POLAROGRAPHIC AND SPECTROPHOTOMETRIC BEHAVIOUR OF SOME N-p-PHENYL SUBSTITUTED BENZAMIDINES

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Five N-p-phenyl substituted benzamidines were studied by DC and DP polarography in a wide pH range. Coulometric results show that the overall processes are four-electron reductions. Logarithmic analysis of the waves indicate that the process are irreversible. The influence of the pH on the polarographic parameters was also studied. A UV spectrophotometric study was performed in the pH range 2–13. In basic media some variations in the absorption bands were observed due to the dissociation of the amidine group. A determination of the pK values was made by deconvolution of the spectra. Correlations of both the electrochemical parameters and spectrophotometric pK values with the Hammett substituent constants were obtained.

The amidine group is the nitrogen analogue of carboxylic acids[1], its biological formation ocurring during the biosynthesis of the purine ring system[2,3]. Furthermore, the biological activity of amidines against pathogenic microorganism or as anti--hypertensive, anti-fibrillatory and anti-cancer agents has also been reported[4,5]. It was reported that amidines were active against human leukemia and lymphoma and that stilbamidines have potentiated the activity of methylglyoxal bis(guanyl-hydrazone) in transplanted mouse leukemia[6]. Terephtanilide derivatives were shown to induce inhibition of DNA synthesis at therapeutic doses[7]. Diarylamidines inhibited DNA polymerase in Moloney murine leukemia virus[8,9]. Afterwards, Dumont et al.[10] reported the effect of a large number of substituted amidines and bis-amidines against a sensitive murine P 388 leukemia in order to detect anti-cancer activity.

Polarography of amidines is represented mainly by a single paper[11], in which it was shown that the behaviour of this group of compounds at the mercury electrode is extremely diverse. In general, aliphatic amidines are not polarographically reducible in buffered solutions; aromatic amidines are reducible only at rather negative potentials at a pH interval from slightly acid to alkaline solutions. Four electrons are transferred in the overall reaction to yield an amine. Cyclic amidines, such as 3,4-dihydroquinazoline[12] and 1,6-dihydropurine[13–15] are reduced in a similar way
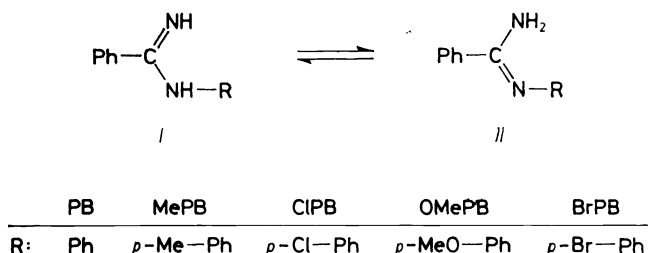
to acyclic amidines. Some amidines such as 2-phenoxyacetamidine[11] which are substituted in the *p*-position with one heteroatom, are polarographically reducible, but the electrode reaction consists of a reductive cleavage of the carbon–heteroatom single bond. Recently, the electrochemical reduction of benzamidine, N-phenyl-benzamidine and N,N′-diphenylbenzamidine were studied in aprotic solvents[16]. The process involved the anion radical formation through two one-electron transfers.

The present study deals with the polarographic and spectrophotometric behaviour of a comprehensive series of N-*p*-phenyl substituted benzamidines in aqueous media in order to report on the substituent effects in both the reduction of the amidine group and the dissociation pK values.

EXPERIMENTAL

Chemicals

The parent structures of the N-*p*-phenyl substituted benzamidines examined in the present study are shown in Scheme 1. The compounds were synthesized following a general procedure described earlier[17]. Physical and spectroscopic characteristics of the obtained compounds were as follows.

	PB	MePB	ClPB	OMePB	BrPB
R:	Ph	*p*-Me—Ph	*p*-Cl—Ph	*p*-MeO—Ph	*p*-Br—Ph

SCHEME 1

N-*Phenylbenzamidine* (*PB*). Yield 65% white crystals from benzene–petroleum ether (40 : 60); m.p. 115—117°C (114—115°C in ref.[18]). IR (KBr) cm^{-1}: 3 472 (m); 3 356 (m); 1 621 (s). ^{1}H-NMR (CCl$_4$) ppm: 8·00—6·70 (m, 10 H); 4·70 (s, broad, 2 H). Signal at 4·70 ppm exchange with D$_2$O.

N-(*p-Methylphenyl*)*benzamidine* (*MePB*). Yield 98·2% white crystals from acetate–petroleum ether (40 : 60), m.p. 101—102°C. IR (KBr) cm^{-1}: 3 571—2 632 (broad); 1 587 (s). ^{1}H NMR (CCl$_4$) ppm: 7·68 (m, 2 H); 7·27 (m, 3 H); 7·00 (d, 2 H, J_d = 8); 6·63 (d, 2 H, J_d = 8); 4·78 (s, broad, 2 H); 2·30 (s, 3 H). Signal at 4·78 ppm exchange with D$_2$O.

N-(*p-Chlorophenyl*)*benzamidine* (*ClPB*). Yield 77·6% white crystals from acetate–petroleum ether (40 : 60); m.p. 121—122°C (114—115°C in ref.[19]) IR (KBr) cm^{-1}: 3 472 (m); 3 448 (m); 1 615 (s); 1 582 (s). ^{1}H NMR (CDCl$_3$) ppm; 7·78 (m, 2 H); 7·60—7·10 (m, 6 H); 6·85 (9 d, 2 H, J_d = 9); 4·70 (s, broad, 1 H). The ^{1}H NMR spectrum of a freshly prepared solution of ClPB in CCl$_4$ shows: 7·73 (m, 2 H); 7·47—7·03 (m, 5·8 H); 6·34 (d, 2 H, J_d = 9); 4·57 (s, broad,

1·3 H). ^{1}H NMR spectrum integration indicates the presence of a 76: 24 (%) = 1 : 2 mixture (Scheme 1). After 30 min the above solution showed the following absorptions: 7·65 (m, 2 H); 7·45—6·90 (m, 5 H); 6·67 (m, 2 H); 4·73 (s, broad, 2 H).

N-(*p-Methoxyphenyl)benzamidine (OMePB)*. Yield 65%; crystals from acetone-petroleum ether (40 : 60); m.p. 117°C (115·5—116·5°C in ref.[19]). IR (KBr) cm^{-1}: 3 488 (m); 3 125 (broad); 1 592 (s); 1 563 (s). ^{1}H NMR (CDCl$_3$) ppm: 7·80 (m, 2 H); 7·57—7·17 (m, 4 H); 6·90 (s, 4 H); 4·77 (s, broad, 1 H); 3·80 (s, 3 H). Signal at 4·77 exchange with D$_2$O.

N-(*p-Bromophenyl)benzamidine (BrBP)*. Yield 59·8%; crystals from petroleum ether–acetone (80 : 20); m.p. 122°C. IR (KBr) cm^{-1}: 3 333 (m); 1 600 (s); 1 565 (s). ^{1}H NMR (CCl$_4$) ppm: 7·58 (m, 2 H); 7·20 (m, 5, 3 H); 6·57 (d, 2 H, J_d = 9); 4·53 (s, broad, 1·7 H). The ^{1}H NMR spectrum integration indicates the presence of a 30 : 70 (%) = 1 : 2 mixture (Scheme 1).

All melting points are uncorrected. All reagents were p.a. grade. As supporting electrolyte buffered solution were used with the following components and concentrations: 0·02M acetic acid and 0·02M phosphoric acid for pH < 8·5 and 0·02M phosphoric acid and 0·02M NaHCO$_3$ for pH > 8·5. The pH was adjusted with NaOH and the ionic strength to 0·3M with KCl. All the N-*p*-phenyl substituted benzamidines were previously dissolved in 1% ethanol and then buffered. All solutions were measured against the saturated calomel electrode (s.c.e.). The solutions were purged with purified nitrogen prior to the electrochemical experiments. The temperature was kept at 25 ± 0·1°C. For UV spectrophotometric measurements buffered solutions consisting of 0·1M H$_3$BO$_3$ — 0·1M NaOH and 0·1M KCl were used.

Apparatus

The ^{1}H NMR spectra were recorded on a VARIAN EM-360 spectrometer, chemical shifts are quoted in ppm from internal TMS. The IR spectra were obtained (KBr) on a LEITZ III-G spectrophotometer, absorption frequencies are quoted in cm^{-1}.

Polarographic data were obtained from an INELECSA assembly equipped with the following elements: a) a generator/potentiostat, b) an interface containing 12 bits A/D and D/A converters attached to a microprocessor with suitable software for fully automated control of the experiments and data acquisition. A MULTITECH, APPLE II PLUS compatible microcomputer was used for data control and acquisition. Experimental data were automatically transferred to an ACER 710 MULTITECH, PC compatible microcomputer which was used for data treatment.

Coulometry was carried out using a conventional two compartment cell. A stirred mercury pool working electrode, platinum wire counter electrode and s.c.e. were also used. A TACUSSEL PRT20-2 potentiostat was used for the potential control and a TACUSSEL EPL-2 with TI-20G plug-in was employed as recorder.

Spectrophotometric measurements were performed on a Hewlett–Packard 8452A diode array spectrophotometer. Absorption spectra have been analyzed by using a computer-assisted method early described[20] on the basis of a lognormal distribution.

RESULTS AND DISCUSSION

The reduction of five N-*p*-phenyl substituted benzamidines was studied in the pH range 0—14. All the compounds show one polarographic wave in the pH range under study. However, in acidic media, below pH 5, the wave is masked by the discharge of the supporting electrolyte. In the pH range 5—9 the wave is distorted by the

presence of polarographic maxima. The maxima turn out to be dependent on the depolarizer concentration, temperature and drop time, increasing when these parameters are increased. The maxima are due to a strong adsorption of the N-*p*-phenyl substituted benzamidines, especially in the halogenates compounds (ClPB and BrPB). In order to obtain reliable values of half-wave or peak potentials we avoided the maxima working at depolarized concentrations below 0.05 mmol l^{-1}, drop time of 1.0 s and a temperature of $25°C$.

The limiting polarographic current (i_1) of the whole family remains constant in the pH range $5-11$. Above pH 11 the current decreases until approximately in half at pH 13. The value of i_1 corresponds to a four-electron process (pH $5-11$), as was confirmed by controlled potential electrolysis at a mercury pool electrode. The peak currents of the differential pulse polarograms behave in a similar way. Four-electron processes have also been previously described[11] for other benzamidines.

The plots of both half-wave and peak potentials vs pH show two linear segments. The potentials are pH-independent at more acidic pH values (except for a slight variation for BrPB, probably due to adsorption interferences). In basic media, the potentials show a marked dependence on pH (Fig. 1). In Table I are shown the slopes of the linear segments. Due to the irreversibility of the processes, only apparent pK values can be obtained from the break of these lines. The polarographic pK are gathered in Table III.

In a previous study, Prevorsek[21] found that N-alkyl monosubstituted amidines exist mainly as the imino tautomers (I) whereas N-phenyl substituted amidines exist as the amino tautomers (II) (Scheme 1). The ^{1}H NMR spectra of the N-*p*-phenyl substituted benzamidines under study also agree with the Prevorsek's conclusion.
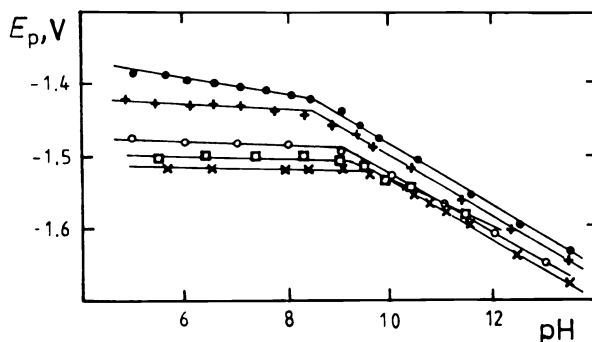

FIG. 1

E_p vs pH plots. $c = 0.025$ mmol l^{-1}: ○ PB; □ MePB; × OMePB; + ClPB; ● BrPB

TABLE I

Polarographic characteristics of N-p-phenyl substituted benzamidines. $c = 0.025$ mmol l^{-1}, pH 12

Compound	$dE/d \log (i/(i_1 - i))$ mV	dE_p/dpH mV	p
BrPB	-42	-43	1·0
ClPB	-41	-43	1·0
PB	-40	-39	1·0
MePB	-40	-35	1·1
OMePB	-40	-42	0·9

TABLE II

Wavelength maxima (in nm) for the best fitting of the deconvolution spectra of N-p-phenyl substituted benzamidines

Band No.	PB	MePB	OMePB	ClPB	BrPB
1	183	182	183	183	183
2	204	203	205	203	202
3	218	217	218	218	219
4	238	237	236	240	241
5	265	268	270	268	273

TABLE III

Polarographic and spectrophotometric pK values for N-p-phenyl substituted benzamidines

Compound	pK	
	polarographic	spectrophotometric
BrPB	$8·4 \pm 0·2$	$9·0 \pm 0·1$
ClPB	$8·5 \pm 0·2$	$9·1 \pm 0·1$
PB	$8·9 \pm 0·2$	$9·4 \pm 0·1$
MePB	$9·2 \pm 0·2$	$9·6 \pm 0·1$
OMePB	$9·4 \pm 0·2$	$9·7 \pm 0·1$

The protonation occurs at the lone pair of the sp^2-hybridized imino nitrogen[22,23] which due to its higher degree of s-character is less basic than the lone pair of the sp^3-hybridzed nitrogen of aliphatic amines. This fact allows us to conclude that the polarographic pK values must be related to the protonation at the imino nitrogen in the corresponding amino tautomer of the N-p-phenyl substituted benzamidines.

On the other hand, we have also obtained the dissociation pK values using the UV spectrophotometric technique with computerized deconvolution of the spectra.

Absorption spectra of the N-substituted benzamidines were obtained in a wide pH range. Several overlapped bands were observed in the $180-350$ nm wavelength range (Fig. 2). In basic media, two or three bands are observed depending on the pH. No variation in the spectra were observed in acid solutions. The changes were relationated with the ionization pK of the amidine group. A suitable characterization of the pK values of these substances requires a deconvolution of the experimental bands and the study of their variation with the acidity of the medium. A logarithmic normal distribution has been used in nitrogen derivatives[24,25].

In our work, five logarithmic normal bands were used in all the cases to resolve the experimental spectra in the absorption range studied (Fig. 3). In the high energy region, the spectra were adjusted to avoid interference with the main bands. Four parameter functions were adjusted by minimizing the residuals. These parameters are: absorption maximum wavelength, absorbance maximum, half-width of the band and the skewness of the band. In Table II the wavelength maxima used in our deconvolution are shown. The fitting show that only for the bands 3 and 4, significative variations with the pH of the absorbance maxima were observed, the rest of the
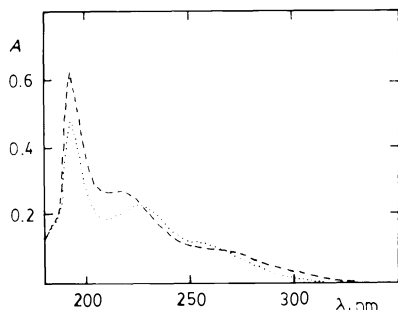

FIG. 2

Experimental UV spectra OMePB. $c =$ $= 0.3$ mmol l^{-1}: $\cdots\cdots$ pH 8.09; $---$ pH 10.87
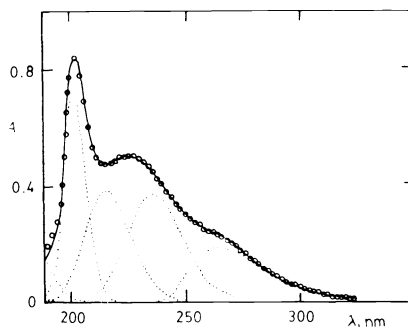
FIG. 3

Best fitting of the lognormal plots to experimental UV spectrum PB. $c = 0.16$ mmol l^{-1}, pH 9.32: ○ experimental data; fitted data: $\cdots\cdots$ individual bands; $---$ overall spectrum

parameters remaining uncharged. As can be seen in Fig. 4a, these variations resemble acid-base dissociation curves. The plots of $\log\left((A - A_{min})/(A_{max} - A)\right)$ vs pH are linear with slopes close to the unity in absolute value as can be expected for protonation equilibria (Fig. 4b). From the intercepts of these lines spectrophotometric pK values were obtained (Table III). The differences between polarographic and spectrophotometric pK values must be due to the irreversible character of the electrochemical processes. Nevertheless, a linear correlation is obtained between the two data sets.

The polarographic behaviour of these compounds have been studied at pH 7 (pH < pK) and pH 11 (pH > pK). The results are consistent with irreversible, diffusion controlled processes. For pH < pK the H$^+$ ions are not involved in the rate determining step. However, for pH > pK the mechanism involves a protonation prior to the rate determining step. The values of logarithmic analysis slopes, d$E_{1/2}$/dpH, and p (H$^+$ ion order) are given in Table I. As can be seen, one proton is
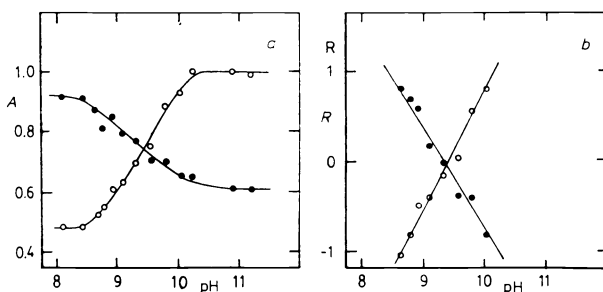

FIG. 4

PB. ● $\lambda = 238$ nm; ○ $\lambda = 218$ nm; $c = 0.3$ mmol l^{-1}. a Variation of the maxima absorbances with the pH. b Plot of $R = \log\left((A - A_{min})/(A_{max} - A)\right)$ vs pH
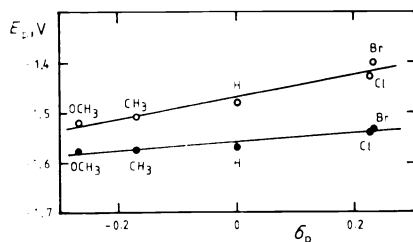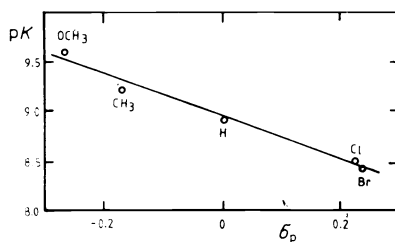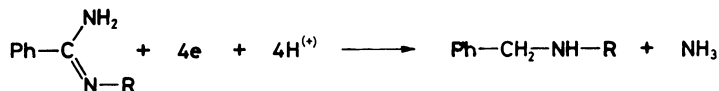

FIG. 5

E_p vs σ_p plots: ○ pH 7; ● pH 11

FIG. 6

Polarographic pK vs σ_p plot

transferred prior to the rate determining step. The proposed overall course for the reduction is shown in Scheme 2. A similar reduction mechanism was also proposed for aromatic amidines[11]. However that paper reports that at pH 9·5 amidines are hydrolyzed very quickly, and polarographic measurement are almost impossible. Our results show that these N-p-phenyl substituted benzamidines are stable and it is possible its study in strong basic media.

$$Ph-C\overset{NH_2}{\underset{N-R}{\big\langle}} \;+\; 4e \;+\; 4H^{(+)} \;\longrightarrow\; Ph-CH_2-NH-R \;+\; NH_3$$

SCHEME 2

For the substituent effect study, the E_p values are plotted against Hammet normal σ_p substituent constant[26] at pH 7 and 11 (Fig. 5). Linear relation with specific reaction constants of $\varrho = 0\cdot218$ (pH 7) and $\varrho = 0\cdot295$ (pH 11) was found. The existence of a correlation between electrochemical data and structural parameters derived from homogeneous reaction indicates that adsorption is not the predominant factor governing the electrochemical behaviour of these N-p-phenyl substituted benzamidines[27]. Thus, the validity of correlations based on linear free energy relationships demonstrated that the electrochemical behaviour of these benzamidines depends primarly on molecular structure and electron density distribution in a similar way to rates and equilibria of homogeneous chemical reactions. Specifically, the electrochemical behaviour of benzamidines depends on electron density distribution in the imino nitrogen. Furthermore, we have obtained a linear correlation between the polarographic pK and σ_p (Fig. 6). A positive value of specific reaction constant $(\varrho = 1\cdot9)$ was obtained. The correlation between the spectrophotometric pK and σ_p gives a ϱ value of 2·2.

In conclusion, this study shows that some correlations can be performed between electrochemical, spectrophotometric and structural parameters of the amidines. These correlations could be used in studies concerning biological activity of these compounds.

REFERENCES

1. Patai S. in: *The Chemistry of Amidines and Imidates* (S. Patai, Ed.). Interscience, London 1975.
2. Mizobuchi K., Buchanan J. M.: J. Biol. Chem. *243*, 4853 (1968).
3. Mizobuchi K., Kenyon G. L., Buchanan J. M.: J. Biol. Chem. *243*, 4863 (1968).
4. Narcisse G., Huguet F., Loisau P., Moreau R. C., Choay P.: Ann. Pharm. Fr. *37*, 325 (1979).

5. Bailer M., Yasen B., Mechoulam R., Becker Y.: J. Med. Chem. *23*, 1144 (1980).
6. Oettgen H. F., Clifford P., Burchenal H.: Cancer Chemother. Rep. *27*, 45 (1963).
7. Burchenal J. H., Purple J. R., Bucholz E., Straub: Cancer Chemother. Rep. *29*, 85 (1963).
8. Yesair D. W., Rogers W. I., Baranowsky P. E., Wodinsky I., Thayer P. S., Kensler C. Y.: Cancer Res. *27*, 314 (1967).
9. de Clercq E., Dann O.: J. Med. Chem. *23*, 787 (1980).
10. Dumont P., Fuks R., Atassi G., Hanocq M.: J. Pharm. Belg. *40*, 373 (1985).
11. Kane P. O.: Z. Anal. Chem. *173*, 50 (1960).
12. Lund H.: Acta Chem. Scand. *18*, 1984 (1964).
13. Smyth D. L., Elving P. J.: J. Am. Chem. Soc. *84*, 1412 (1962).
14. Janik B., Elving P. J.: Chem. Rev. *68*, 295 (1968).
15. Kwee S., Lund H.: Experientia Suppl. *18*, 387 (1971).
16. Hess V.: Z. Chem. *28*, 144 (1988).
17. Cooper F. C., Partridge A. W.: Org. Synth., Coll. Vol. *IV*, 769 (1963).
18. Sandler S. R., Karo W. in: *Organic Functional Groups Preparations*, Vol. III. Academic Press, New York 1972.
19. Cooper F. C., Partridge A. W.: J. Chem. Soc. *1953*, 255.
20. Sevilla J. M., Dominguez M., Garcia-Blanco F., Blazquez M.: Comput. Chem. *13*, 197 (1989).
21. Prevorsek D. C.: J. Phys. Chem. *66*, 769 (1962).
22. Kotera A., Morita T., Aoyagi S., Kabiuchi Y., Nagakura S., Kume K.: Nippon Kagaku Zashsi *82*, 302 (1961).
23. Kiro Z. B., Telerin Yu. A., Nikolenko L. N., Stepanov B. I.: Zh. Org. Khim. *8*, 2573 (1972).
24. Metzler D. E., Harris C. M., Johnson R. J., Siano D. B., Thomson J. A.: Biochemistry *12*, 5377 (1973).
25. Blazquez M., Sevilla J. M., Perez J., Dominguez M., Garcia-Blanco F.: J. Chem. Soc., Perkin. Trans. 2 *1989*, 1229.
26. Leffler J. E., Grunwald E.: *Rates and Equilibria of Organic Reactions*. Wiley, New York 1963.
27. Zuman P. in: *The Elucidation of Organic Electrode Processes*. Academic Press, London 1969.