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Electrochemical Reduction of Purine, Pyrimidine, and Imidazole in Aqueous Media. Kinetics and Mechanisms

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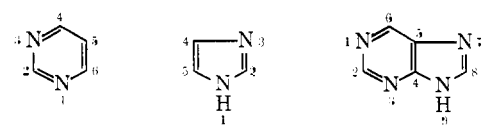
Abstract: The detailed steps in the mechanisms for the electrochemical reduction of purine and pyrimidine in aqueous solution and the kinetic parameters for some of these steps were determined, and the differences between the two compounds were examined in terms of their structure; imidazole itself is not reducible. Reduction of purine involves two pH-dependent processes, corresponding to the overall irreversible reduction of the 1,6 and 3,2 N=C bonds (two electrons and two protons are added per bond). Each reduction stage consists of a very rapid preprotonation of the nitrogen atom at the electroactive site, rapid successive one-electron transfers to produce a carbanion, and, finally, protonation of the latter which results in the overall irreversibility of the electrode process. The heterogeneous rate constants for the electron-transfer steps exceed 0.1 cm sec^{-1}. The pseudo-first-order rate constants, activation energies, and entropies for the follow-up protonation reactions are 4.9×10^3 sec^{-1}, 1.8 kcal mol^{-1}, and -4×10^1 eu for the first reduction process and 1.5×10^4 sec^{-1}, 1.3 kcal mol^{-1}, and -4×10^1 eu for the second. The equilibrium constant for dimerization of the pyrimidine free radical produced on the initial electron addition is estimated to exceed 10^5 l. mol^{-1}. The rate constant for the protonation of the pyrimidine carbanion, which corresponds to the first purine reduction stage, is approximately 5×10^4 sec^{-1}. The diffusion activation energies for the two compounds have also been determined.

In recent years, many of the factors in the electrochemical reduction of the purines and pyrimidines in aqueous media have been identified and evaluated.[2] Interest is increasing in the electrochemical behavior of purine and pyrimidine nucleosides, nucleotides, and polynucleotides;[3a] the compounds investigated have included many biologically important species ranging in complexity to the nucleic acids.[3b] However, there are two aspects, in which the information obtained has been particularly deficient and which need to be understood in order to have a satisfactorily complete description of the redox behavior of the various species involved; these lacunae involve (a) the kinetics of the individual electron transfer and accompanying chemical steps composing the overall redox process, and the exact sequence of these individual steps in the latter process, and (b) the effects on the kinetics and energetics of the overall redox process of orientation of the compound at the electron-transfer interface, adsorption of the compound and other species in the redox mechanism at that interface, and association in the adsorbed state. Information of the type indicated would be especially useful in attempting to use knowledge of the mechanisms of electrochemical redox processes involving purines and pyrimidines better to understand biological processes involving these compounds.

In connection with the systematic exploration of the areas enumerated in the previous paragraph, the present paper covers (a) an examination of the comparative behavior of the three fundamental nitrogen heterocyclic compounds involved (pyrimidine (I), imidazole (II), and purine (III), where the latter base represents a



I II III

fusion of the two previous compounds) and (b) a detailed analysis of the reduction of purine. Purine is known to be irreversibly reduced in aqueous media in two steps; each step involves the addition of two elec-

(1) National Science Foundation Predoctoral Fellow, 1970–1971.

(2) B. Janik and P. J. Elving, *Chem. Rev.*, **68**, 295 (1968); (b) P. J. Elving, J. E. O'Reilly, and C. O. Schmakel, in "Methods of Biochemical Analysis," D. Glick, Ed., Interscience, New York, N. Y., in press.

(3) (a) V. Vetterl, *Collect. Czech. Chem. Commun.*, **34**, 673, (1969); *J. Electroanal. Chem.*, **19**, 169 (1968); E. Palecek, *ibid.*, **22**, 347 (1969); V. Brabec and E. Palecek, *ibid.*, **27**, 145 (1970); B. Janik and P. J. Elving, *J. Amer. Chem. Soc.*, **92**, 235 (1970); J. W. Webb, B. Janik, and P. J. Elving, *J. Amer. Chem. Soc.*, in press; (b) E. Palecek, *Progr. Nucl. Acid Res. Mol. Biol.*, **9**, 31 (1969).

trons and two protons to hydrogenate first the 1,6 N=C bond and then the 3,2 N=C bond.[2,4] The present investigation is particularly concerned with the kinetics of both the heterogeneous electron-transfer steps and the homogeneous chemical-reaction steps in the overall two-electron electrode processes indicated, since irreversible redox behavior may be exhibited by electrode processes involving a slow electron transfer or a rapid electron transfer coupled to a subsequent (follow-up) rapid irreversible chemical reaction, and a distinction in respect to the situation is crucial for proper mechanistic evaluation of the reduction of purine species.[5a]

Because of their importance to the arguments used, certain aspects of ac polarographic theory will be introduced[5b] before summarizing the evidence for the reaction mechanisms and kinetics of purine and pyrimidine.

Ac Polarography. Ac faradaic current magnitudes lower than expected for diffusion-controlled reversible processes are generally attributed to a slow electron-transfer process or to slow chemical equilibria involving the reactant and/or rapid equilibria involving the product of the electron-transfer process. The latter case (the one applicable in the present study) may be depicted by the equation

$$O + ne \underset{}{\overset{k_{s,h}}{\rightleftharpoons}} R \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Z \qquad (1)$$

where O represents the electroactive species, n the number of electrons transferred, R the primary reduction product, and Z a chemically altered form of R, which is electrochemically inactive at the potential at which O is reduced; $k_{s,h}$ is the standard formal heterogeneous rate constant for the limiting electron-transfer (charge-transfer) step, and k_f and k_b are the rate constants for the homogeneous chemical transformation of R to Z.

When both k_f and the equilibrium constant (k_f/k_b) are very large, the overall electrode process is irreversible even if $k_{s,h}$ is large. For the case where k_b approaches zero and $k_f \gg \omega$ (the angular frequency of the applied alternating voltage), expressions for the ac summit potential E_s and peak current i_s have been obtained[6]

$$E_s = E_{1/2}^{r} + \frac{RT}{nF} \ln [1.349 k_f^{1/2} t^{1/2}] - \frac{RT}{nF} \ln Q \qquad (2)$$

$$i_s = \frac{1.644 n^2 F^2 A C_0 (\omega D_0)^{1/2} \Delta E}{RT Q^{1/2} (1 + Q^{-0.545})[1 + (1 + Q^{1/2})^2]^{1/2}} \qquad (3)$$

where $Q = 1.907(\omega t)^{1/2}$, ΔE is the amplitude of the applied alternating voltage, $E_{1/2}^{r}$ is the reversible half-wave potential, *i.e.*, that in absence of a perturbing chemical reaction, t is the dme drop-time, and the other symbols have their usual electrochemical meaning.

The dependence of i_s and E_s on ω, t, ΔE, C_0, and T can be used as diagnostic criteria in respect to the magnitude of $k_{s,h}$ or "reversibility" and to the nature of the coupled chemical reaction. Thus, the frequency dependencies of ac polarographic current, potential, and phase angles may be used at low frequencies to study relatively slow coupled chemical reactions and at high frequencies to evaluate heterogeneous rate parameters;[7] *e.g.*, although the ac current for reversible processes is linearly proportional to $\omega^{1/2}$ with an associated phase angle of 45°, it may be possible at high frequencies to obtain currents and phase angles which are proportional to $k_{s,h}$.[8,9]

In connection with the determination of k_f, irreversible electrode reactions of the type summarized by eq 1 display frequency-independent currents when $k_f \gg \omega$ (*cf.* eq 3) except at very low frequencies where a relatively minor deviation from ω dependence is expected. At high frequencies, *i.e.*, when $k_f < \omega$, the chemical reaction involving the product R occurs only to an inappreciable extent during the period of the alternating voltage and, due to the increased contribution by the reoxidation of species R, the current increases and becomes dependent on $\omega^{1/2}$.

A plot of $i_s/i_{d,s}$ *vs.* $\omega^{1/2}$, where $i_{d,s}$ is the calculated diffusion-controlled current peak for a reversible redox system,[9,10] reaches a limiting value at high frequencies, from which k_f for the irreversible follow-up chemical reaction can be calculated.[7b,11,12]

$$k_f^{1/2} = \frac{1}{1.387 t^{1/2}} [(4.78 i_{d,s}/i_s)^{1/2} - 1]^2 \qquad (4)$$

Imidazole and Pyrimidine[13]

Imidazole does not show any faradaic reduction in aqueous media within the normally available potential range. This lack of redox activity is to be associated with the aromatic nature of the five-membered system which can exist in a large number of resonance forms,[14] as shown by the stability of imidazole to chemical reduction.[15] Pyrimidine, on the other hand, is easily reduced to 1,4,5,6-tetrahydropyrimidine;[16] purine is catalytically hydrogenated, apparently to the 1,6-dihydro derivative.[17]

(4) (a) D. L. Smith and P. J. Elving, *J. Amer. Chem. Soc.*, **84**, 1412 (1962); (b) B. Janik and P. J. Elving, *J. Electrochem. Soc.*, **116**, 1087 (1969); (c) G. Dryhurst and P. J. Elving, *Talanta*, **16**, 855 (1969).

(5) (a) Kinetic steps in electrode processes may be resolved and measured by the use of suitably rapid relaxation methods, in which a perturbing impulse, *e.g.*, a controlled potential increment, is applied to the system under investigation, when it is at equilibrium, and the resulting transient relaxation or response of the system is measured, *e.g.*, as a current flow. Cyclic voltammetry and phase-selective alternating current polarography have been used in the present investigation. (b) For an excellent revew of ac polarography, see D. E. Smith, *Crit. Rev. Anal. Chem.*, **2**, 247 (1971).

(6) D. E. Smith and T. G. McCord, *Anal. Chem.*, **40**, 474 (1968).

(7) (a) T. G. McCord and D. E. Smith, *ibid.*, **40**, 1959 (1968); (b) T. G. McCord, H. L. Hung, and D. E. Smith, *J. Electroanal. Chem.*, **21**, 5 (1969).

(8) P. Delahay, "Double-Layer and Electrode Kinetics," Interscience, New York, N. Y., 1965.

(9) D. E. Smith, in "Electroanalytical Chemistry," A. J. Bard, Ed., Vol. 1, Marcel Dekker, New York, N. Y., 1966, pp 1–155.

(10) B. Breyer and H. H. Bauer, "Alternating Current Polarography and Tensammetry," Interscience, New York, N. Y., 1963.

(11) G. H. Aylward and J. W. Hayes, *Anal. Chem.*, **37**, 195, 197 (1965).

(12) The only unknown in the computation of $i_{d,s}$ is the factor $AD_0^{1/2}$, which is evaluated from dc polarography using the Ilkovic equation. Equation 4, which is for a first-order reaction, can be applied to the follow-up reaction of eq 1, when any solution species, which reacts with R to produce Z, is present at constant activity, *e.g.*, protons in buffered solution or the solvent itself.

(13) The experimental results and certain aspects of the theoretical background for the interpretation of these results are described in the Experimental Section.

(14) D. J. Cram and G. S. Hammond, "Organic Chemistry," McGraw-Hill, New York, N. Y., 1959.

(15) A. Albert, "Heterocyclic Chemistry," 2nd ed, Oxford University Press, New York, N. Y., 1968.

(16) D. J. Brown, "The Pyrimidines," Interscience, New York, N. Y., 1962, p 450; R. F. Evans, *Rev. Pure Appl. Chem.*, **15**, 23 (1965); R. F. Evans and J. S. Shannon, *J. Chem. Soc.*, 1406 (1965); D. J. Brown and R. F. Evans, *ibid.*, 527, 4039 (1962).

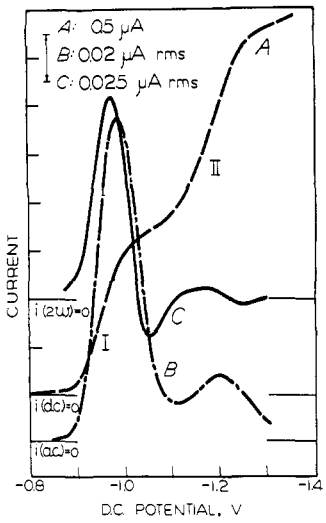


Figure 1. Relation of dc (dme), fundamental frequency ac, and second harmonic ac polarograms for 0.50 m*M* pyrimidine in pH 3.9 acetate buffer. (A) Dc polarogram; (B) phase-selective ac polarogram (in-phase current component), frequency 50 Hz; (C) phase-selective second-harmonic polarogram, frequency 50 Hz (10.6 mV rms), response at 100 Hz. Roman numerals refer to the two pyrimidine waves or peaks seen at this pH.

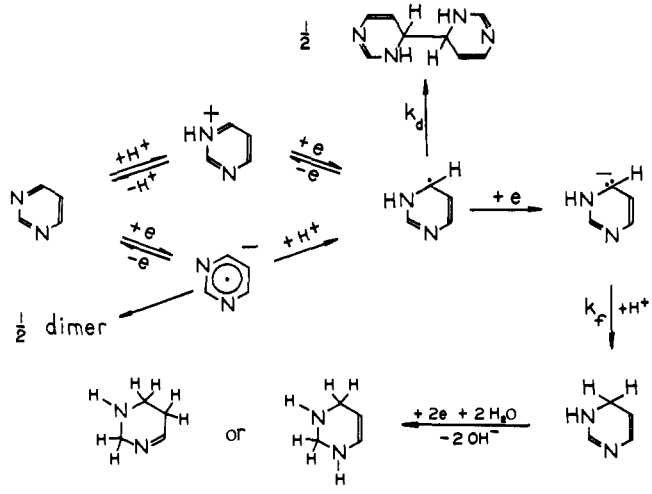


Figure 2. Interpretation of the electrochemical behavior observed for pyrimidine in aqueous media. The lower reaction sequence involving electron addition to unprotonated pyrimidine is that in aprotic media. The reactions involved in the reduction of the 1,2 N=C bond are analogous to those indicated for the 3,4 N=C bond reduction.

Figure 1 presents comparative dc and ac polarograms for pyrimidine waves I and II. The mechanism for the reduction of pyrimidine at mercury electrodes over the available pH range, based on current knowledge, is summarized in Figure 2 (*cf.* Table I and ref 4c, 18, and 19). In order to minimize duplication, certain numerical data and conclusions concerning pyrimidine are given in the section on Purine, where they can be readily compared with the data and conclusions relative to purine.

Comparative Reduction of Purine and Pyrimidine

Although imidazole itself shows little electroactivity, the presence of the imidazole function as part of the purine molecule has a profound effect on the behavior of the latter. This is to be expected from the alteration in the electron densities in the pyrimidine ring when it is fused with the imidazole ring to form purine; *e.g.*, comparison of the calculated electron densities (π charges) for the ground state at the nine positions in purine[20a] and the corresponding positions in pyrimidine (π-deficient N-heterocycle) and imidazole (π-excessive N-heterocycle) indicate a flow of electrons from the imidazole region to the pyrimidine region of the purine (*cf.* Table 2 in ref 20b).

An analogous effect due to electron donation from the imidazole moiety to the pyrimidine moiety of purine is evident in the experimentally measured dipole moments: 2.42 D (in dioxane solution) and 2.4 D (in benzene) for pyrimidine,[15] 3.84 D (in benzene) and 4.84 D (in dioxane; hydrogen bonding may occur between compound and solvent) for imidazole,[15] and 4.3 D for purine (9-butyl derivative in dioxane[21]).

The increases in base strength of N(1) in purine (comparable in structure to N(3) of pyrimidine) and in acid strength of purine N(9) (comparable to N(1) of imidazole) as a result of the electron shift are evident on comparing the pK_a values for proton acquired (basic pK_a) and for proton lost (acidic pK_a): 7.1 and 14.52 for imidazole, 1.23 for pyrimidine, and 2.52 and 8.92 for purine.[22]

Ease of Reduction.[23] One result of the increased electron density is to make the initial electrochemical reduction in aqueous media of purine more difficult than that of pyrimidine, even though the pyrimidine ring is involved in both processes. The potential required for the initial addition of an electron to purine is so much greater than in pyrimidine that the free-radical species (or one derived from it by an exceedingly rapid chemical reaction; *cf.* subsequent discussion) is immediately reduced, resulting in an initial two-electron reduction wave. The fact that the initial pyrimidine reduction (one-electron wave I) is always easier, *i.e.*, at more positive potential, than the initial purine reduction (two-electron wave I), may reflect the assisting action of the pyrimidine free-radical dimerization as well as electronic energy levels.

The first purine wave observed (two-electron wave I) has nearly the same half-wave potential ($E_{1/2}$) as the initially observed two-electron pyrimidine wave (wave III), *i.e.*, $E_{1/2} = -0.686 - 0.086$pH for purine (average of two sets of data) and $-0.680 - 0.089$pH for pyrimidine (Table I). If these two reduction processes

(17) A. Bendich, A. Giner-Sorolla, and J. J. Fox in "Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little Brown, Boston, Mass., 1957, p 11.

(18) (a) D. L. Smith and P. J. Elving, *J. Amer. Chem. Soc.*, 84, 2741 (1962); (b) *Anal. Chem.*, 34, 930 (1962).

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

(20) (a) Calculations for purine cited in the present paper refer to its H(9) tautomer since this is the predominant species in aqueous solution; *e.g.*, *cf.* ref 20b; (b) I. Fischer-Hjalmars and J. Nag-Chaudhuri, *Acta Chem. Scand.*, 23, 2963 (1969).

(21) H. DeVoe and I. Tinoco, *J. Mol. Biol.*, 4, 500 (1962).

(22) (a) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solutions," Butterworths, London, 1965; (b) G. Kortüm, W. Vogel, and K. Andrussaw, "Dissociation Constants of Organic Acids in Aqueous Solutions," Butterworths, London, 1961.

(23) In comparing the behavior of pyrimidine, it must be kept in mind that pyrimidine waves III and IV correspond to purine waves I and II, respectively, in being due to the two-electron reductions of similar sites in the pyrimidine moiety. The fact that purine does not produce one-electron waves similar to pyrimidine waves I and II reflects a distinct difference in mechanism, which results from the fusion of the supposedly inert imidazole ring to the pyrimidine ring.

Table I. pH Dependence of the Potentials for the Reduction of Purine and Pyrimidine in Aqueous Media

pH range[a]	Wave	Potential,[b] V	I_d[c]	Ref
		Pyrimidine		
0.5–5	I	$E_{1/2}$ = −0.576 − 0.105 pH	2.0–2.5	18
3–5	II	−1.142 − 0.011 pH	2.2–2.7	
5–8	III	−0.680 − 0.089 pH	4.3–4.7	
7–8	IV	−1.600 − 0.005 pH	4.8	
9–13	V	−0.805 − 0.079 pH	8–9	
0–5.5	I[d]	E_p = −0.52 − 0.125 pH		4c
		−0.57 − 0.113 pH		
4.5–5.5	II[d]	−1.11 − 0.036 pH		
		−1.22 − 0.031 pH		
6–9	III[d]	−0.73 − 0.082 pH		
		−0.74 − 0.087 pH		
8	IV[d]	1.66		
		−1.71		
8.5–13	V[d]	−0.76 − 0.094 pH		
		−1.90		
0.5–4.8	I	E_s = −0.575 − 0.108 pH		19
2.8–4.8	II	−1.226 − 0.003 pH		
4.8–8.0	III	−0.766 − 0.078 pH		
7.0–8.3	IV	−1.982 + 0.023 pH		
8.3–13.0	V	−1.365 − 0.039 pH		
4.2	I	E_s = −1.024 to −1.037		*f*
	II	−1.190 to −1.20		
4.2	I	E_q = −1.075		*f*
4.4	I	E_0 = −1.06 to −1.10		*f*
		Purine		
2–6	I	$E_{1/2}$ = −0.697 − 0.083 pH	3.8	4a, 18b
	II	−0.902 − 0.080 pH	6.3	
1.4–5.2	I	$E_{1/2}$ = −0.680 − 0.089 pH		*f*
	II	−0.940 − 0.075 pH		
1.4–5.2	I	$E_{1/2}^r$ = −0.748 − 0.089 pH		*f*
	II	−1.016 − 0.075 pH		
0–9	I[d]	E_p = −0.72 − 0.085 pH		4c
		−0.74 − 0.086 pH		
4.2	I[e]	$E_{p/2}$ = −1.407 to −1.057		*f*
0–9	II[d]	E_p = −1.02 − 0.075 pH		4c
		−1.10 − 0.064 pH		
1.4–5.2	I	E_s = −0.714 − 0.087 pH		*f*
	II	−1.055 − 0.061 pH		
4.2–5.2	I	E_q = −0.741 − 0.086 pH		*f*
	II	−0.94 − 0.10 pH		
1.4–5.2	I	E_0 = −0.741 − 0.088 pH		*f*
	II	−1.037 − 0.080 pH		

[a] Buffers used in the various studies may differ somewhat in composition and ionic strength for similar pH regions, which may cause small differences in polarographic parameters. [b] Potentials are *vs.* sce at 25° unless otherwise stated. Equations, where given, indicate the potential, when extrapolated to zero pH, and the variation with pH. Potential parameters: $E_{1/2}$ = half-wave potential on dc polarography at dme; E_s = summit potential on ac polarography; E_q = quadrature peak potential on ac polarography; E_p = peak potential on cyclic voltammetry at hmde; E_0 = second harmonic null potential on ac polarography. Experimental factors such as depolarizer concentration, perturbation frequency in ac polarography and scan rate in cyclic voltammetry may affect the potential. [c] Diffusion current constant: $I_d = i_d/Cm^{2/3}t^{1/6}$. In cases where the current may have had a kinetically controlled component, I was still calculated using i_l, in order to have available comparative values. [d] The upper equation for each wave refers to a scan rate of 0.026 V/sec; the second equation to a scan rate of 0.26 V/sec. [e] Scan rates of 0.024 to 0.192 V/sec. [f] Present investigation.

were truly equivalent, *i.e.*, if they were uninfluenced by kinetic effects due to accompanying chemical reactions, the half-wave potentials would be expected to be different due to the increased electron density in the pyrimidine moiety of purine resulting from the fused imidazole moiety as discussed.

Once the 1,6 and 3,4 N=C sites in purine and pyrimidine, respectively, have been hydrogenated, the situation is drastically altered and 1,6-dihydropurine is more readily reduced than 3,4-dihydropyrimidine; *e.g.*, $E_{1/2}$ values at pH 7, where the latter process is seen, are −1.46 V for two-electron wave II of purine and −1.64 V for two-electron wave IV of pyrimidine (Table I).

Catalytic Hydrogen Reduction. Many nitrogen heterocyclic compounds participate in so-called catalytic hydrogen discharge processes as a result of the easier reduction of the protonated base than of the proton itself. Such processes can be schematically described as follows where RN: represents the nitrogen base.

$$RN: + H^+ \rightleftharpoons RN:H^+ \quad (5)$$

$$RN:H^+ + e \longrightarrow RN: + H\cdot \quad (6)$$

The base liberated in eq 6 can immediately react further with protons.

Imidazole and pyrimidine solutions of pH 4.2 show no shift in the potential of the background discharge (hydrogen-ion reduction). The pyrimidine waves show normal diffusion current constants (I) (Table I). Purine, however, shifts the background discharge to more positive potential; its second wave process contains considerable catalytic hydrogen-ion reduction as revealed by the abnormally large I value for an expected two-electron process (Table I). The effective catalytic agent is the adsorbed four-electron reduction

product (tetrahydropurine and/or its hydrolysis product).[4a]

Purine. Reaction Mechanism and Kinetics[13]

The relationship between the dc and ac polarograms for purine is indicated in Figure 3. The dc half-wave potentials, $E_{1/2}$, the ac summit potentials, E_s, and the second-harmonic null potentials, E_0, for waves I and II show the same pH dependence with one possible exception (Table I). Since E_0 and E_s for wave II are clearly affected by an adjacent catalytic hydrogen evolution, the intercepts and slopes given in Table I are only approximations (*cf.* Experimental Section). Representative data for the dc and ac polarographic reduction of purine are given in Tables II–V.

Table II. Ac and Dc Polarographic Currents and Potentials for Purine (Variation with Concentration[a])

Concn, mM	I_1	i_s/C, μA/mM	i_q/C, μA/mM	$-E_{1/2}$, V	$-E_s$, V	$-E_q$, V
			Wave I			
0.11	4.74	0.82	0.182	1.055	1.085	1.115
0.22	4.39	0.85	0.205	1.060	1.091	1.116
0.55	4.35	0.85	0.191	1.065	1.101	1.132
0.77	4.48	0.84	0.182	1.073	1.105	1.135
1.10	4.34	0.83	0.182	1.080	1.110	1.140
2.20	4.19	0.76	0.164	1.087	1.122	1.152
4.40	4.26	0.69	0.145	1.103	1.135	1.170
			Wave II			
0.11	7.68	0.51	0.227	1.257	1.320	1.400
0.22	7.43	0.52	0.136	1.257	1.320	1.385
0.55	6.28	0.51	0.136	1.262	1.325	1.400
0.77	6.03	0.49	1.169	1.263	1.325	1.420
1.10	5.21	0.47	0.155	1.275	1.325	1.420
2.20	5.20	0.43	0.136	1.280	1.330	1.450
4.40	5.55	0.31	0.082	1.300	1.330	1.460

[a] System: pH 4.7 acetate buffer. $I_1 = i_1/Cm^{2/3}t^{1/6}$, where i_1 is the dc polarographic limiting current (undamped); i_s is the in-phase ac polarographic peak current at 100 Hz; i_q is the ac polarographic quadrature current.

Table III. Ac and Dc Polarographic Currents and Potentials for Purine (Dependence on DME Drop-Time)

Drop-time, sec	Ac Polarography[a]: $i_s t^{1/2}$, μA sec$^{1/2}$ I	II	E_s, V I	II
3.86	2.40	1.41	−1.080	−1.305
4.60	2.36	1.39	−1.079	−1.300
5.59	2.36	1.38	−1.077	−1.300
7.02	2.33	1.35	−1.076	−1.305
9.43	2.28	1.39	−1.074	−1.300
	Dc Polarography[b]: $i_d/h^{1/2}$, μA cm$^{-1/2}$		$(E_{1/4} - E_{1/2})$, mV	
Drop-time,	I	II	I	II
3.31	0.37	0.61	43	53
4.36	0.37	0.64	47	51
7.14	0.38	0.69	57	47

[a] System: 1.54 m*M* purine in pH 4.2 acetate buffer; 100 Hz. Roman numerals refer to peaks. [b] System: 0.5 m*M* purine in pH 4.2 acetate buffer. Roman numerals refer to waves.

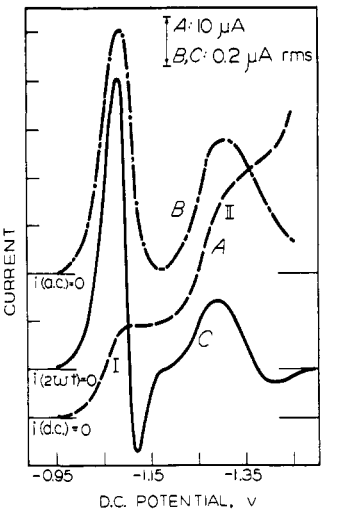


Figure 3. Relation of dc (dme), fundamental frequency ac, and second harmonic ac polarograms for 1.54 m*M* purine in pH 4.2 acetate buffer: (A) dc polarogram; (B) phase-selective ac polarogram (in-phase current component), frequency 100 Hz, scan rate 50 mV/min; (C) phase-selective second-harmonic polarogram, frequency 100 Hz, response at 200 Hz, scan rate 50 mV/min. Roman numbers refer to the two purine waves or peaks.

Table IV. Ac Polarographic Potentials for Purine and Pyrimidine (Dependence on Frequency)

Freq, Hz	$E_{1/2} - E_s$, mV: Purine[b] Calcd[a]	Purine[b] I	Purine[b] II	Pyrimidine[c] I
10	22	26	35	
20	25	26	40	
30	26			36
50	28	28	42	38
100	30	30	45	44
250	33			50
500	35	34	50	54
1000	37	36	50	56
2000	39	40	50	

[a] Calculated from eq 2 and 10; t = 4.64 sec. [b] System: 1.54 m*M* purine in pH 4.2 acetate buffer. Roman numerals refer to peaks. $E_{1/2}$ values for purine waves I and II are −1.052 and −1.250 V, respectively. [c] System: 0.50 m*M* pyrimidine in pH 3.9 acetate buffer. $E_{1/2}$ for pyrimidine wave I is −0.953 V.

Table V. Second Harmonic Currents and Potentials for Purine (Dependence on Frequency[a])

Freq, Hz	$i(2\omega)$, μA: I_a	I_c	II_a	II_c	$-E_0$, V I	II	$(E_{1/2} - E_0)$, mV I	II
25	0.38	0.13	0.10	0.02	1.180	1.41	42	80
50	0.51	0.30	0.18	0.05	1.175	1.41	37	80
100	0.88	0.24	0.24	0.03	1.193	1.43	55	100
200	0.76	0.20	0.20	0.02	1.200	1.47	62	140
400	0.95	0.22	0.26	0.02	1.203		65	
800	1.24	0.30	0.33	0.02	1.205		67	
1200	1.30	0.43	0.53	0.05	1.208	1.45	70	110

[a] System: 1.54 m*M* purine in pH 4.2 acetate buffer. Roman numbers refer to the corresponding peaks and waves; subscript a refers to current on more positive potential side of curve and subscript c to that on the more negative potential side.

The potential indices become more negative in the order of $E_{1/2} > E_s > E_0$ and $E_s > E_q$ (quadrature peak potential); the potential indices for pyrimidine show the same order. The constant differences between the three purine potentials listed can be ascribed to the experimental factors of dme drop-time and perturbation frequency (*cf.* eq 2).

The essential overall reaction sequence for each two-electron electrode process can be formulated as in eq 1, with the qualifications that $k_{s,h}$ is large, $k_b \rightarrow 0$, and

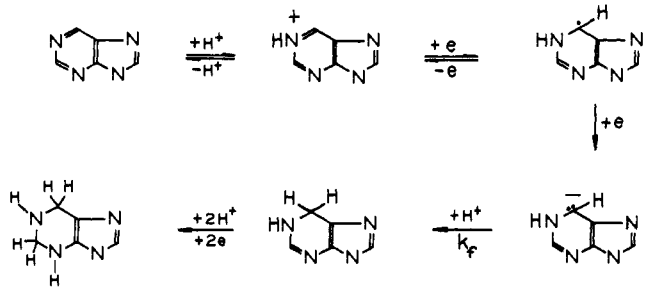


Figure 4. Interpretation of the electrochemical behavior observed for purine in aqueous media. The reactions involved in reduction of the 3,2 N=C bond are similar to those indicated for the 1,6 N=C reduction.

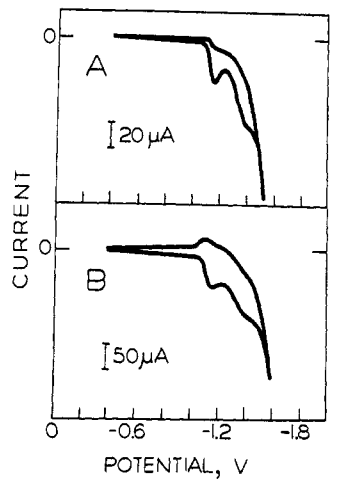


Figure 5. Cyclic voltammograms of 1.54 mM purine in pH 4.2 acetate buffer. Scan rate: (A) 2.4 V/sec; (B) 24 V/sec.

$k_f \gg \omega$. Purine also undergoes a preliminary preprotonation reaction (Figure 4), whose rate is extremely rapid and which does not materially affect the equilibria of eq 1.

Evidence for Associated Chemical Reactions. The similar linear pH dependence of the three potential indices for wave I (Table I) probably indicates that the potential-determining step involves one proton or a species in equilibrium with a proton. Since d(E)/d(pH) values do not change with frequency, the protonation reaction is likely to be a preprotonation step, which is sufficiently rapid so as not to influence kinetically the overall reduction process.

The influence of an associated or coupled chemical reaction on the reduction process was detected from the dc polarographic log current–potential plots for wave I. Cyclic voltammetry (Figure 5) then confirmed the presence of a rapid follow-up reaction. The second-harmonic ac polarogram (Figure 3) is characteristic of reduction processes influenced by chemical reactions involving the reduction product. Faradaic ac current magnitudes for both reduction processes are generally low compared to reversible process expectations; the sharper peaks and larger current magnitudes for the first reduction process (wave or peak I) indicate that the reoxidation reaction is more prevalent for this process than for the second (wave or peak II).

The shape of the $i_s - \omega^{1/2}$ curve for both peaks is indicative of an irreversible electrode process of the type depicted by eq 1; i_s, which is only slightly dependent on frequency within the range of 100 to 500 Hz, increases abruptly above 500 Hz. At frequencies below 500 Hz, the half-life of the electroactive intermediate is short compared to the time scale of the electrochemical experiment; however, as the latter is decreased by increasing the frequency, the chemical reaction cannot keep pace with the much faster electron-transfer process and an approach toward reversibility is observed.

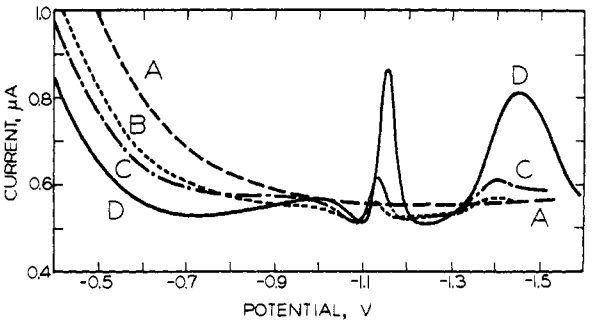


Figure 6. Effect of purine on quadrature component of phase-selective ac polarograms: frequency 100 Hz; scan rate 50 mV/min. (A) pH 4.7 acetate buffer (0.5 M ionic strength); (B) after addition of 0.22 mM purine; (C) after addition of 0.55 mM purine; (D) after addition of 2.20 mM purine.

Another strong proof for the presence of an irreversible follow-up chemical reaction is the dependence of the faradaic phase angle, ϕ, on the dc potential. If the cause of overall irreversibility of an electrode process is a slow chemical reaction accompanying a rapid electron-transfer process, cot ϕ will increase at potentials more positive than $E_{1/2}$ for a chemical reaction involving the reduced species and at potentials more negative than $E_{1/2}$ for one involving the oxidized species. Total overall irreversibility is associated with phase angles approaching zero. In the case of both purine waves, cot ϕ increases to very large values at potentials more positive than $E_{1/2}$, indicating very small angles; ϕ at the $E_{1/2}$ for each process is 0 ± 2° at 100 Hz. For pyrimidine wave I at 100 Hz, ϕ is also near zero.[24]

Adsorption. Fusion of the pyrimidine and imidazole rings causes a major change in adsorptive behavior at the mercury–aqueous solution interface. In the absence of faradaic processes and under an alternating voltage perturbation, the deviation of the quadrature current response of a solution of a compound from the response of the same solution in its absence would be due to the alteration of the interfacial electrical double layer by the presence of the compound; essentially, the quadrature current is then an indirect measure of the differential capacity of the double layer.

At pH 4.2, the presence of imidazole has negligible effect on the quadrature current. Pyrimidine also has generally very little effect except for a small but distinct depression of the quadrature current in the potential region in which the faradaic in-phase current is flowing. However, the quadrature ac polarogram for purine, *e.g.*, Figure 6, reveals a general depression of the capacitive current throughout the potential range, indicating adsorption of purine species; adsorption is also demonstrated by a general lowering of the electrocapillary curve.[4c] The fact that the capacitive current

(24) Cot ϕ is quite large and possibly even negative, depending on choice of background current, at potentials more positive than E_s.

is lower than background current between the two quadrature peaks (associated with the faradaic processes) indicates that the first reduction product is also adsorbed.

Reaction Mechanism. The irreversible reduction of eq 1 may be considered as a simplification of the more general mechanism shown in eq 7-9. The fact that

$$O + e \rightleftharpoons R_1 \qquad E_1^\circ \tag{7}$$

$$R_1 + e \rightleftharpoons R_2 \qquad E_2^\circ \tag{8}$$

$$R_2 \xrightarrow{k_f} Z \tag{9}$$

only one two-electron cathodic wave or peak is observed for each purine bond reduction indicates that E_1° is equal to or more negative than E_2°; *i.e.*, the initially produced free radical corresponding to R_1 is as readily or more readily reduced than the protonated purine, O. This sequence is generally parallel to that observed for the reduction of pyrimidine itself in acetonitrile media, where the neutral free radical, produced by one-electron reduction of pyrimidine and protonation, is more readily reduced than pyrimidine itself.[25] It is possible that there may be an exceedingly rapid chemical change in the nature of the species R_1 between the reactions of eq 7 and 8.

Combination of all available data suggests for the initial reduction step for purine (wave I) the mechanism outlined in Figure 4. Supporting evidence is provided by the pH dependence of the first-electron addition and the pH independence of the second-electron addition in the reduction of pyrimidine itself in aqueous media.[18,19] Protonation of purine is an exceedingly rapid process;[4a,b,26,27] the similar linear pH dependencies of $E_{1/2}$, E_s, and E_0 indicate that addition of the second electron does not effect the protonation equilibrium involving purine.

Reduction of the 3,2 N=C bond, which produces purine wave II, apparently follows a path similar to that of the 1,6 N=C reduction; the final reduction product can hydrolyze to cleave the tetrahydropyrimidine portion of the ring.[4a]

The electron-transfer processes for both reduction steps are very rapid since the ac current increases at perturbation frequencies in the range of 500 Hz to 2 kHz; the standard heterogeneous rate constants ($k_{s,h}$) probably exceed 0.1 cm sec^{-1}.

The rates of deactivation of the reaction intermediate, *i.e.*, protonation of the carbanion (Figure 4), are dependent on the effective concentrations as well as stabilities of the carbanions and, hence, indirectly on the stabilities of the free radicals and their rates of oxidation and reduction. Thus, the sharper fundamental ac and second harmonic polarographic peaks observed for the first reduction process indicate that the initial free radical or carbanion intermediate is sufficiently stable to be at least partially reoxidized before being further reduced or otherwise reacting; *e.g.*, at 100 Hz, the perturbation is rapid enough for a fraction of the intermediate to be reoxidized. Protonation of the carbanion in the second reduction step to form the tetrahydropurine may be slightly assisted by the hydrolysis of the latter; however, the rate of hydrolysis is so slow compared to that of protonation that this effect would be expected to be minimal.

The relative magnitudes of the k_f values for the carbanion protonation, 4.9×10^3 sec^{-1} for the 1,6 N=C bond reduction process and 1.5×10^4 sec^{-1} for the 3,2 N=C reduction, are in accord with observations mentioned in the previous paragraph. The low activation energies of 1.8 and 1.3 kcal mol^{-1} calculated for the two processes are consistent with the rapid reactions seen with purine, which would be expected to be enhanced by the coulombic attraction between the carbanion and hydronium ions. Adsorption of the various species may also be a contributing factor. The similar activation entropies of 4×10^1 entropy units for the two processes indicate their similar natures.

The rate constant, k_f, for protonation of the pyrimidine carbanion after the second one-electron addition, about 5×10^4 sec^{-1}, is an order of magnitude faster than the corresponding protonation rate for purine (4.9×10^3 sec^{-1}) as would be expected for a more highly reactive monocyclic compound. The equilibrium constant for dimerization of neutral pyrimidine free radicals is estimated to be greater than 10^5 l./mol, on the basis of E *vs.* log $[i/(i_d - i)]$ plots for polarographic wave I of pyrimidine. Since the dimerization rate constant for the forward process would be expected to be greater than that for pyrimidine radical anions in acetonitrile solvent[25] of 8×10^5 l./(mol sec), because of the electrostatic repulsion between two anionic species, this large value for the equilibrium constant is not unexpected.

Experimental Section

To minimize the effects of ionic strength, double layer structure alteration, and cell resistance, the test solutions were generally of 0.5 *M* ionic strength (adjusted with KCl where necessary).

All data reported are at 25° unless otherwise stated. All potentials are referred to the sce.

Chemicals. The chromatographic grade purine and pyrimidine (Mann Research Laboratories) used exhibited no polarographically detectable impurity; imidazole (Eastman) was recrystallized. Nitrogen used to deoxygenate the test solution was purified by bubbling it successively through two acidic V(II) solutions, saturated calcium hydroxide solution, and, finally, distilled water. All solutions were prepared with distilled and deionized water of 1.4 megohm specific resistivity. The mercury was triply distilled; all other chemicals were of reagent grade.

Apparatus. The temperature of the water-jacketed, three-compartment cell employed[28] was controlled to ±0.1°. The electrodes consisted of (a) a dme (marine barometer tubing) in the test solution compartment, (b) a coil of platinum wire immersed in a solution of supporting electrolyte as the counter electrode in one end compartment, and (c) an aqueous sce as reference electrode in the other end compartment. The dme flow rate, m, was 1.59 mg sec^{-1} at a column height, h (corrected for back pressure), of 64.8 cm for capillary A and 1.15 mg sec^{-1} at 75.9 cm for capillary B; drop-times (t) at potentials of interest were generally 4 to 5 sec (capillary B was used to obtain the pyrimidine data and the following purine data: Figure 6, the data in Table II, and the ac data in Table III). Cyclic voltammetric measurements were made in a water-jacketed single compartment cell, fitted with a Teflon top through which the hanging mercury drop electrode (hmde), a commercial sce, a fitted sleeve with the counter electrode, and a gas bubbling tube could be inserted.

Dc and ac voltammograms were obtained with an instrument assembled from solid-state operational amplifiers and standard electronic components. The ac polarographic arrangement followed Hayes and Reilley[29a] and Smith;[29b] a Princeton Applied

(25) J. E. O'Reilly and P. J. Elving, *J. Amer. Chem. Soc.*, **93**, 1871 (1971).
(26) B. Janik and P. J. Elving, *ibid.*, **92**, 235 (1970).
(27) R. J. Pugmire and D. M. Grant, *ibid.*, **93**, 1880 (1971).
(28) J. E. Hickey, M. S. Spritzer, and P. J. Elving, *Anal. Chim. Acta*, **35**, 277 (1966).
(29) (a) J. W. Hayes and C. N. Reilley, *Anal. Chem.*, **37**, 1322 (1965); (b) D. E. Smith, *ibid.*, **35**, 1811 (1963).

Research Model 122 lock-in amplifier was used to effect phase-selective measurement. A Hewlett-Packard 202A function generator was used for the triangular wave input for cyclic voltammetry and as the external sinusoidal perturbation source for the second-harmonic experiments. Curves were recorded on a Moseley 7005B X–Y recorder. Signal monitoring and calibration were performed with a Tektronix 502A oscilloscope; a Hewlett-Packard 3430A digital voltmeter was used to monitor the applied dc potential. A Beckman Model G pH meter was used to measure the pH of test solutions.

Procedures. Stock solutions of depolarizer and supporting electrolyte (generally acetate buffer) were prepared by diluting weighed quantities to known volume. Test solutions were prepared by diluting appropriate amounts of stock solutions in volumetric flasks. Nitrogen was bubbled through the test solution in the electrolytic cell for at least 10 min, the dme was inserted, and nitrogen was passed over the test solution during the experiment.

The desired in-phase and quadrature components of the ac polarographic signal were selected by the use of a dummy cell composed of 1% precision RC components. This procedure compensated for phase changes due to the external circuit by directly phasing the current response with the reference signal of the lock-in amplifier, which is also the perturbation signal. The potential drop caused by the resistance uncompensated by the potentiostat (*ca.* 100 ohms) was corrected by positive feedback;[30] this was generally accomplished by adjusting the feedback resistor in the potentiostat to minimum in-phase current at a potential where no faradaic current was flowing. The resistance at the latter thus selected generally agreed with that determined on the solution system with a Wheatstone bridge, using a 1-kHz signal. Phase selection and resistance compensation for second-harmonic experiments were accomplished similarly, except that the response signal was tuned to twice the frequency of the applied perturbation. All polarograms were recorded at natural drop-lives; *i.e.*, current magnitudes are those at the end of the drop-life. Dc current measurements are undamped; ac currents were recorded with damping equivalent to a 1-sec RC time constant, except for the data in Table II and Figure 6 where the time constant was 0.3 sec.

Current was customarily measured to ±1%. Ac and dc potentials were generally measured to ±1 mV; due to the broadness of the various ac purine peaks II, these and the cyclic voltammetric peaks were measured to ±5 mV. The closeness of purine wave II (and its corresponding peaks) to background discharge introduces an appreciable uncertainty in measurements made on it.

Amplitudes for the applied alternating voltage perturbation signal were 2 mV (rms) for fundamental frequency experiments and 20 mV (peak-to-peak), *i.e.*, 7.1 mV rms, for second-harmonic experiments, unless otherwise specified; all alternating current magnitudes are in rms values.

Purine. Experimental

Most of the data were obtained at pH 4.2 and 4.7, where minimum complications of chemical and electrochemical origins are to be expected.[4a,b]

Dc Polarography and Voltammetry. In the pH range of 2–6, purine produces two diffusion-controlled two-electron reduction waves at the dme.

Plots of E *vs.* $\log [i/(i_d - i)]$ for wave I at pH 4.2 and 5.2 and at purine concentrations from 0.31 to 3.1 mM are nonlinear with $(E_{1/4} - E_{3/4})$ values greater than the 28 mV expected for reversible processes; similar plots of E *vs.* $\log [i^2/(i_d - i)]$ are linear with slopes of 28 mV, indicating a two-electron process (*cf.* ref 31a). A two-electron process is thus supported by the logarithmic current–potential relationships with the deviations from Nernstian reversibility being attributed to processes associated with the electron-transfer reaction proper (*cf.* ref 31). An analysis for wave II is unwarranted because of the effect of catalytic hydrogen evolution on the latter portion of the wave.

Single scan (sweep to negative potential) voltammograms at a hmde at pH 4.2 show two well formed cathodic peaks corresponding to the dc polarographic waves at a very slow scan rate (v), *e.g.*, 24 mV/sec; with increasing v, peak II merges with the following catalytic hydrogen wave and cannot be properly analyzed. The variation in $i_p/v^{1/2}$ and $E_{p/2}$ values at slow scan rates (24 to 192 mV/sec) for single cathodic scan voltammograms is consistent with that prescribed by theory;[32] the $i_p/v^{1/2}$ ratio should vary by only about 10% for a variation in v of about three orders of magnitude, and E_p should shift to more positive potential by $29.6/n$ mV for a tenfold decrease in v. Over the range of 0.024 to 0.192 V/sec scan rate, $i_p/v^{1/2}$ for purine peak I is essentially independent of v, and E_p shifts 13 ± 3 mV/decade.

Cyclic voltammetry at low scan rates does not reveal any anodic peaks in the accessible potential range; however, at faster scan rates (corresponding to shorter switching times or time gates, τ) an anodic peak is observed corresponding to the oxidation of the peak I reduction product (Figure 5); a τ of 70 msec or less is needed to detect effectively the anodic peak complementary to cathodic peak I. The anodic peak increases in magnitude with decreasing switching time at v = 160 V/sec; above this rate, the charging current becomes too large to allow meaningful faradaic current measurement.

Observation of an anodic peak only at rapid scan rates indicates that the irreversibility is due not to a slow electron-transfer process, but to fast deactivation of the reduction product by a chemical reaction.

The homogeneous reaction rate constant k_f for a follow-up chemical reaction may often be determined from cyclic voltammetric i_a/i_c ratios. Unfortunately, measured i_a/i_c ratios for peak I exceeded the limit of the i_a/i_c *vs.* $k_f \tau$ relationships summarized by Nicholson and Shain,[32] so that k could only be estimated to exceed 2×10^2 sec^{-1} for τ in the range of 7 to 70 msec.

Since an anodic peak corresponding to cathodic peak II is not observed even for a τ of 7 msec at a v of 160 V/sec, it can be concluded that the electroactive intermediate is much less stable than that in the first wave process; *i.e.*, k_f for the wave II process exceeds that for the wave I process.

Ac Polarography.[33] The fundamental frequency and second-harmonic current magnitudes are proportional to ΔE and ΔE^2, respectively, as expected from theoretical considerations. A slight deviation of the $i_s - \Delta E$ plot from linearity at perturbation amplitudes greater than 5 mV is due to the significant contribution of higher harmonic current at the larger amplitudes; because a perturbation voltage of 2 mV is sufficient to yield adequate current responses, it was used.

Ac peak I is sharper than peak II, although the potential widths at half-height of both peaks are greater than the $90/n$ mV expected for a reversible process (Figure 3B). A plot of $\log \{(i_s/i)^{1/2} - [(i_s - i)/i]^{1/2}\}$ *vs.* E for peak I at 100 Hz (Figure 7) is quite linear on the positive potential side but nonlinear on the negative side with slopes of 104 and −74 mV, respectively (theoretical slope for a reversible process, ±120/n mV). The very

(30) (a) E. R. Brown, T. G. McCord, D. E. Smith, and D. D. DeFord, *Anal. Chem.*, **38**, 1119 (1966); (b) E. R. Brown, H. L. Hung, T. G. McCord, D. E. Smith, and G. L. Booman, *ibid.*, 40, 1424 (1968).

(31) (a) G. J. Hoijtink, J. van Schooten, E. de Boer, and W. Y. Aalbersberg, *Recl. Trav. Chim. Pays-Bas*, **73**, 355 (1954); (b) A. A. Vlcek, *Progr. Inorg. Chem.*, **5**, 257 (1967).

(32) R. S. Nicholson and I. Shain, *Anal. Chem.*, **36**, 706 (1964).

(33) Most of the considerable amount of ac polarographic data on purine collected in the course of the present study has not been presented for reasons of length.

low current magnitudes of both peaks suggest irreversibility; the experimental values[34] of 1.89 and 1.00 μA for peaks I and II, respectively, compare well with the maximum i_s value of 1.45 μA for a totally irreversible ac peak calculated from eq 3.

The asymmetries of the second-harmonic curves (Figure 3C) are characteristic of reduction processes influenced by a chemical reaction involving the reduction product. Current magnitudes for both reduction processes are low compared to reversible process expectations; current magnitudes for the peaks at more negative potential are less than those for the corresponding precedent peaks. The sharper peaks for the first process indicate it to be more reversible than the second. The potential separation between the positive and negative peaks of the second-harmonic curve for both processes is considerably greater than the 34 mV expected for a reversible two-electron process; *i.e.*, the more positive second-harmonic peak potential for peak I is 35 mV positive of E_0 while the more negative peak is 17 mV more negative. The magnitude, broadness, and virtual disappearance of the more negative peak of the second harmonic for peak II are indicative of the faster deactivation of the electroactive intermediate compared to that of the first process.

The following subsections summarize the effects of experimental variables primarily on the ac polarographic behavior of purine and indicate the use of these effects as diagnostic probes in mechanism elucidation and for kinetic measurement.

Effect of Concentration. The ratios of dc polarographic i_l and ac i_s to depolarizer concentration are essentially independent of concentration as expected for diffusion controlled processes (Table II). The deviations at concentrations exceeding 2 mM may be due to the increasing effect of purine adsorption on the electrode,[10] which results in a leveling off of the amount adsorbed with increasing concentration, and/or to a breakdown in the diffusion concentration gradient of a type frequently encountered at higher concentrations. The negative shift in potential with increasing purine concentrations is probably due to increasing purine adsorption, which may diffuse the double layer and, correspondingly, increase the ψ potential; the net effect is that more negative potentials are required to produce the same currents as at lower ψ potentials.

Effect of Ionic Strength. E_s for both ac peaks is independent of ionic strength. A small positive shift in peak I observed when KCl is added to a purine solution in acetate buffer is probably a result of restructuring of the diffuse double layer with a resultant decrease in the ψ potential;[8] the precision of measurement of peak II ($\pm$5 mV) is inadequate to detect a similar shift. Dependence of E_s on ionic strength would be expected if the depolarizing species were ionic; *i.e.*, its activity coefficient would be affected. Since pK_a for purine (proton addition) is 2.5, the neutral purine molecule would be the primary species present in the bulk solution at pH 4.2, which is in conformity with the lack of ionic strength effect.

The data do not exclude protonation of adsorbed purine or a concerted electron transfer–protonation process. For example, the behavior of purine would be explicable if the molecule were protonated at N(1), which is part of the initial reduction site, as a result of increasing electron density at N(1) on adsorption of purine on the negatively charged electrode. The peak current is not significantly affected by the ionic strength.

(34) The experimental conditions are C_0 = 1.54 mM, n = 2, T = 25°, ΔE = 2.0 mV rms, ω = 628 radians sec^{-1}, t = 4.64 sec, and $AD^{1/2}$ = 8.83 × 10^{-5} cm^2 sec$^{-1/2}$.

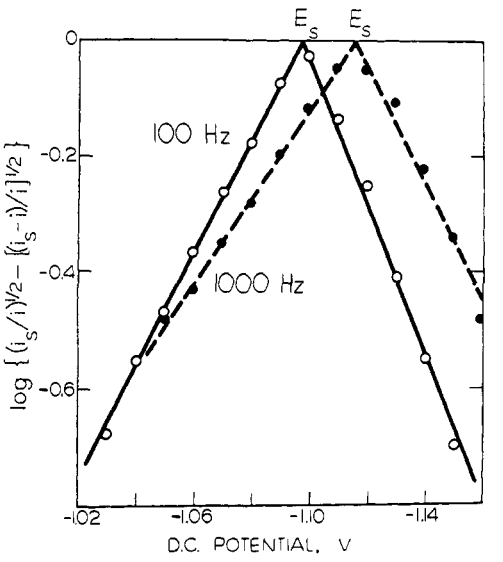


Figure 7. Plot of log $\{(i_s/i)^{1/2} - [(i_s - i)/i]^{1/2}\}$ *vs.* dc potential for ac polarographic peak I of Figure 3B at 100 and 1000 Hz; i is faradaic ac current component in phase with the applied perturbation at end of the natural mercury drop-life.

Effect of Drop Time. The ac polarographic current shows a dependence on dme drop-time whenever the faradaic process is kinetically influenced by some process slower than diffusion,[35] *e.g.*, coupled chemical reactions, slow electron-transfer processes, slow adsorption processes, and amalgam formation. Peak currents for irreversible processes[6,36] are linearly dependent on $t^{-1/2}$, *e.g.*, eq 3; the product $i_s t^{1/2}$ is independent of t for purine (Table III). The constancy of the dc i_l to $h^{1/2}$ ratio for wave I confirms diffusion control of the dc process. The large values of $i_d/h^{1/2}$ for wave II as compared to wave I are due to the influence of the catalytic hydrogen reaction; the latter is also manifest in increase of the ratio with decreasing h.

Equation 2 indicates that the difference (E_s − $E_{1/2}$) should become more negative with increasing t; however, the shift, which would only amount to 7.1/n mV for a threefold increase in t, is not easily detectable experimentally. Hence, although E_s is virtually independent of t, no definite conclusions can be drawn. However, the diffusion control of the dc current eliminates the possibility of a preceding chemical reaction which is slow, and the proportionality of the ac current to $t^{1/2}$ indicates irreversibility without identifying its nature.

Frequency Dependence. Plots of i_s *vs.* $\omega^{1/2}$ for purine at pH 4.2 and 5.2 are similar (Figure 8). The shape of the ac curve is in agreement with theory, *e.g.*, the negative shift in E_s with increasing frequency (eq 2) (Table IV).

(35) G. H. Aylward and J. W. Hayes, *J. Electroanal. Chem.*, **8**, 442 (1964); *Anal. Chem.*, **36**, 2218 (1964); H. L. Hung and D. E. Smith, *ibid.*, **36**, 922 (1964); D. E. Smith and H. L. Hung, **36**, 2219 (1964).

(36) B. Timmer, M. Sluyters-Rehbach, and J. H. Sluyters, *J. Electroanal. Chem.*, 14, 169, 181 (1967); B. G. Dekker, M. Sluyters-Rehbach, and J. H. Sluyters, *ibid.*, 21, 137 (1969).

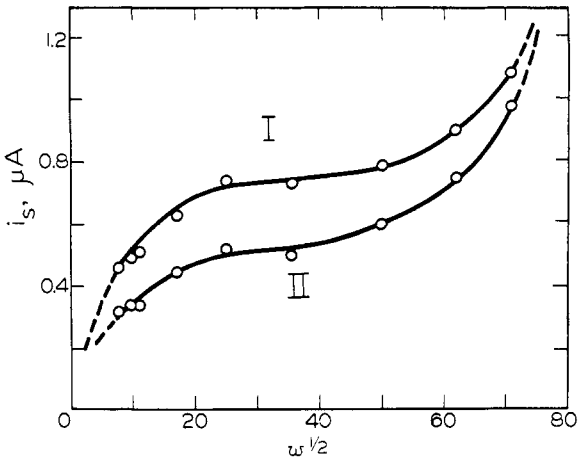


Figure 8. Variation of i_s with frequency for 1.00 mM purine in pH 4.2 acetate buffer; ω is the angular frequency of the applied alternating voltage. Roman numbers refer to the two purine peaks.

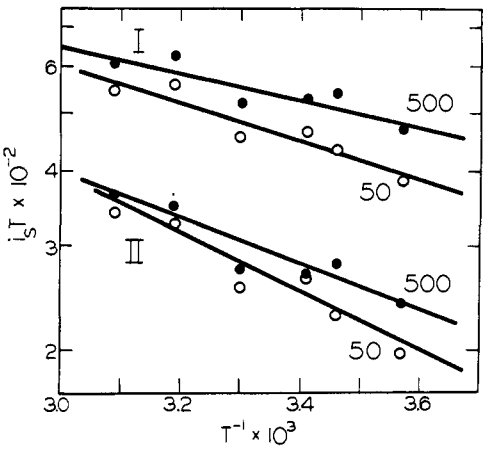


Figure 10. Variation of log (i_sT) with T^{-1} for 1.54 mM purine in pH 4.7 acetate buffer. Roman numbers refer to the two purine peaks, arabic numbers to the frequency in Hz.

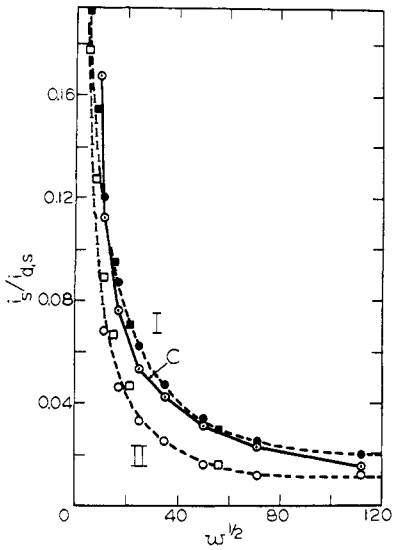


Figure 9. Variation of the ratio of $i_s/i_{d,s}$ with frequency for 1.54 mM purine. Circles correspond to i_s values at pH 4.7 and squares at pH 5.2. Roman numbers refer to the two purine peaks; C refers to ratios based on values of i_s calculated by eq 3.

The second-harmonic current varies with frequency in the same general manner as i_s (representative values in Table V), *i.e.*, the currents do not vary appreciably except at high frequencies and the second-harmonic wave I is more symmetrical and larger than wave II throughout the frequency range; E_0 also becomes more negative with increasing frequency.

The i_s values for purine do not change appreciably in the pH range of 3.7 to 5.2, but E_s and $E_{1/2}$ became linearly more negative. Such behavior is in agreement with a rapid equilibrium involving protons, which should, therefore, not affect the rate of the overall reaction.

Typical plots of $i_s/i_{d,s}$ *vs.* $\omega^{1/2}$ are shown in Figure 9; the frequency-independent asymptotes can be used in conjunction with eq 4 to determine k_f. The k_f values, thus determined at pH 4.7 and 5.2, are 4.9×10^3 sec^{-1} for the first reduction process and 1.5×10^4 sec^{-1} for the second.

At low frequencies, where i_s is independent of k_f, there is general agreement between the experimental $i - \omega$ curves of Figure 9 and the theoretical curve for ratio of i_s (calculated by eq 3, using the parameters corresponding to peak I) to calculated $i_{d,s}$.

On the basis of the variation of i_s at high frequencies, $k_{s,h}$ for the limiting heterogeneous electron-transfer process probably exceeds 0.1 cm sec^{-1}, based on measurement at 2.0 kHz, since the current is still increasing sharply with no apparent tendency to level off.

The reversible half-wave potentials at 25° may be estimated from the measured k_f values.[37]

$$E_{1/2} = E_{1/2}^{r} + \frac{RT}{nF} \ln [1.349(k_f t)^{1/2}] \qquad (10)$$

The reversible half-wave potential for purine wave I is 68 mV more negative than its $E_{1/2}$; that for wave II is 74 mV more negative. Equations for the pH dependency of $E_{1/2}^{r}$ may then be written (Table I).

Temperature Dependence. The temperature dependence of the ac faradaic current can also be used for mechanistic elucidation,[38] *e.g.*, log (i_sT) *vs.* $1/T$ plots for charge-transfer reactions uncomplicated by slow coupled chemical reactions are linear with the slope dependent on the charge-transfer rate; the plots are nonlinear in the presence of slow coupled chemical reactions. A totally irreversible process of the eq 1 type should result in linear plots, since i_s is dependent on temperature only through the diffusion coefficient (eq 3), provided that $k_f \gg \omega$ within the temperature range investigated.

The observed ac currents for purine increase with temperature; the i_s temperature coefficients $[(1/i_sT) \cdot (d[i_sT]/dT)]$ at 50 Hz are 0.89% for peak I and 1.36% for peak II, based on equations fitted to the data (Figure 10) by a least-squares procedure. Similarly, the second-harmonic current peaks usually increase with temperature. The dc current temperature coefficient $[(1/i_l) \cdot (di_l/dT)]$ for wave I is 1.2%, indicating diffusion control; that for wave II is larger due to the adjacent catalytic hydrogen wave (the plot for wave II is curved; if the two extreme temperature points are used, the coefficient is 2.1%).

Log (i_sT) plots (Figure 10) should give, for situations

(37) It is implicitly assumed in eq 10 that variation in pH will not affect k_f values, although this assumption was only verified in the pH range of 4.2 to 5.2.

(38) G. H. Aylward and J. W. Hayes, *Bull. Chem. Soc. Jap.*, **38**, 1794 (1965).

where eq 3 is applicable, slopes corresponding to diffusion activation energies at low frequencies, *e.g.*, 50 Hz (ω = 314 radians sec^{-1}), where the reduction process is expected to be totally irreversible since $k_f \gg \omega$. The experimentally determined activation energies of 2.9 and 4.5 kcal/mol for peak I and peak II, respectively, are in reasonable agreement with the expected 4 to 6 kcal/mol.[38,39] The activation energy for dc wave I is 3.7 kcal/mol; that for dc wave II could not be calculated due to the curvature in the log i_l *vs.* T^{-1} plot caused by the catalytic hydrogen evolution.

At high frequencies where ω is comparable to k_f, *e.g.*, at 500 Hz where ω = 3140 radians sec^{-1}, eq 3 no longer holds and account has to be taken of the temperature dependence of k_f. Thus, the apparent temperature coefficients and activation energies for peak I and peak II at 500 Hz are 0.64 and 1.03% and 2.1 and 3.4 kcal/mol, respectively. With increasing temperature, k_f increases so as to render the overall process more irreversible; this effectively lowers the ac current, whereas the increase in diffusion coefficient with temperature increases the current. Since the temperature coefficient for diffusion-controlled current is *ca.* 1.3%/deg and that for a chemical reaction rate is about 10%/deg, the two opposing effects will tend to lessen the slopes of the log ($i_s T$) plots.

The temperature dependence of k_f may accordingly be determined at high frequencies and an Arrhenius plot constructed for the homogeneous reaction, *e.g.*, Figure 11 (the temperature dependence of the diffusion coefficient was determined from dc polarographic diffusion-current temperature coefficients).[4a] Activation energies ($\Delta H^{\pm}$) were calculated from the slopes of the best straight lines and activation entropies ($\Delta S^{\pm}$) from the intercepts for the processes producing both peaks I and II. The activation energies are 1.8 kcal/mol for peak I and 1.3 kcal/mol for peak II. The activation entropies for peaks I and II, based on the k_f values obtained in the limiting high frequency region at 25° (Figure 9), are identical within experimental error at -4×10^1 entropy units.

The E_s values for purine waves I and II exhibit a linear negative shift with increase in temperature; over the interval from 5 to 50° the two waves have temperature coefficients of −0.2 and −1.0 mV/° at both 50 and 500 Hz (when corrected for the change in potential of the sce reference electrode). Although the temperature coefficient of $E_{1/2}$ for a reversible wave is usually between −2 and +2 mV/°C, that for an irreversible process is usually positive with increasing temperature, which would lead to an expected positive shift in E_s, not to the negative shift obtained. This observed shift could then be due to the extent or ease of adsorption of purine decreasing relatively less than that of its reaction product.

Pyrimidine. Experimental

Since the electrochemical behavior of pyrimidine in aqueous media has been extensively reviewed,[4c,18,19] the present discussion is limited to factors which will particularly provide comparison to the purine reduction mechanism or to new insights gained, for example, from the application of phase-selective ac polarography to the pyrimidine system.

(39) A. A. Vlcek, *Collect. Czech. Chem. Commun.*, 24, 3538 (1959).

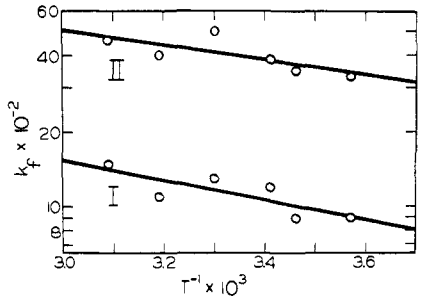


Figure 11. Arrhenius plots for k_f for 1.54 m*M* purine in pH 4.7 acetate buffer. Rate constants determined from ac peaks at 500 Hz. Roman numbers refer to the two purine peaks.

Dc Polarography and Voltammetry. Polarograms for pyrimidine wave I in pH 0.33 HCl (0.5 *M*) were obtained in order to see if the recently published theory[40] for dimerization following electron transfer could be used to determine various kinetic and electrochemical parameters. Over the concentration range of 0.05 to 2.0 m*M*, i_d was constant at 2.35 ± 0.02, indicating a diffusion coefficient $D = 1.50 \times 10^{-5}$ cm^2/sec. A plot of $E_{1/2}$ *vs.* log *C* has a slope of +24.7 mV per decade concentration; the theoretical expectation[40] for a dimerization following a one-electron transfer is 28.8 mV.

Plots of *E vs.* log [$i/i_d - i$)] are nonlinear, resembling two linear segments with some curvature near the $E_{1/2}$. The slope for the initial linear segment, at the foot of the wave, averages −28.1 ± 2.4 mV and for the second linear segment is −54.7 ± 3.2 mV. The theoretical expectations for the general case of dimerization are −29.6 to −59.2 and −118.4 mV, respectively, for the two segments. Plots of *E vs.* log [$i^{2/3}/(i_d - i)$] are nonlinear at all concentrations, indicating that the dimerization is not an irreversible process.

Plots of *E vs.* log [$i/(i_d - i)^2$] are remarkably linear, with an average slope of −27.7 ± 1.4 mV; the theoretically expected slope for a reversible dimerization following a fast electron transfer is −29.8 mV. Unfortunately, the pyrimidine concentration could not be lowered sufficiently to enable determination of the values for the dimerization equilibrium constant, and for E_0 and $k_{s,h}$ for the electron transfer process. Since this log plot is still linear at 0.05 m*M* pyrimidine, the theory[40] indicates that the equilibrium constant for dimer formation is greater than about 10^5 l./mol, and $k_{s,h}$ exceeds 0.1 cm/sec.

Although the postulation of reversible dimerization for pyrimidine is somewhat unpalatable on chemical grounds, since this involves reversible cleavage of an unstrained C–C single bond, polarographic analysis of pyrimidine wave I is in accord with the assumptions that both the chemical and electrochemical reactions are very fast, and that the equilibrium greatly favors the dimeric form.

Cyclic voltammetry of pyrimidine at a hmde in the pH range of 0.3 to 9 did not show anodic peaks corresponding to any of the five pyrimidine reduction waves at scan rates up to 150 V/sec, indicating that the rates for the follow-up chemical reactions for pyrimidine are much greater than for purine and that the rate for the deactivation process following the initial electron

(40) R. Bonnaterre and G. Cauquis, *J. Electroanal. Chem.*, 32, 199 (1971).

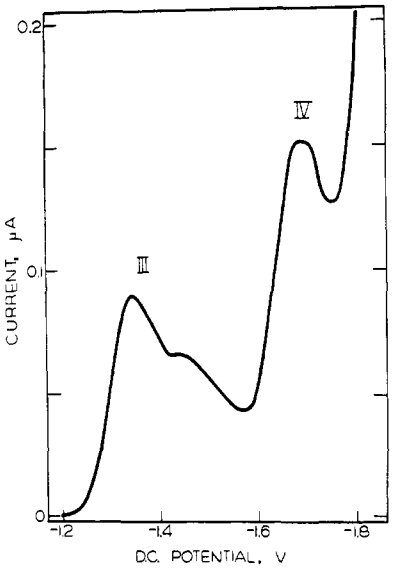


Figure 12. Phase-selective alternating current polarogram (in-phase component) of 0.5 m*M* pyrimidine in pH 7.5 phosphate buffer, where peaks III and IV appear, showing the splitting of peak III into its component parts, frequency 30 Hz.

transfer is greater in aqueous than in acetonitrile medium.[25]

Ac Polarography. One consequence of the application of phase-selective ac polarography is that the in-phase peaks seen are much sharper and more detailed, and not generally smoothed-out and distorted by the large capacitive charging current included in total ac polarography. For example, Figure 12 shows the resolution of two-electron peak III of pyrimidine into its two component one-electron processes at a pH value much more basic than that at which either dc polarography[18a] or total ac polarography[19] indicates merging of waves I and II (*cf.* Figure 1B in ref 19). Since two-electron waves III and IV of pyrimidine correspond formally to the two two-electron waves I and II of purine, and since two-electron purine wave I gives no indication of being split or otherwise ill-shaped (Figure 3), this is a further indication of the differences in reduction mechanism of these two compounds and of the influence of the imidazole group on the base pyrimidine ring.

Plots of log $\{(i_s - i)^{1/2} - [(i_s - i)/i]^{1/2}\}$ *vs.* E for peak I (pH 0.3 and 4.2) and peak III (pH 7.5) are distinctly nonlinear on the positive potential side of E_s (Figure 13), approaching in shape two linear segments intersecting near (±15 mV) the value for $E_{1/2}$ of the wave (at that pH), in sharp contrast to the linearity exhibited by the purine system. On the negative potential side of E_s, the slope is reasonably linear. These results are expected on the basis of the "bent" nature of polarographic log $[i/(i_d - i)]$ plots discussed previously. It is expected that, when the ac polarographic theory for dimerization following electron transfer is published, log plots such as these will be predicted.

The second harmonic curves for pyrimidine are highly asymmetric, much in the same manner as for purine (Figure 3C) but generally of lesser magnitude

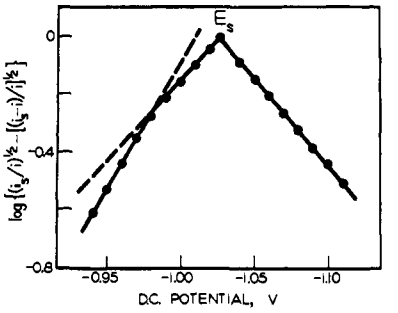


Figure 13. Plot of log $\{(i_s/i)^{1/2} - [(i_s - i)/i]^{1/2}\}$ *vs.* dc potential for ac polarographic peak I of 1 m*M* pyrimidine in pH 4.2 acetate buffer at 100 Hz. The slopes of the three linear segments (solid line) are 119, 169, and −168 mV, respectively.

under parallel experimental conditions, indicating much faster deactivation processes for pyrimidine. The second harmonic currents for peak II are very small (under 0.01 μA) and generally less than $^1/_{20}$th the magnitude of those for peak I. The potential separation between the positive and negative peaks of the second harmonic curve for peak I averages about 90 mV, substantially larger than the 68 mV expected for a reversible one-electron process.[9]

Frequency Dependence. Plots of $i_s/i_{d,s}$ *vs.* $\omega^{1/2}$ for pyrimidine peaks I and II in pH 3.9 acetate buffer have shapes very similar to those of purine (Figure 9). Using the limiting value of $i_s/i_{d,s}$ for peak II and eq 4, a value for k_f for the irreversible chemical reaction following transfer of the second electron can be calculated to be on the order of 5×10^4 sec^{-1} (the small magnitude of the alternating current observed and the proximity of peak I introduce considerable experimental error into this value).

Unfortunately, the problems encountered in doing ac polarography at pH 7.5 made it impossible to secure adequate meaningful numerical rate-constant data on pyrimidine waves III and IV for direct comparison with the corresponding waves I and II of purine.

Temperature Dependence. The observed ac currents for pyrimidine increase with temperature; the i_s temperature coefficients $[(1/i_sT)(d[i_sT]/dT)]$ at 250 Hz are 1.38%/deg for peak I and 2.41%/deg for peak II. Dc temperature coefficients $[(1/i_l)(di_l/dT)]$ for waves I, II, III, and V are 1.38, 1.35, 1.23, and 1.43%/deg, respectively, indicating diffusion control over the entire pH range.[41] Activation energies for ac peaks I and II can therefore be calculated to be 4.6 and 8 kcal/mol, respectively; because of the small magnitude of currents for peak II and because of its proximity to peak I, the value of 8 kcal/mol should be regarded as only an approximation. The activation energies for the dc limiting currents average out at 4.5 kcal/mol. These values are in good agreement with the 4 to 6 kcal/mol expected for both dc and ac currents for diffusion-controlled processes.[38,39]

Acknowledgment. The authors thank the National Science Foundation for helping to support the work described and Mr. James W. Webb for assistance with some of the experimental measurements.

(41) D. L. Smith, Ph.D. Thesis, University of Michigan, 1962.