (BuOH), kK	$\Delta\nu^a$ (H^+, OH^-), kK	$\Delta\nu^b$ (H_2O, ROH), kK
OH	38.91	33.00	34.50	36.77	4.41	2.14
NH_2	44.44	34.25	36.83	32.00	7.61	2.25
OCH_3	38.31	38.76	40.00	38.02	−1.69	0.74
Br	41.15	41.15	42.37	40.48	−1.22	0.67
Cl	41.93	41.93	42.55	41.49	−0.62	0.44
F	43.29	43.29	44.25	43.10	−0.96	0.19
CH_3	41.15	41.67	42.73	41.49	−1.58	0.18
H	43.86	43.86	44.05	43.29	−0.19	0.57
NO_2	39.06	39.06	37.31	39.37	1.75	−0.31
COOR	41.98	41.98	42.12	41.75	−0.14	0.23
COO^-	42.00	42.00	42.20	41.60	−0.20	0.40

[a] $\Delta\nu$ (H^+, OH^-) is obtained by subtracting the values of ν_{max} (pH 13) from ν_{max} (pH 1). [b] $\Delta\nu$ (H_2O, ROH) is obtained by subtracting the values of ν_{max} (BuOH) from ν_{max} (pH 8) except for OH, which is ν_{max} (pH 1) minus ν_{max} (BuOH).

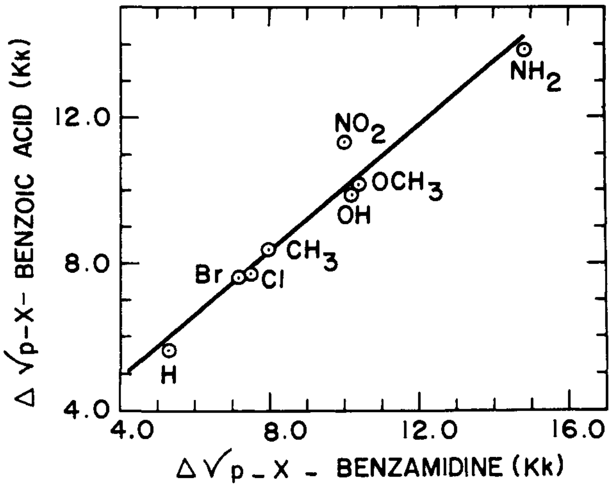


Figure 2. Correlation of the bathochromic shifts, relative to the ${}^1A-{}^1L_a$ band (202 nm) of benzene, of para-substituted benzoic acids with para-substituted benzamidinium ions, $r = 0.9794$.

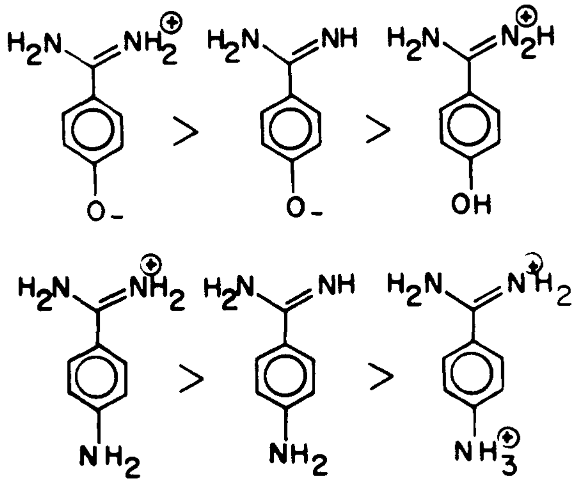
Table III. Comparison of the Experimental $\Delta\nu_{max}$ and the Calculated Values Obtained from Eq 1, with Respective Parameters

Substituent X	l_3 cm^{-1}	x_3 $cm^{-1/2}$	$\Delta\nu$ calcd, kK	$\Delta\nu^a$ exptl, kK
O^-	6.58	1.62	16.93	16.10
OH	1.63	0.68 ± 0.10	8.82	10.19
NH_2	5.65	1.42 ± 0.08	15.16	14.85
OCH_3	3.05	0.80 ± 0.10	10.40	10.34
CH_3	0.71	0.12 ± 0.20	6.47	7.43
$C(NH)NH_2$	4.49	−0.82	8.26	5.05
$C(NH_2)_2^+$	5.65	−1.03	5.64	5.24
F	0.55	0.00	6.08	5.81
Cl	1.40	0.20 ± 0.15	7.22	7.17
Br	1.51	0.64 ± 0.20	8.59	7.59
COOR	5.65	−1.03 ± 0.15	9.15	7.17
NO_2	11.89	−1.50 ± 0.06	14.43	10.04

[a] ${}^1A-{}^1L_a$ (benzene) 49.10 kK.

ductive effect (I^-), they would cause a hypsochromic shift relative to the benzamidinium ion; this is not consistent with the observed order. The observed order of the bathochromic shifts caused by the halogens, Br, Cl and F, is the same as the decreasing order of atomic radii, suggesting that for these substituents, it is the polarizability or π-inductive effect which stabilizes the excited state more than the ground state. Indeed, the order of the bathochromic shifts observed for the halogens and hydrogen (Table III) agrees with the tabulated polarizability for the C-X bond,[12] Br > Cl > F > H. Also, Schubert and coworkers,[13] studying para-halo-substituted anisoles and nitrobenzenes, indicated polarizability and solvent polarization as stabilization factors in these compounds. Thus, the stabilization by a polarizability or π-inductive effect seems to be important in the para-halo-benzamidines as well as in CO_2H, CO_2R, and NO_2 substitution. Although $\Delta\nu_{max}$ for the nitro group does not correlate well with $-\sigma_R$, it correlates well with the total σ, as shown by the line of positive slope in Figure 3, suggesting that this group stabilizes the excited state (structures II and IV, eq 2 and 3) via a combined resonance and predominant polarity effect. As for the methyl group (Figure 3), the greater stabilization of the excited state may be attributed to an inductive effect or to hyperconjugation. Schubert, Robins, and Haun[14] studied *p*-alkyl-substituted nitrobenzenes and acetophenones and showed that the bathochromic shifts for these derivatives correlate with the inductive effects of the substituents.

Effect of pH on the Spectra. The ${}^1A-{}^1L_b$ band, which appears in the spectra of the benzamidinium and *p*-carbethoxybenzamidinium ions, disappears with deprotonation. At least in part, this may be attributed to the reduction of symmetry of the amidinium groups through deprotonation. The values of λ_{max} for the ions derived from *p*-hydroxybenzamidine and *p*-aminobenzamidine correspond to the sequences



These are exactly the sequences expected as a consequence of the greater resonance stabilization of the excited states in relation to the ground states. The hypsochromic shifts of the ${}^1A-{}^1L_a$ band, caused by deprotonation of the amidinium group, can be rationalized on the basis of valence bond theory as shown in Figure 4. One may infer from this diagram that the order of decreasing hypsochromic shift caused by deprotonation of the amidinium group should parallel the order of decreasing substituent reso-

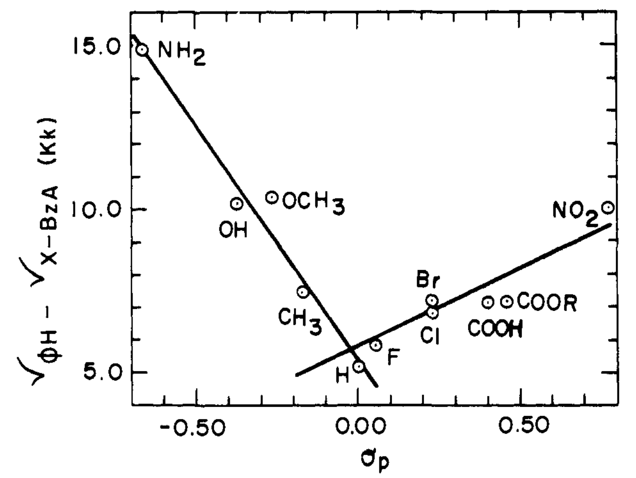


Figure 3. Correlation of the bathochromic shifts of para-substituted benzamidinium ions, relative to the $^1A-^1L_a$ band (202 nm) of benzene with σ_p, $r = 0.8953$.

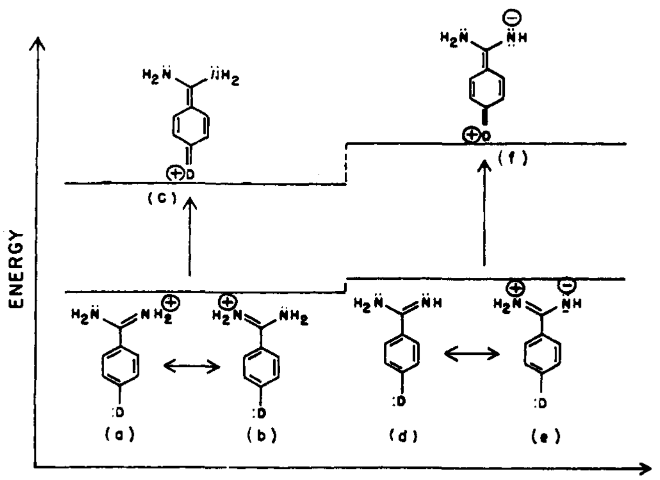


Figure 4. Resonance structures indicating the effects of protonation of the benzamidinium ion on the relative stabilities of the ground and excited states.

nance. The substituent exerts a greater effect on the stability of the excited state than on the ground state since c and f contribute little to the stability of the ground states because of the loss of aromaticity in these structures. The difference in energy between the ground and the excited states of the benzamidinium ion would not be so great because c has the same charge and number of double bonds as a and b. Although the ground state of the unprotonated amidine would be less stable than the protonated form because of the small contribution of e to the stability, there would be an even greater difference between the two excited states because of the greater separation of charge in f, all other factors being equal. Roughly speaking, the blue shift reflects the difference in charge separation between structures e and f. Thus, the effect of protonation on the stability of the excited state would be greater than its effect on the ground state, provided that resonance was the predominant factor contributing to the stability of the molecule.

Figure 5 confirms that, with the exception of the *p*-nitro-, *p*-hydroxy-, and *p*-aminobenzamidine derivatives, resonance is an important feature of the hypsochromic effects caused by a change in pH. However, valence bond theory does not appear to explain the bathochromic shifts of the *p*-nitro-, *p*-hydroxy-, and *p*-aminobenzamidine derivatives. The *p*-hydroxy- and *p*-aminobenzamidines may deviate from the regression line of Figure 5 because their charged substituents undoubtedly interact strongly with solvent.

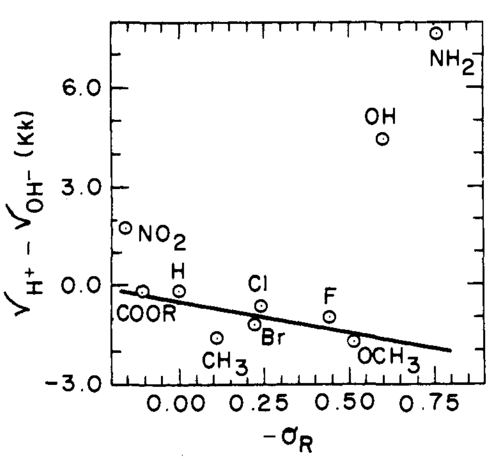


Figure 5. Correlation of the hypsochromic shifts caused by deprotonation of the para-substituted benzamidinium ions with $-\sigma_R$, $r = 0.6797$.

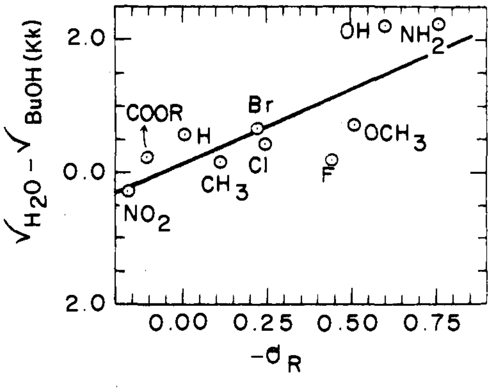


Figure 6. Correlation of the bathochromic shifts caused by a decrease in solvent polarity (for para-substituted benzamidinium ions) with $-\sigma_R$, $r = 0.8072$.

Effect of Solvent on the Spectra. The solvent change from water (Tris 0.1 *M*, pH 8.0) to 1-butanol causes a bathochromic shift in λ_{max} for nearly all the para-substituted benzamidinium ions; the *p*-nitro derivative exhibits a hypsochromic shift. This suggests that, with the exception of the *p*-nitrobenzamidinium ion, the ground state is more polar than the excited state, which is consistent with the resonance structures shown in eq 2. The formal positive charge is more dispersed in the excited than in the ground state and, consequently, the latter should be more polar. The ground state will be less stabilized by resonance than the excited state as the solvent polarity decreases. The greater the electron-donating power of the substituent, the greater will be the difference in polarity between the ground and excited states and greater will be the bathochromic shift.

Figure 6 illustrates the correlation between the shift in absorption frequency with change of solvent polarity and $-\sigma_R$. This correlation reinforces the importance of resonance in the ground and excited states. Since the correlation with $-\sigma_R$ is not very strong, other effects such as induction and ion-dipole, induced ion-dipole, dipole-dipole, and hydrogen bond interactions with the solvent, as well as σ-inductive effects appear to play an important role in the stabilization of the ground and excited states. In fact, a strong correlation with σ_I was found for NO_2, F, Cl, Br, and NH_2. In addition, Schubert and coworkers[15] showed that the polarizability of the molecule and solvent polarization greatly affect the absorption frequencies of para-halo-substituted benzenes. This polarizability effect would tend to mask the resonance and inductive effects of the halo groups.

Table IV. Analyses and Melting Points of New Para-Substituted Benzamidinium Chlorides

Substituent X	Mol wt	H_2O (η)	Mp, °C	Calculated, % C	H	N	Cl	Br	Found, % C	H	N	Cl	Br
OCH_3	186.65		218–220 (122–124)[a]	51.48	5.94	15.01	19.00		51.65	6.04	14.92	19.16	
Br	271.55	2	267–269	30.96	4.45	10.31	13.06	29.42	31.03	4.53	10.19	12.91	29.34
Cl	201.07	½	243–245	41.81	4.51	13.93	17.63		42.28	4.63	14.04	17.81	
F	192.63	1	212.5–214.5	43.72	5.23	14.54	18.40		42.91	5.42	14.70	18.55	

[a] Free base.

With the data available, it is not possible to distinguish between these other effects. The deviation of the *p*-hydroxybenzamidinium ion from the regression line of Figure 6 may be attributed to the existence of an equilibrium with the *p*-oxybenzamidinium form at pH 8.0, although the unprotonated form should predominate. The nitro group, having greater electron-withdrawing power than the amidinium group, inverts the polarity of the excited state and a hypsochromic shift with reduction of solvent polarity would be expected. This is consistent with the experimental observations.

The association of small molecules of appropriate optical properties with macromolecules may yield valuable information about the structure of the macromolecule. The small molecule may act as a probe; that is, the value of one of its physical parameters will reflect its microscopic molecular environment. The information obtained in this study of the effects of substituents, pH, and polarity of the environment on the spectra of benzamidines has been used in the interpretation of the interactions of these compounds with trypsin and will soon be published.[19]

Conclusion

We have found the following. (1) There is a good correlation between the ν_{max} of para-substituted benzamidines and the frequencies of the ${}^1A$-${}^1L_a$ band calculated using perturbation theory. (2) In the majority of the spectra of the para-substituted benzamidines studied, the principal band is a composite of the superimposed ${}^1A$-${}^1L_a$ and ${}^1A$-${}^1L_b$ bands. (3) Resonance is an important factor in the bathochromic shifts caused by substitution and by changing solvent polarity. (4) Resonance is an important factor in the hypsochromic shifts caused by a change in pH. (5) The fact that the shifts of the ${}^1A$-${}^1L_a$ band correlate with the reactivity parameters (σ's) is an indication that the changes that occur in the electron distribution in the molecule as a result of electronic excitation are similar to those that occur in the formation of the transition state during a chemical reaction.

Experimental Section

Materials and Methods. The following substituted benzamidines were studied: *p*-fluoro, *p*-chloro, *p*-bromo, *p*-methyl, *p*-methoxy, *p*-hydroxy, *p*-amino, *p*-nitro, *p*-carboxy, and *p*-carbethoxy. *p*-Hydroxybenzamidine was prepared from *p*-cyanophenol by the method of Partridge and Short.[16] *p*-Aminobenzamidine was prepared by catalytic hydrogenation of *p*-nitrobenzamidine, according to the method described by Shaw and Wooley.[17] *p*-Methylbenzamidine was described earlier by Mares-Guia.[2b] The *p*-carboxy derivative was prepared by hydrolysis of the corresponding ethyl ester. All other para-substituted benzamidines were prepared from the corresponding benzonitriles by the classical Pinner[18] method. Benzamidinium chloride and para-substituted benzonitriles were obtained from Aldrich. All other reagents were analytical grade. Table IV gives the elemental analyses and some physical properties for the benzamidine derivatives.

For each derivative, solutions of at least seven different concentrations were prepared in Tris 0.10 *M*, pH 8.0, at 25°, such that the absorbancies fell within the range of 0.100–1.600. The spectra were recorded with a Perkin-Elmer Model 450 double beam spectrophotometer. The base line from 400 to 220 nm was obtained using two 1.000-cm quartz cells filled with 3.00 ml of Tris 0.10 *M*, pH 8.0. The sample cell was then washed, dried, and filled with the inhibitor solution. The resolution and response time were adjusted such that a slit width of less than 1 mm could be maintained, and the spectrum was run at a speed compatible with the response time of the instrument. The absorbancies at a given wavelength for different concentrations of inhibitor were analyzed according to the Lambert–Beer law using a Fortran IV computer program, "CEXMO". This program analyzes the data following the linear regression model by the method of least squares, providing a graph of the absorbancies as a function of solute concentration and showing the regression line as well as the confidence limits. It also provides the molar extinction coefficient and its standard deviation.

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