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Correlation of Electrochemical Reduction of Adenine Nucleosides and Nucleotides with Structure and Orientation in Solution

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Abstract: The electrochemical behavior over the available pH range of the fundamental sequence of adenine nucleosides and nucleotides at mercury electrodes is similar, in principle, to that of the parent adenine; up to pH 4–5, all exhibit a diffusion-controlled 4e wave due to reduction of the 1,6 and 3,2 N=C bonds in the protonated species; the wave is kinetically controlled at higher pH where it begins to disappear. $E_{1/2}$ becomes more negative with increasing pH. Attachment of a ribose or ribosophosphate moiety decreases the ease of reducibility; electrostatic effects, association, and adsorption overcome the electron-withdrawing effect of the ribose. The compounds are strongly adsorbed at *ca.* −0.6 V, which involves an uncharged portion of the molecule; at more negative potential, the species gradually desorb and may be readsorbed *via* the protonated portion of the molecule. The marked decrease of the experimental diffusion coefficients with concentration, and their being 3–4 times greater than those calculated on the basis of the Stokes–Einstein relation, are interpreted in terms of association, preferential orientation, and conformation of the species diffusing to the electrode. The latter involves planar arrangement of the rings and vertical stacking, which results in the effective barrier to diffusional transport being essentially the minimal cross-sectional area of the planar purine moiety; these factors also influence the ease of reduction. The variation in diffusion coefficient mentioned may also provide a test for association at the millimolar concentration level.

The enormous growth in nucleic acid research has resulted in a need for methods suitable for the study of nucleic acid structure, physiochemical properties, and allied features. Thus, polarographic methods have been successfully applied to the study of native DNA and its polymeric degradation products,[2] conformation changes in DNA,[3–5] genetic relationships of bacterial DNA,[6] and the estimation of denatured DNA.[3,7]

Since the electrochemical behavior of polymeric nucleic acids and synthetic polynucleotides is essentially determined by that of the constituent purine and pyrimidine bases, detailed knowledge of the behavior of the monomeric units, *i.e.*, purine and pyrimidine nucleosides and nucleotides, will facilitate correct interpretation of results obtained with polymers and will, in turn, allow estimation of the effects of polymer secondary structure and sugar and sugar–phosphate moieties on the behavior of the parent bases. Furthermore, polarographic methods furnish data, *e.g.*, with respect to nature in solution and adsorption, which may be correlated with some biological, physical, and chemical properties.[8]

While the electrochemical behavior of adenine has been extensively studied,[9,10] and its reduction mecha-

(1) To whom correspondence should be addressed.
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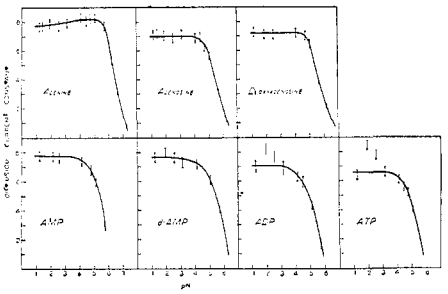

Figure 1. Variation with pH of diffusion current constants, I, of adenine and its nucleosides and nucleotides for 0.25 mM depolarizer in chloride and McIlvaine buffers of 0.1 M ionic strength at 1.5°. Values of I are plotted with a range of the maximum estimated experimental error.

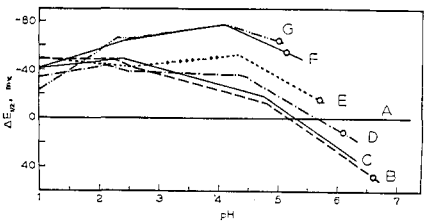

Figure 2. Variation with pH of $\Delta E_{1/2}$ values obtained by subtracting $d(E_{1/2})/d(pH)$ values for adenine from those for the adenine nucleosides and nucleotides. (A), adenine; (B), deoxyadenosine; (C), adenosine; (D) dAMP; (E) AMP; (F), ADP; (G), ATP, Experimental details are given in Table I. An inflection point on the $E_{1/2}$–pH plot for adenine occurs at pH 2.0–2.5 due to change from chloride to McIlvaine buffer. Circles denote a potential of −1.44 V, near which the waves merge with background.

nism at the dropping mercury electrode (dme) has been established,[11] little attention has been given (*cf.* ref 12) to its nucleosides and nucleotides, except for four papers,[13–16] which provide only scant dc polarographic data for adenosine and its phosphates in $HClO_4$ or unspecified media, and two oscillopolarographic studies.[17] Consequently, the fundamental sequence of adenine nucleosides and nucleotides has been studied by dc and ac polarography in order to define the essential features of their electrochemical reduction and the effects of substituent and structure. Although electrochemical reduction in nucleosides and nucleotides occurs primarily in the pyrimidine ring of the purine moiety, the sugar or sugar–phosphate group may influence association, conformation, orientation, and adsorption, as well as electron density at electroreactive sites, which effects may then be manifest in the current–potential patterns.

Discussion

Each adenine derivative examined[18] shows one generally well-defined cathodic polarographic wave; the overall behavior pattern is fundamentally similar to that of adenine itself.[11] At higher ionic strength, *e.g.*, 0.5 M at 25°, the upper portion of the wave may be distorted by a second more negative wave, whose behavior resembles in some respects that of a maximum of the second kind; this abnormal wave does not appear at the dme at low ionic strength (0.1 M) and temperature (1.5°), or at the hanging mercury drop electrode at 0.5 M ionic strength and 25°. Data obtained in the presence or absence of the abnormal wave are, in principle, identical; however, due to the proximity of the waves, data obtained in its absence are more accurate and are those primarily discussed. A detailed study of the abnormal wave, which has been seen with other purine derivatives, is in progress.[19]

Nature of the Reducible Species

The need for protonation of the adenine nucleosides and nucleotides prior to reduction, *i.e.*, the presence of the conjugate acid of the purine as the electrochemically reducible species, is supported by the following facts: (1) Plots of limiting current *vs.* pH (Figure 1) exhibit a sigmoidal current decrease centering at 1.2–2.2 pH units higher than the dissociation constant (pK_a) for the protonated purine. Such behavior is characteristic of polarographic reduction of the acid form of a conjugate acid–base system. (2) The wave is kinetically controlled in the pH region where it decreases, indicating combination of the nonprotonated form with protons. (3) Rapid protonation prior to polarographic reduction occurs with 6-substituted purines, including adenine.[8] (4) Plots of half-wave potential ($E_{1/2}$) *vs.* pH usually change slope at 0.1–0.3 pH unit higher than pK_a (Table I; Figure 2); such behavior frequently indicates a change in the extent of protonation.[20,21]

In protonation, the proton would be predominantly added at N(1), which is the most likely protonation site in the adenine moiety;[22,23] although each adenine ring is only monoprotonated in the pH range 1.5–4.0, equilibria involving monoprotonated species with the proton at N(1), N(3), or N(7) are possible.[24]

Effect of Substituent on Reducibility. The negative shift of $E_{1/2}$ for the series with increasing pH is related to an acid–base equilibrium and to consumption of hydrogen ion in the reduction process. To isolate the pH effect due to ribose and ribosophosphate substituents on the adenine nucleus, $d(E_{1/2})/d(pH)$ values for adenine itself may be subtracted from those for the nucleosides and nucleotides in corresponding pH regions. The resulting plots (Figure 2) indicate that attachment of a sugar or sugar–phosphate moiety decreases the ease of reducibility except above pH 5, where the waves show kinetic control due to protonation equilibria. The general pattern does not change with depolarizer concentration, although the order of ease of reducibility may vary somewhat due to the effect of concentration on $E_{1/2}$; *e.g.*, the order at pH 4.1 and zero concentration—adenine > deoxyadenosine, adenosine, dAMP > AMP

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(20) P. J. Elving, *Pure Appl. Chem.*, **7**, 423 (1963).
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Table I. Variation with pH of $E_{1/2}$ for Reduction of Adenine Nucleosides and Nucleotides

Compound (pK_a)[a]	1.5° [b] pH	1.5° [b] $-E_{1/2}$, V	25° [c] pH	25° [c] $-E_{1/2}$, V
Adenine	1.0–2.5	0.985 + 0.083pH	1.0–6.5	0.975 + 0.084pH
(4.20)	2.5–7.0	1.015 + 0.072pH		
Adenosine	1.0–2.5	1.020 + 0.089pH		
(3.60)	2.5–4.8	1.095 + 0.059pH	2.0–4.5[f]	1.040 + 0.070pH
	4.8–6.3	1.195 + 0.038pH	4.5–6.0	1.180 + 0.041pH
Deoxyadenosine	1.0–2.4	1.035 + 0.082pH		
(3.7)	2.4–4.8	1.090 + 0.059pH	2.5–4.6[d,e]	1.060 + 0.069pH
	4.8–6.7	1.190 + 0.038pH	4.6–6.5	1.205 + 0.037pH
AMP	1.0–4.3	1.040 + 0.078pH	1.0–4.3	1.015 + 0.083pH
(3.80)	4.3–5.7	1.185 + 0.045pH	4.3–5.5	1.115 + 0.060pH
dAMP	1.0–2.1	1.010 + 0.092pH		
(4.40)	2.1–4.4	1.055 + 0.071pH	2.0–6.5[e,f]	0.985 + 0.080pH
	4.4–6.3	1.175 + 0.044pH		
ADP	1.0–2.5	1.010 + 0.099pH		
(3.9)	2.5–4.1	1.060 + 0.080pH		
	4.1–5.5	1.180 + 0.050pH		
ATP	1.0–2.3	0.975 + 0.116pH		
(4.05)	2.3–4.1	1.060 + 0.080pH	2.5–4.5[f]	1.035 + 0.083pH
	4.1–5.1	1.155 + 0.057pH	4.5–5.5	1.175 + 0.052pH

[a] Dissociation constants are taken from: D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solutions," Butterworth and Co., London, 1965. [b] Data for 0.25 m*M* depolarizer in chloride and McIlvaine buffers at 0.1 *M* ionic strength. The average estimated experimental error is less than ±5 mV. [c] Average data for 0.125 and 0.50 m*M* depolarizer in chloride, acetate, and McIlvaine buffers at 0.5 *M* ionic strength; for 0.50 m*M* ATP, only $E_{1/2}$ data above pH 4.0 are included since below this pH the normal and abnormal waves merge. The average estimated experimental error is ±12 mV. [d] $E_{1/2}$ data at pH below the lower limit deviate, being more positive. [e] Maximum at pH 1. [f] $E_{1/2}$ in pH 3.0 acetate buffer deviates from relation shown.

> ADP > ATP—gradually changes with increasing concentration to that at 1.0 *M:* adenine > adenosine, deoxyadenosine > dAMP, AMP > ADP > ATP.

The net effect of addition of the ribose and ribosophosphate groups is more complex than in the case of simple substituents such as alkyl, amino, alkylamino, arylamino, and hydroxyl, which decrease the ease of reducibility of the parent pyrimidine or purine,[8,11,25–27] *i.e.*, there are the conflicting effects of (a) substitution by ribose or ribosophosphate, which involves an electron-withdrawing effect,[28–30] increasing the reducibility of the purine ring, and (b) adsorption[31,32] and intermolecular association,[30,33,34] which can be expected to decrease the reducibility (*cf.* subsequent discussion). The simultaneous manifestation of these two opposing effects accounts for the relatively small differences in $E_{1/2}$ in the adenine series, which do not exceed 50 mV (involving base, nucleosides, and nucleoside monophosphates), compared to *ca.* 120 *m*V in the cytosine series. In the latter series, the electron-withdrawing effect of the ribose ring would be expected to predominate due to (a) the relative proximity of sugar and reduction site, and (b) the decreased tendency to adsorbability[32] and intermolecular association compared to the adenine series; the ease of reducibility does increase in the order of base < nucleotide < nucleoside.[26] The tendency of adenine nucleosides and nucleotides to become more easily reducible than adenine at higher pH, *i.e.*, as the extent of protonation decreases, may indicate that the electron-withdrawing effect of ribose and 2′-deoxyribose is weakened, on protonation, due to the competitive electrostatic attraction of electrons by the positive charge.

Lower values of pK_a[34a,35] and of apparent polarographic pK_a (Figures 1 and 2) for ribosyl than for 2′-deoxyribosyl derivatives in the adenine series indicate that the base in the 2′-deoxyribosyl derivatives is more readily protonated; this may be due to the weaker electron attraction of 2′-deoxyribose.[29]

An important factor in decreasing reducibility is the presence of negatively charged phosphate groups, which cause electrostatic repulsion of the compound from the similarly charged dme surface and weaken electron-withdrawal by ribose; this factor is negligible at low pH where the phosphate group is screened by protons (Figure 2). The condensed structure, which ATP assumes in aqueous media,[36] also decreases reducibility; the molecule is so folded that the two terminal dissociated phosphate groups approach the protonated N(1) and amino groups to form a closely packed zwitterion; the N(1)=C(6)—NH_2 reduction site is thus surrounded by a protective cloud of negatively charged phosphate groups. A similar situation may occur with ADP.

While $E_{1/2}$ for simple 6-substituted purines can be correlated with experimental and theoretical structural and reactivity indices,[8] the lack of data and available computational approaches for the present series prevents a similar examination. Electron density at the reduction site, which is the essential factor determining the reducibility of simple 6-substituted purines,[8] probably

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(36) B. Pullman and A. Pullman, "Quantum Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1963, pp 364 and 374–376.

Table II. Alternating Current Polarographic Data for Adenine Nucleosides and Nucleotides[a]

Compound	Depression		Broad peak		Main peak		
	$-E_{min}$, V	i_{min},[b] μA	$-E_s$, V	i_s,[b] μA	$-E_s$, V	$E_{1/2} - E_s$, mV	i_s, μA
Adenine	0.62	−0.11	0.85	0.01	1.260	75	0.46
Adenosine	0.61	−0.22	0.98	0.02	1.285	65	0.40
Deoxyadenosine	0.63	−0.20	0.90	−0.40	1.270	40	0.48
AMP	0.58	−0.32	1.40	0.10	1.295	70	0.36
dAMP	0.63	−0.18	0.85	−0.02	1.250	65	0.47
ATP	0.62	−0.24	1.00	0.12	1.295	55	0.23

[a] 0.25 m*M* depolarizer in pH 2.5 McIlvaine buffer (0.5 *M* ionic strength) at 25°. [b] Current was measured from the background electrolyte base current; negative values denote current below that of the background. See Experimental Section for details.

cannot be expected to be predominant in the adenine nucleosides and nucleotides, due to the more important role of such factors as adsorption, solvation, and association, quantitative estimation of whose effects is at present impossible. Thus, $E_{1/2}$ for 6-substituted purines correlates linearly with electron-acceptor properties as represented by the calculated energy of the lowest empty molecular orbital (k_i coefficient),[8] *e.g.*, $E_{1/2} = 0.34 + 1.72k_i$ at pH 2.5; ATP ($E_{1/2} = -1.260$; $k_i = -0.913$) fits the latter equation, while ADP (−1.260; −0.985) and AMP (−1.235; −1.144) deviate markedly. Again, $E_{1/2}$ correlates with pK_a for the simple 6-substituted purines, but not for the present series (Table I).

Adsorption on Mercury

The present discussion is based on ac polarography at pH 2.5, where the positively charged protonated species predominates (pK_a values are between 3.7 and 4.4), and of dAMP between pH 2.0 and 6.4.

The deep depression of the base current near the dme potential of zero charge (Figures 3 and 4; Tables II and III) indicates strong adsorption involving an uncharged portion of the molecule, which is likely to be the imidazole ring;[37] this is supported by the increasing magnitude of the depression with increasing pH, *i.e.*, with increasing solution concentration of uncharged species. The order of uncharged-site-controlled adsorbability, based on the magnitude of the depression as an approximate measure of extent of adsorbability of the depolarizer, agrees with the order of surface activities calculated from differential capacity data obtained with 1.85 m*M* solutions in 1 *M* NaCl: adenine < dAMP < deoxyadenosine.[32]

At more negative potential, gradual desorption occurs (*cf.* the broad desorption peak around −1.0 V); variation of the differential capacity in 1 *M* NaCl solution supports such desorption.[32] Similar to the simple 6-substituted purines,[8] the depolarizer seems to be readsorbed just before the main faradaic peak. Actually, the adsorbed molecules probably reorient themselves on the electrode surface as the potential becomes more negative, so that the positively charged site of the molecule is attached to the mercury surface at potentials just preceding reduction. Adsorption of the depolarizer in the potential region of the faradaic peak is supported by an ac polarographic study[38] of nucleosides at pH 7, which

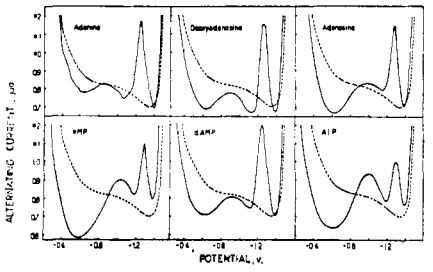

Figure 3. Alternating current polarograms for 0.25 m*M* solutions of adenine and its nucleosides and nucleotides in pH 2.5 McIlvaine buffer (0.5 *M* ionic strength) at 25°. Dashed line is the background electrolyte base current.

Table III. Variation with pH of Alternating Current Polarographic Data for dAMP[a]

pH	Depression		Broad peak		Main peak		
	$-E_{min}$, V	i_{min},[b] μA	$-E_s$, V	i_s,[b] μA	$-E_s$, V	$E_{1/2} - E_s$, mV	i_s, μA
2.0					1.255	110	0.56
2.5	0.61	−0.03	0.87	0.01	1.280	95	0.35
3.7	0.60	−0.06	0.95	0.06	1.360	80	0.48
4.5	0.60	−0.10	0.89	0.04	1.410	65	0.39
5.5	0.53	−0.12	0.78	0.04	1.470	45	0.15
6.4	0.48	−0.14	0.82	0.07			

[a] 0.50 m*M* dAMP in McIlvaine buffer except for pH 2.0 (chloride buffer); 0.5 *M* ionic strength at 25°. [b] Current was measured from the background electrolyte base current; negative values denote current below that of the background. See Experimental Section for details.

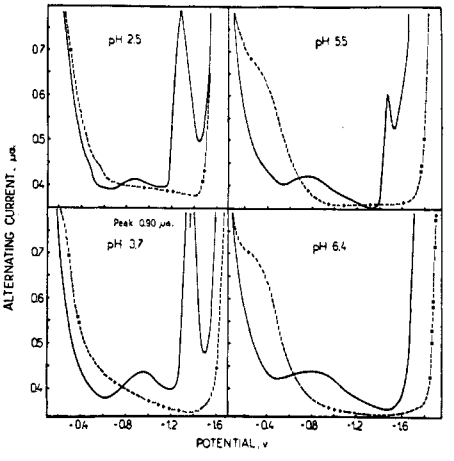

Figure 4. Variation in alternating current polarographic behavior of 0.50 m*M* dAMP with pH in McIlvaine buffer (0.5 *M* ionic strength) at 25°. Dashed line is the background electrolyte base current.

(37) G. Dryhurst and P. J. Elving, *Talanta*, **16**, 855 (1969).

(38) V. Vetterl, *J. Electroanal. Chem.*, **19**, 169 (1968).

Table IV. Variation with Concentration of Diffusion Parameters for Adenine Nucleosides and Nucleotides

Compound	Concn, mM	Experimental: I[a]	Experimental: D,[c] 10^6 cm^2 sec^{-1}	Experimental: V_m,[d] cm^3 mole^{-1}	Calculated: V_m,[e] cm^3 mole^{-1}	Calculated: D_0,[f] 10^6 cm^2 sec^{-1}	Calculated: I_0[g]
Adenine	0.0	9.5[b]					
	0.1–0.5	8.5	12.3	3.6	96*	3.5	4.5
	0.5–1.0	9.5–2.0C					
Adenosine	0.0–1.0	7.5–2.5C	9.5	7.7	170	2.9	4.1
Deoxyadenosine	0.0–1.0	7.9–2.4C	10.6	5.6	166	2.9	4.2
AMP	0.0	8.3[b]					
	0.1–1.0	7.9–2.1C	10.6	5.6	211	2.7	4.0
dAMP	0.0–1.0	8.0–2.6C	10.8	5.2	207*	2.7	4.0
ADP	0.0–1.0	7.3–2.8C	9.0	9.0	248*	2.6	3.9
ATP	0.0	7.0[b]					
	0.1–1.0	6.4–1.8C	6.9	20	290*	2.4	3.8

[a] Variation with concentration of the diffusion current constant, $I = i_d/Cm^{2/3}t^{1/6}$, was calculated from average current data for 0.01–1.0 mM depolarizer in pH 4.1 McIlvaine buffer of 0.1 M ionic strength at 1.5°. The maximum estimated deviation of experimental points from the calculated line is 4% (8% for AMP). C = mM concentration. [b] Obtained by extrapolating the I–C plot, which is nonlinear below the lowest concentration shown, to zero concentration. [c] Calculated from the experimental I value by eq 2. [d] Calculated from the experimental D value (decreased by 15%) by eq 1. [e] Calculated from molecular weight and density given in the literature. "Crystal Data Determinative Tables," 2nd ed, J. D. H. Donnay, Ed., American Crystallographic Association, 1963; J. Kraut and J. N. Jensen, *Acta Cryst.*, **16**, 79 (1963). If a density was not reported, a reasonable value based on that of structurally similar compounds was used. The latter values are starred. [f] Calculated from the calculated V_m value by eq 1. [g] Calculated from the D_0 value by eq 2.

indicated strong adsorption of adenosine and deoxyadenosine near −1.2 V at high enough concentration, *i.e.*, above 3.3 and 4.7 mM, respectively. At the low concentrations (0.25 mM) used in the present study, only relatively weak adsorption may be expected at the ac peak or more negative potentials, *e.g.*, *i–t* curves do not indicate strong adsorption of depolarizer.

The summit potential, E_s, of the ac faradaic reduction peak at pH 2.5 is more negative than the corresponding $E_{1/2}$ (Table II). Adsorption of a depolarizer frequently inhibits the electrolytic process and, consequently, shifts the wave to more negative potential,[39,40] with the effect increasing with depolarizer concentration; $E_{1/2}$ does become more negative with increasing depolarizer concentration (association in solution may also be a factor; *cf.* subsequent discussion). The decrease in $(E_{1/2} - E_s)$ with increasing pH (Table III) indicates that the reduced form becomes more strongly adsorbed relative to the oxidized form in the E_s potential region.[41] An obvious factor is destruction of the oxidized form with concomitant formation of the reduced form.

The ac measurements do not provide unequivocal evidence for adsorption at potentials more negative than that of the faradaic peak due to closeness of the latter and background discharge.

Relation of Diffusion Coefficient to Structure, Orientation, and Association

Current-Controlling Processes. The polarographic limiting current, i_l, for the normal wave of adenine and its nucleosides and nucleotides is diffusion-controlled below pH 4–5, where i_l is essentially independent of pH; it is kinetic-controlled in the pH region where the wave is about to disappear.

Experimental and Calculated Diffusion Coefficients. The diffusion current constants, I, vary from 6.5 to 8.1 below pH 4 or 5 for 0.25 mM solutions (Figure 1) and from 6.4 to 8.5 at pH 4.1, for zero concentration (Table IV). Therefore, since reduction of adenine at the dme is a 4e process,[11] the same number of electrons is involved in each normal wave process. The magnitude of I generally corresponds to that expected on the basis of the apparent molecular volume, V_m (molecular weight/density), of the solid compounds.[42] V_m is used in the Stokes–Einstein equation[43,44] for the diffusion coefficient, D_0, of a spherical particle at infinite dilution

$$D_0 = k/(V_m)^{1/3} \text{ cm}^2 \text{ sec}^{-1} \quad (1)$$

where k is a numerical constant (1.60×10^{-5} for aqueous solution at 1.5°). D_0 can be used to calculate the limiting I value at zero concentration, I_0, from the Ilkovic equation for average currents

$$I = 607nD^{1/2} \quad (2)$$

where n is number of electrons transferred per molecule in the faradaic electrode reaction. Calculated and experimental values of these parameters are given in Table IV.

Calculated D_0 values for the present series are comparable with experimental diffusion coefficients of similar molecular weight compounds,[35,45] *e.g.*, sucrose and FMN. However, experimental I values for zero concentration, being 1.7–2 times that of calculated I_0 values, produce diffusion coefficients which are 2.8–4 times the calculated D_0 values; commonly, experimental diffusion coefficients calculated from the Ilkovic equation are only about 15% higher than the actual values.[43] Thus, even considering the latter error, the effective barrier to diffusional mass transport for the adenine series seems to correspond to an effective molecular volume which is

(39) Since adsorbed molecules are in a lower free energy state than those in solution, they are more difficultly reduced: R. Brdička, *Collect. Czech. Chem. Commun.*, 12, 522 (1947).

(40) J. Volke, *Talanta*, 12, 1081 (1965).

(41) B. Breyer and H. H. Bauer, "Alternating Current Polarography and Tensametry," Interscience Publishers, New York, N. Y., 1963, pp 69–74; B. Breyer, T. Biegler, and H. H. Bauer in "Modern Aspects of Polarography," T. Kambara, Ed., Plenum Press, New York, N. Y., 1966, p 50.

(42) A plot of I *vs.* $(V_m)^{1/3}$ gives a line with an inflection with AMP, dAMP, ADP, and ATP on one branch, and dAMP and deoxyadenosine near the branch determined by AMP and adenine; adenosine deviates markedly.

(43) L. Meites, "Polarographic Techniques," John Wiley and Sons, Inc., New York, N. Y., 1965, p 133.

(44) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd ed, Interscience Publishers, New York, N. Y., 1952, pp 56–59.

(45) "International Critical Tables," Vol. 5, E. W. Washburn, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1929.

only about $^1/_{15}$ to $^1/_{40}$ of that expected for a spherical type of molecule.[43,44]

The inability to calculate the effective molecular volume of nucleosides and nucleotides with respect to diffusion in solution in a simple fashion can be considered and rationalized, at least in part, on the basis of the preferred conformation of the nucleosides and nucleotides in solution (*cf.* subsequent discussion).

Some factors, which might possibly cause high values for the experimental diffusion current constants, can be shown to be unimportant. Electromigration of a positively charged electroactive species would be a negligible factor in i_l in view of the molar ratio of buffer electrolyte to depolarizer exceeding 100. On the basis of validity of the Stokes–Einstein law and the 15% excessive contribution of the Ilkovic equation to diffusion coefficient, n would have to exceed 6 to make the experimental and calculated I values comparable; however, the reduction of adenine under polarographic conditions does seem to be a 4e process.[11] Participation of some other process in addition to diffusion, *e.g.*, a catalytic current, is not supported by the concentration, pH, temperature, and mercury height dependencies of i_l.

Additional insight is provided by the decrease of I with increasing depolarizer concentration (Table IV), which is much greater than normally encountered. A frequently observed decrease of diffusion coefficients of organic molecules with increasing concentration and ionic strength,[44] usually by a factor less than 0.7 between zero and 1 M concentration,[44,45] is due to a decrease in mobility of the diffusing molecules (this phenomenon has not been fully explained). The latter effect can only partially account for the much greater decrease of diffusion coefficients in the adenine series, *e.g.*, by a factor of 0.5 between zero and 1 mM concentration.[46]

Preferred Orientation in Solution. The considerably larger experimental diffusion coefficients (D) for the adenine series compared to the calculated ones (D_0) (Table IV), particularly for nucleosides and nucleoside monophosphates, suggest that the average preferred orientation of the diffusing species, which approaches the electrode, is with the purine ring plane perpendicular to the surface and with the protonated reduction site facing the surface. The preferred conformation of purine nucleosides and nucleotides, which is *anti*[30,47–49] in solution, results in a relatively planar arrangement of the molecule; consequently, the effective barrier to diffusional transport of the species is the minimal barrier, being the cross-sectional areal bulk of the planar purine moiety, plus those portions of the sugar or sugar–phosphate moiety which protrude from the purine ring plane. The fact that the molecular volume of ATP, calculated from polarographic current data, deviates least in the series from that calculated from eq 1, indicates that ATP exhibits a structure which is the closest to spherical, *e.g.*, a folded structure[36] as discussed. (The ratio of calculated to experimental V_m values for AMP, ADP, and ATP is 37, 28, and 15, respectively.)

Association in Solution. Nmr[24b,30,34b,53] and vapor pressure osmometric[34,54,55] measurements (largely between pH 5 and 7) indicate association of all purines and their nucleosides and nucleotides in aqueous solution; the extent of association increases with concentration. The mode of the association is essentially that of vertical stacks with hydrophobic interaction of bases.[34b,54] Since the standard free energy for this association is of the order of the thermal energy,[34b] the stacks must break and re-form rapidly, and higher stability can be expected at lower temperature. Nmr measurements do indicate a greater degree of association at lower temperature;[56a] correspondingly, the decrease of I with increasing concentration is about 2–3 times greater at 1.5° than at 25°, indicating the considerably greater association at the lower temperature. The decreasing reversibility of the electrode process with increasing concentration (*cf.* calculation of αn_a and p) is in agreement with additional energy being involved in the electrode process with increasing concentration, as would be the case for increased association and/or adsorption.

As a general phenomenon, diffusion of vertical stacks to the electrode should be in agreement with the model proposed for monomers, *i.e.*, with the purine ring plane perpendicular to the electrode. Although the mode of arrangement of individual molecules in the stacks is still a matter for speculation, some models are favored, *e.g.*, 9-alkyl purines could be so arranged that a pyrimidine ring faces an imidazole ring;[56b] for purine nucleosides, it is proposed[34a] that pyrimidine faces pyrimidine with the ribose moieties being opposite to one other in the stack. The presence of protonated N(1) would tend to favor alternate stacking (pyrimidine facing imidazole) due to the reduced electrostatic repulsion. The alternate stacking model would also better explain a relatively strong concentration effect—even at relatively low concentrations—on D and I; blockage of reduction sites might occur in stacked molecules that approach the electrode with alternate pyrimidine rings oriented away from the surface.

Unfortunately, the literature evidence on the inter- and intramolecular interaction of protonated purine derivatives is ambiguous. Thermodynamic data[57] indicate ring–chain interaction in nucleotides only at low pH where the ring has a net positive charge, while nmr[48] indicates such interaction only at pH 5.0–8.2. Another

(46) Decrease of I with increasing concentration is not an artifact due to concomitant presence of background discharge current in the measured normal wave height; the decrease is observed with nucleoside and nucleotide waves, which are close to background discharge, as well as with the adenine wave which is well separated from the latter. Actually, increase of I with increasing concentration might be expected if background current were involved, because of the pronounced positive shift of background discharge with increasing depolarizer concentration.

(47) A. E. V. Haschemeyer and A. Rich, *J. Mol. Biol.*, 27, 369 (1967).

(48) S. S. Danyluk and F. E. Hruska, *Biochemistry*, 7, 1038 (1968).

(49) The *anti* conformation is characterized[50] by the C(1′)–O(1′) bond of the sugar moiety being rotated about the glycosidic bond C(1′)–N(9) to a position which is *ca.* 6–20° anticlockwise, when viewed along C(1′)–N(9) from the purine ring plane (this torsion angle, ϕ_{CN}, has a negative sign and is zero when O(1′) lies in the purine ring plane opposite to C(8)). The angle between the purine and sugar ring planes can be expected to be similar to that in the solid state, *i.e.*, between 55 and nearly 90°.[51,52]

(50) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, 2, 363 (1960).

(51) S. Furberg, *Acta Chem. Scand.*, 4, 751 (1950).

(52) M. Sundaralingam, *Acta Cryst.*, 21, 495 (1966).

(53) (a) J. H. Prestegard and S. I. Chan, *J. Am. Chem. Soc.*, **91**, 2843 (1969); (b) M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *ibid.*, **87**, 5241 (1965).

(54) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *ibid.*, **85**, 1289 (1963).

(55) S. J. Gill, M. Downing, and G. F. Sheats, *Biochemistry*, **6**, 272 (1967).

(56) (a) G. K. Helmkamp and N. S. Kondo, *Biochim. Biophys. Acta*, **145**, 27 (1967); (b) *ibid.*, **157**, 242 (1968).

(57) R. Phillips, P. Eisenberg, P. George, and R. J. Rutman, *J. Biol. Chem.*, **240**, 4393 (1965), and local citations.

nmr study[24b] indicates that stacking is apparently reduced in monoprotonated ApA so that self-intercalation also becomes possible. Monoprotonated ApA forms an intercalated complex with neutral purine, in which the latter interacts with the two adenine bases. Similar behavior may occur in solutions of adenine derivatives at pH 4, in which *ca.* 50% or less of the molecules are protonated, *e.g.*, stacks with alternating neutral and protonated molecules are possible. Association of purines even at low pH is apparently possible.

Mixtures of adenosine and guanosine were briefly examined by dc polarography (guanosine is not reducible under normal polarographic conditions[58]) to see whether the experimental I of adenosine would decrease due to the expected association with guanosine. No such decrease or noticeable shift in $E_{1/2}$ was observed on adding up to 0.5 mM guanosine to 0.12 mM adenosine. However, an apparent capacity wave appeared at 150 to 250 mV more positive than the adenosine $E_{1/2}$; no such wave was observed with either compound alone. (The height of the capacity wave increased and its $E_{1/2}$ became more negative up to a ratio of 2 guanosine to 1 adenosine; above this ratio, there was no further change.) Such a wave indicates sudden changes in the double-layer capacity, such as might arise from dissociation and/or desorption of associated adenosine–guanosine.

Even though inability to evaluate fully the effects of orientation and conformation prevent any attempt—at least at present—to formulate quantitative statements regarding stacking on the basis of polarographically measured diffusion coefficients, the relative variation of the latter with concentration would seem to provide a means of identifying the existence of stacking at very low concentrations, *e.g.*, at 1 mM and lower. While this polarographic approach to determining the presence and extent of association may be limited in the case of adenine derivatives by the effect of protonation, it would be quite applicable to species reducible at higher pH, where association would be more pronounced, *e.g.*, cytosine species.

Experimental Section

Chemicals. Calbiochem A grade adenine, adenosine, deoxyadenosine, adenosine 5′-monophosphate hydrate, deoxyadenosine 5′-monophosphate disodium salt tetrahydrate, monosodium adenosine 5′-diphosphate–2.5-water, and dipotassium adenosine 5′-triphosphate–1.5-water were generally used for measurements at 0.1 M ionic strength and 1.5°. Mann Research Laboratories M.A. grade adenine, adenosine, deoxyadenosine monohydrate, adenosine 5′-monophosphate dihydrate, deoxyadenosine 5′-monophosphate monohydrate, and adenosine 5′-triphosphate dipotassium salt were largely used at 0.5 M ionic strength and 25°. The reported analytical data indicated sufficient purity for polarographic study. Stock solutions, 5 mM in distilled water, were refrigerated. Concentrations were checked spectrophotometrically at pH 1.0 or 2.0 and 7.0; when molar absorptivities were not supplied with an individual lot of material, literature values were used.[35]

The following buffer systems, prepared from analytical grade chemicals (J. T. Baker) and kept at room temperature with chloroform added to prevent microorganism growth, were used (pH region in parentheses): KCl + HCl (1–2); constant ionic strength McIlvaine buffer[59] consisting of Na_2HPO_4 + citric acid + KCl (2.5–7.8); NaOAc + HOAc (3.9–5.9). The chloroform, which was removed on nitrogen purging, did not affect the wave pattern of background or test solutions.

Nitrogen used for deoxygenating was purified and equilibrated by bubbling it successively through vanadous chloride in dilute HCl solution containing heavily amalgamated mossy zinc,[60] NaOH solution, and water.

Apparatus. Polarograms were recorded on a Metrohm E261 Polarecord and a Sargent Model XV polarograph, using a water-jacketed H-cell, maintained at 1.5 ± 0.3° or 25.4 ± 0.1°, respectively, and containing a saturated calomel reference electrode (sce) separated from the test solution by a glass frit and agar plug. The mercury reservoir height was 64 cm, unless otherwise indicated. Capillaries had the following m values in distilled water (open circuit; h = 64 cm): A, 2.22 mg sec^{-1} (at 25°); B, 1.67 (25°); C, 1.42 (5°); D, 2.11 (3.5°). Drop times, measured at potentials of interest, were generally between 3 and 4 sec. For depolarizer concentration studies at 25° and 0.5 M ionic strength, the capillary end was coated with Beckman Desicote to minimize drop-time irregularities at higher concentrations.

Current-time (i-t) curves on a single drop (capillary B) were observed on a Tektronic Type 502 oscilloscope and were photographed with a Tektronic C-12 Polaroid camera and 3000 speed Type 47 Polaroid film.

An operational amplifier-based polarograph[37] was used in conjunction with capillary A and a 50-Hz signal of 4-mV peak-to-peak voltage for ac polarography on 0.25 mM depolarizer solutions. Polarograms were recorded manually; the alternating current (rms) was measured with a Hewlett-Packard Model 400D vacuum tube voltmeter. A modular solid state unit,[61] capillary B (h = 73 cm), and a 50-Hz signal of 10-mV peak-to-peak voltage were used for measurements on 0.50-mM dAMP solutions; polarograms were recorded on a Moseley Model 7001A(S) X–Y recorder.

Procedures. Conventional procedures were generally used. Wave height was measured by a technique recommended for waves with ill-defined plateaus,[62] based on selecting the inflection point, where the slope of the plateau is smallest, by means of two lines, drawn with the smallest possible slope and passing through the oscillation maxima and minima.

The manually noted ac magnitudes (Figure 3 and Table II) are those measured at the end of the drop life or before an abrupt current increase just previous to fall of the drop, and are attenuated by a factor of 1.11. The recorder current magnitudes (Figure 4 and Table III) are attenuated by 1.93, which factor tends to be higher at higher currents and higher di/dt values. Small differences in shape and current magnitudes of ac polarograms of dAMP at pH 2.5 (Figures 3 and 4) are due to the different means of current measurement and concentrations employed, *e.g.*, the increase in base current after a main peak at higher concentration (Figure 4) is presumably due to the presence of abnormal wave and background current components.

Mercury height, h, data are corrected for back-pressure. Test solution pH was measured with a Beckman Model G pH Meter after polarography. All potentials are reported *vs.* sce.

In view of the known lability of the adenine nucleosides and nucleotides in acidic solution, exposure of these compounds to low pH at 25° was minimized.

Dc Polarography: Normal Wave Characteristics. $E_{1/2}$ of the normal reduction wave of the adenine series becomes more negative with increasing pH (Table I); $E_{1/2}$–pH plots consist of two to three linear segments; d($E_{1/2}$)/d(pH) generally varies from 0.04 to 0.08 V/pH, compared to a theoretical value of 0.06 for an electrode process involving the same number of electrons and protons in the potential-determining step. Increasing concentration usually makes $E_{1/2}$ more negative, *e.g.*, at pH 4.1, by an average of 5–20 mV (below 0.1–0.3 mM) and 2–4 mV (above that and up to 1.0 mM) per each 0.1-mM concentration increment.

The wave is constant in height up to pH 4 or 5, when it begins to decrease sharply with increasing pH and disappears by pH 6 or 7 (Figure 1); the ADP and ATP waves deviate at pH 2.0 (chloride buffer) and 2.5 (McIlvaine buffer) in being greater in height (Figure 1) and in ($E_{1/4} - E_{3/4}$) values (*cf.* calculation of αn_a and p). The current, i_l, in the pH region of constant height is linearly proportional to depolarizer concentration, C; under conditions favoring appearance of the abnormal wave, the i_l–C plot may seem to consist of two linear segments. The diffusion current constant, I, decreases linearly with increasing C (*cf.* ref 11 and 63) (Table IV),

(58) B. Janik and E. Paleček, *Z. Naturforsch.*, *B*, 21, 1117 (1966).
(59) P. J. Elving, J. M. Markowitz, and I. Rosenthal, *Anal. Chem.*, 28, 1179 (1956).
(60) Reference 43, p 89.
(61) G. Dryhurst, M. Rosen, and P. J. Elving, *Anal. Chim. Acta*, 42, 143 (1968).
(62) Reference 43, p 155.
(63) D. L. Smith and P. J. Elving, *Anal. Chem.*, 34, 930 (1962).

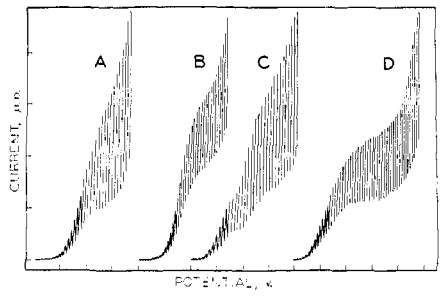

Figure 5. Variation in the shape of the normal and abnormal waves of AMP (A and B, 0.45 mM) and adenine (C and D, 0.50 mM) with temperature and ionic strength. Temperature, ionic strength, capillary, and current scale (μA/division): (A): 25°, 0.1, C, 5.0; (B): 1.5°, 0.1, D, 2.5; (C): 25°, 0.5, C, 10.0; (D): 1.5°, 0.1, D, 5.0. Each curve starts at −1.2 V; the potential scale is 100 mV/division.

e.g., 13% for adenine and 27–38% for the nucleosides and nucleotides, between extrapolated zero concentration and 1.0 mM at pH 4.1 and 1.5°, and about 10% at 25°. Up to 0.5 mM adenine, I is almost independent of concentration. A sudden increase in I, which is often seen below 0.1 mM (Table IV), may be due to a positive shift of the background discharge potential in the presence of adenine derivatives, which does not permit proper correction for the residual current at high current sensitivities.

Buffer composition has little or no influence on i_l; at 25° and 0.5 M ionic strength, the apparent greater height of the wave in chloride and acetate than in McIlvaine buffers is probably due to the abnormal wave masking the normal wave limiting portion in the latter buffer. $E_{1/2}$ values in acetate buffer generally fall on the $E_{1/2}$–pH plot for McIlvaine buffer, while those in chloride buffer, *i.e.*, pH 2.0 and below, are usually more positive (Table I), resulting in an inflection on the $E_{1/2}$–pH plot.

Plots of i_l *vs.* $h^{1/2}$ in the pH region of constant i_l are linear and pass through the origin; the corresponding $i_l:h^{1/2}$ ratios are nearly independent of $h^{1/2}$. (For diffusion-controlled currents, theory[43] predicts that the $i_l:h^{1/2}$ ratio is independent of $h^{1/2}$ or decreases slightly with increasing $h^{1/2}$.) Under conditions producing the abnormal wave or an additional current increment (apparently part of the abnormal wave) on the normal wave limiting portion, $i_l/h^{1/2}$ *vs.* $h^{1/2}$ plots vary from an 11% increase to a 9% decrease for h between 40 and 80 cm; obviously, the measured i_l is uncertain when the abnormal wave impinges on the normal wave limiting portion. The wave height is independent of h in the pH region where it is one-tenth or less of that in the pH 1–4 region, showing kinetic control of the current.

Temperature coefficients of i_l for adenine and AMP at pH 3–4 are between 1.1 and 2.2%.

Polarization rate has only a slight effect on wave height, *e.g.*, i_l for 0.125 mM adenine at pH 3.0 increases by *ca.* 9% between 50 and 250 mV/min.

The background discharge is shifted to more positive potential in the presence of adenine and, particularly, its nucleosides and nucleotides, even in the pH region where no reduction wave is seen. The shift is proportional to depolarizer concentration, pH, and background composition, *e.g.*, *ca.* 50 mV for 0.5 mM depolarizer in pH 1.0 chloride, 200 mV in pH 5.0 McIlvaine, and 160 mV in pH 5.0 acetate solutions of 0.5 M ionic strength at 25°. The limiting portion of the normal wave may be ill-defined, even in the absence of the abnormal wave, due to merging with the background discharge at concentrations above *ca.* 1.0 mM and at pH where the wave begins to disappear; complete merging in the latter case limits appearance of the wave of nucleosides and nucleotides to pH where $E_{1/2}$ is *ca.* −1.44 V or less negative (Figure 2).

A maximum, which may appear on the crest of the wave at higher buffer concentration at pH 1, is easily suppressed by addition of 0.0003–0.0005% Triton X-100.

Abnormal Wave. Under certain conditions, adenine, AMP dAMP, and ATP produce a more negative, so-called "abnormal" wave. Adenosine and deoxyadenosine produce no such wave; however, the limiting portion of the normal wave above *ca.* 0.5 mM depolarizer solution and below pH 3.5 may be steeply inclined, indicating the presence of an additional faradaic process. The abnormal wave is usually hard to define due to its merging with background discharge and/or normal wave limiting portion; the latter effect makes the analysis of the behavior of both waves difficult. The abnormal wave or the additional current increment affect the measured normal wave height usually in such a way that the latter is lower in their presence. In general, the definition of the two waves is better with adenine than with AMP, dAMP, and, particularly, ATP, *e.g.*, both waves merge completely for 0.50 mM ATP at pH 2.5–4.0 for 0.5 M ionic strength and 25°.

The abnormal wave pattern changes markedly with the usual experimental conditions.[19] It appears only in a limited pH region, since it merges with background discharge below pH 2.0 and above pH 4.0–5.5. It decreases with increasing ionic strength and temperature, *e.g.*, neither the wave nor an additional current increment appears at 0.1 M ionic strength and 1.5° (Figure 5). With decreasing depolarizer concentration, the abnormal wave height decreases more rapidly than that of the normal wave. Below 0.2 mM depolarizer at 0.5 M ionic strength and 25°, the abnormal wave is small and ill-defined, and may often appear as an additional contribution on the normal wave limiting portion.

The abnormal wave may be at least partially eliminated by Triton X-100 addition, which is generally more effective at higher pH, as well as where the normal and abnormal waves are relatively well separated, where the latter merges with background discharge, or where only an additional current is superimposed on the normal wave limiting portion.

Calculation of αn_a and p. Since cyclic voltammetry at the hanging mercury drop electrode gives no evidence of reversibility for adenine, and ac polarography indicates only slight reversibility,[37] the following equations[64] can be used to calculate αn_a (product of transfer coefficient and number of electrons involved per molecule of reactant in the rate-determining step) and p (number of hydrogen ions involved per molecule of reactant in the rate-determining step).

$$E_{1/4} - E_{3/4} = 0.05172/\alpha n_a \qquad (3)$$

$$\frac{d(E_{1/2})}{d(pH)} = \frac{-0.05915}{\alpha n_a} p \qquad (4)$$

Calculation of αn_a for adenine at 26° using the equation proposed by Oldham and Parry[65]

$$\frac{0.0592}{\alpha n_a} \log \left[\frac{2X(3 - X)}{5(1 - X)}\right] = E_{1/2} - E \qquad (5)$$

where X is the ratio of the current at potential E to i_l, gave values identical within experimental error with those calculated from eq 3.

The following αn_a and p values are based on data obtained at 0.1 M ionic strength and 1.5°; deviations shown represent an average maximum estimated deviation of $(E_{1/4} - E_{3/4})$. For 0.25 mM adenosine, deoxyadenosine, AMP, and dAMP, αn_a is essentially constant (0.85 ± 0.05 to 1.01 ± 0.06) between pH 1 and 5; however, αn_a for adenine decreases linearly from 1.3 ± 0.1 to 0.72 ± 0.03, and for ADP and ATP increases from 0.75 ± 0.05 to 1.08 ± 0.10. As previously mentioned, αn_a values for the latter two compounds deviate from the line shown between pH 2.0 and 2.5, *i.e.*, αn_a for both is 0.62 at pH 2.0 and 0.75 at pH 2.5. For all compounds, αn_a increases with decreasing concentration, *e.g.*, from 0.76 ± 0.05 at the 1.0 mM level to 0.94 ± 0.09 at 0.25 mM at pH 4.1 (from 0.52 ± 0.02 to 0.77 ± 0.03 for adenine); below 0.1 mM, αn_a usually abruptly increases up to 1.3 ± 0.1 at zero concentration (1.8 ± 0.2 for deoxyadenosine).

Values of p calculated for adenosine, deoxyadenosine, AMP, and dAMP, based on the relatively constant αn_a values between pH 1 and 5, average 1.20 ± 0.25 between pH 1 and 4, and 0.64 ± 0.10 at pH 5.

The number of electrons and protons involved in the rate-determining step, n_a and p, cannot be unambiguously estimated from the calculated αn_a and p values because of the relatively large experimental error and of the uncertainty in estimating α. However, on the basis of α being in the neighborhood of 0.5, as seems reasonable, the data indicate an involvement of 1 to 2 electrons and 1 proton in the rate-determining steps; a decrease of αn_a with increasing pH for adenine, and with increasing concentration for the whole series, may indicate decreasing reversibility, *i.e.*, a decreasing value of α and/or a change in the number of electrons involved from 2 to 1. The involvement of two electrons in the rate-determining step

(64) Reference 43, pp 244–248.
(65) K. B. Oldham and E. P. Parry, *Anal. Chem.*, 40, 65 (1968).

need not necessarily mean that this number of electrons is actually added simultaneously, but, most probably, that there are two successive steps too nearly simultaneous to be distinguished.[64] These results are in accord with the shift of $E_{1/2}$ with pH and with the controlling processes in the electrochemical reduction of pyrimidine itself[9,66] (the pyrimidine ring is the site of reduction in the adenine series).

Current-Time Curves. The variation of current with time during the life of a single drop (i–t curves) was recorded for sequential drops from a vertical capillary. Due to depletion of the solution surrounding the drop,[67] i–t curves so obtained produce log i *vs.* log t plots, which are curves convex to the time axis, so that the choice of the slope is ambiguous. Although such plots cannot be used to calculate the amount of diffusion control, they can show the depression of the instantaneous current expected for a strongly adsorbed depolarizer;[40,67] i–t curves recorded on the limiting portion of the normal wave for 0.25 mM solutions of adenine, adenosine, and AMP at pH 2.5 do not show any such depression.

Ac Polarography. The general pattern for an ac polarogram for a 0.25 mM solution of an adenine nucleoside or nucleotide in pH 2.5 McIlvaine buffer (0.5 M ionic strength) at 25° consists of a depression of the base current with a minimum at *ca.* −0.6 V, a broad peak at −0.85 to −1.09 V, and a narrow main peak at −1.25 to −1.29 V (Figure 3 and Table II). The summit potential, E_s, of the latter, which is the ac faradaic peak, is 40–45 mV more negative than $E_{1/2}$ of the corresponding dc polarographic wave. The broad peak and the foot of the main peak at less negative potential are above the background base current for adenosine, AMP, and ATP, and below for deoxyadenosine and dAMP. No depression of the base current is observed at potentials more negative than that of the main peak.

This pattern is essentially unchanged for 0.50 mM dAMP solution with increasing pH up to *ca.* pH 4.5, where the main peak begins to decrease sharply and disappears by pH 6 (Figure 4 and Table III). With increasing pH, E_s becomes more negative ($E_s = -1.120 - 0.064\text{pH}$), ($E_{1/2} - E_s$) decreases linearly (*ca.* 15 mV/pH), the background discharge shifts to more positive potential, and the magnitude of the base current depression at *ca.* −0.6 V increases. The broad peak at *ca.* −1.0 V is above background base current over the whole pH range. No depression of the base current below that of the background electrolyte is observed on either side of the main peak except for a very small depression on the positive side at pH 2.0.

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(66) J. E. O'Reilly and P. J. Elving, *J. Electroanal. Chem.*, 21, 169 (1969).

(67) J. Kůta and I. Smoler in "Progress in Polarography," Vol. 1, P. Zuman and I. M. Kolthoff, Ed., John Wiley and Sons, Inc., New York, N. Y., 1962, p 43.

The Electrochemistry and Electron Spin Resonance Spectroscopy of 9,10-Di(α-naphthyl)anthracene

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Abstract: Both the anodic and cathodic electrochemistry of 9,10-di(α-naphthyl)anthracene were studied. It was found to be oxidized in methylene chloride to a cation radical which slowly decomposed to another electroactive species. The reduction proceeded through two one-electron steps, the first reversible, the second irreversible. The esr spectra of both the cation and anion radicals were obtained and interpreted. The angle between the anthracene and naphthalene nuclei was found to be 75° by both electrochemical and esr techniques. This latter result is discussed in the context of previously determined angles for other molecules.

The relief of steric strain by twisting about essential single bonds is a chemical phenomenon that has been studied for many years in many ways. The goal of these studies is normally to determine the extent of the departure from coplanarity exhibited by the parts of the molecule in question, and the techniques directed to this end are most often spectroscopic. A frequently cited and studied example is provided by biphenyl.[1] Aryl-substituted polycyclic aromatic hydrocarbons have also received a great deal of attention. These latter-named molecules are of special interest because the magnitude of the steric repulsion energy is very much a function of the position in which the aryl substitution occurs. Compare for example the different amounts of steric interaction encountered in the three isomeric phenylanthracenes.

The structures of 9,10-diphenylanthracene (9,10-DPA), 9,10-di(α-naphthyl)anthracene (9,10-DNA), and 9,9′-bianthryl have been discussed on the basis of their ultraviolet absorption spectra.[2] The ultraviolet spectra of several di- and polyphenyl anthracenes have also been reported.[3] Molecules such as rubrene and 1,8-diphenylnaphthalene have been the subject of several studies because of the possible existence of a spiroconjugative interaction.[4–6] The advent of sophisticated SCF techniques has made the quantitative interpretation of electronic spectra possible. Tinland has applied calculations of this type to naphthyl- and phenyl-substituted naphthalenes, anthracenes, and phenanthrenes.[7,8] A similar approach has also been followed for 2-phenylnaphthalene and some of its derivatives.[9,10]

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