Table VI

	log k_r, M^{-1} sec^{-1}	T, °K	Technique
1	9.5 ± 0.2	700–800	VLPP, this work
2	8.6 ± 1.1	415	Radical buffer (includes thermochemistry)
3	8.8 ± ?	~1100	Single-pulse shock tube, reverse reaction, plus thermochemistry

the reaction coordinate, the lengthening of that bond by ~1 Å with the internal rotation about it being rendered free, and the weakening of the four bending modes, which are destined to become free rotations of the isopropyl radicals, to a frequency value anywhere from a fifth to a tenth of the values in DMB. If one uses this sort of model, one cannot fit the data, such as to predict essentially no temperature dependence for recombination of isopropyl radicals over the 700° temperature range represented by values 2 and 3 above. In fact, if one assigns the transition-state parameters, such that the value for k_f agrees with Tsang's parameters at 1100°K (leading to value 3 above), one predicts a value of log k_r (M^{-1} sec^{-1}) = 9.2 at 400°K. On the other hand, an assignment of transition-state parameters, such that the value of k_f agrees with the value of k_f at 700°K derivable from this work (value 1 above), leads to a value of log k_r (M^{-1} sec^{-1}) = 8.6 at 400°K. It should also be pointed out that since ΔE_{100} = 78.1 kcal/mol, both results have to be accommodated with a negative value of activation energy for the radical combination, a phenomenon whose origins are extremely unclear. More than likely the transition-state model is not correct, and the assignment of the low-frequency bending modes is open to question. An alternative model in which these motions are treated as restricted external rotations having less heat capacity than vibrational modes is considered elsewhere.[15]

In conclusion, the value for k_r measured here is $10^{9.5 \pm 0.2}$ M^{-1} sec^{-1}, which is in seeming disagreement with the value derivable from Tsang's[13] single-pulse shock tube pyrolysis of DMB and with the radical buffer[3c] value by a factor of ~5. Compatibility with either value depends on transition-state models, since the temperatures differ.

It should also be noted in conclusion that the single biggest difficulty in this work, namely the propensity for alkyl radials to react in a first-order manner at the walls of the reactor, gives us cause to be concerned for some of the extant values of radical reaction rate constants.

Acknowledgments. Discussions with S. W. Benson were, as always, incisive and inspiring. J. I. Brauman's contributions, while specifically mentioned in the text, deserve special acknowledgment for their spiritual quality as well.

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Redox Pattern for Purine and 6-Substituted Purines in Nonaqueous Media. Free Radical Behavior

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Abstract: The redox behavior of purine, adenine (6-aminopurine), 6-methylpurine, 6-methoxypurine, 6-methylaminopurine, and 6-dimethylaminopurine has been examined in nonaqueous media (*N*,*N*-dimethylformamide, acetonitrile, and dimethyl sulfoxide) at mercury electrodes by a variety of electrochemical techniques. The purines undergo an initial one-electron (1e) reduction to form the corresponding anionic free radicals, which dimerize (rate constant of 10^3 to 10^5 l. mol^{-1} sec^{-1}). The dimers are oxidized at considerably more positive potential to regenerate the original purines. The rate constant for protonation of the purine anion radical is 1 sec^{-1}. On the addition of weak proton donors, the 1e reduction product is further reduced at the potential of its formation as the result of formation of a more readily reduced protonated species; the reduction attains the level of a 4e process at a mole rate of acid to purine of 3.8–4.0 (total faradaic n for the purines in aqueous media is 4). In the presence of strong acid (perchloric), purine and 6-methylpurine exhibit two 2e waves and the other 6-substituted purines a single 4e wave. The effect of substitution in the 6 position on ease of reducibility is the same in the neutral purines (nonaqueous media) and in positively charged (protonated) purines (aqueous media).

The electrochemistry of biologically important compounds has been the subject of increasing interest in recent years.[2–4] This has been particularly true of purines and pyrimidines related to the constituent bases of the nucleic acids, which direct the synthesis of proteins and are responsible for transfer of genetic infor-

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mation. The important biological purines are 6 substituted; *e.g.*, adenine (6-aminopurine) forms base pairs with thymine or uracil in the helical structure of DNA, as well as being involved as ATP (adenosine triphosphate) in energy-transfer processes.

Electrochemical studies[4-8] on purine and 6-substituted purines in aqueous media in the pH range of 1–9 show that, whereas purine and 6-methylpurine normally undergo two successive 2e reductions of the 1,6 and 3,2 N=C bonds (the 6-methylpurine waves fuse to a single 4e wave at pH 6), other 6-substituted purines undergo a single 4e reduction of these two double bonds. In the case of adenine, relatively slow elimination of NH_3 from the reduced form regenerates the 1,6 N=C bond which can be reduced in a further 2e process.

The polarographic half-wave potentials ($E_{1/2}$) are pH dependent (Table I) due to proton participation in the electrode process. At higher pH, the polarographic reduction is inhibited, *e.g.*, at pH 9 for purine and at pH 5–6 for adenine, and is presumed to be due to lack of protonation; prior protonation (probably at N(1)) is necessary before reduction can occur.[4,5,7,9] The basic question of the electrochemical reducibility of the unprotonated purines (neutral form) and the mechanistic patterns involved has not been answered. Pyrimidine, whose moiety is the site of electrochemical reduction in the purines, is itself electrochemically reduced in the neutral form in acetonitrile[10] at −2.3 V. Under aqueous conditions, pyrimidine is electrochemically reduced in the pH range of 1–13 with proton participation in the electrode process.[7,11]

Table I. Polarographic Reduction of Purines in Nonaqueous Media

Compound	Solvent[a]	$-E_{1/2}$,[b] V	Wave slope,[c] mV	I_d[d]	X[e]
Purine	DMF	1.89	66	1.92	0.50
	DMSO	1.89		1.09	
	AN	1.88	66	3.54	
	H_2O[f]	0.697 + 0.083 pH	31	5.05[g]	0.50
6-Methylpurine	DMF	2.06		2.72	0.49
	H_2O[f]	0.745 + 0.091 pH	31	4.25[g]	0.50
6-Methoxypurine	DMF	2.12	140	1.78	
	H_2O[f]	0.85 + 0.10 pH	43	8.5	0.50
6-Aminopurine (adenine)	DMF	2.41	66	2.3	0.50
	DMSO	2.41	66	1.5	
	AN	2.50	68	4.0	
	H_2O[f]	0.975 + 0.084 pH	47	9.8	0.50
6-Methylaminopurine	DMF	2.46	66	2.0	0.50
	H_2O[f]	0.995 + 0.081 pH	51	9.8	0.50
6-Dimethylaminopurine	DMF	2.30		2.3	
	H_2O[f]	0.930 + 0.089		8.9	0.50

[a] DMF = *N*,*N*-dimethylformamide; AN = acetonitrile; DMSO = dimethyl sulfoxide. The background is 0.1 *M* n-Bu_4NClO_4. [b] Potentials, reported *vs.* aqueous saturated calomel electrode, are for the main wave in nonaqueous media; a prewave appears at more positive potential, depending on the concentration of the electroactive species. Potentials for aqueous media are for the first wave if two appear. [c] Wave slope = $E_{1/4} - E_{3/4}$. A value of 56 mV is theoretically expected at 25° for an uncomplicated reversible 1e process and of 28 mV for a corresponding 2e process. [d] Diffusion current constant, $I_d = i_d/Cm^{2/3}t^{1/6}$. Magnitudes for I_d of 2 in DMF, 3 in AN, and 1.3 in DMSO correspond to a 1e faradaic process. [e] X = column height dependence, *i.e.*, slope of the plot of log i *vs.* log h_{Hg}. The theoretical slope for a diffusion-controlled wave is 0.50; *i.e.*, the current is proportional to the square root of the mercury column height. The slopes are for the main wave in nonaqueous media and for the first wave in aqueous media. [f] Data for aqueous media are taken from ref 6. [g] I is calculated for the first of the two 2e waves seen in aqueous media.

Further, the nonreducibility of purine bases in double stranded polynucleotides in aqueous media[12] has been ascribed to the inaccessibility of reducible groups and to the existence of the purines in neutral form and the nonavailability of the potential range for their reduction. To clarify this and the preceding hypotheses, it would be necessary to know the potential at which neutral purine bases are reduced. If nonavailability of potential range is the cause, then the use of nonaqueous solvents such as *N*,*N*-dimethylformamide (DMF), acetonitrile (AN), and dimethyl sulfoxide (DMSO) should at least provide a limited answer as the available potential range is 1.0 V or more greater than in aqueous alkaline medium.[13] From knowledge obtained by electrochemical studies of the purines, it would be possible at a later date to examine in meaningful fashion the double stranded polynucleotides.

A further problem involves conflicting reports concerning the mechanistic pathways for adenine in aqueous media. While the overall large scale electrochemical reduction product of adenine is agreed to be tetrahydropurine (or its hydrolysis product), following a 6e process, disagreement exists as to the sequence of steps in the reduction. Kwee and Lund[14] suggest that adenine undergoes a 2e reduction to dihydroadenine, which deaminates to give purine with the latter undergoing 4e reduction as it is formed. Previously, deamination had been shown[5,6] to be a slow process and had been considered to occur after the 4e reduction of adenine to tetrahydroadenine, which would deaminate to dihydropurine; the latter, in turn, would undergo 2e reduction as formed.

In order to help clarify the various questions indicated, a systematic study was undertaken of purine, adenine, and selected other 6-substituted purines in nonaqueous media. It has, consequently, been possible to formulate a generalized redox pattern for the purines, in nonaqueous media, which would make more explicit the sequence of steps in aqueous reduction, and to report on the effects of substituents on the electrochemical reduction and free radical stabilities.

Results and Discussion

The purines reported in the present study are soluble in DMF, DMSO, and, with difficulty, AN.

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Polarographic dme data are summarized in Table I. The purines exhibit a well defined 1e diffusion-controlled wave in nonaqueous media (wave height $\propto h^{1/2}$ (height of mercury column)).

A prewave appears as the purine concentration is increased; *e.g.*, purine itself at 2.8 m*M* shows a prewave of $E_{1/2} = -1.58$ V. Temperature coefficients for the limiting current (i_l) are 0.6% per degree for the prewave and 2.2% for the normal wave (a temperature coefficient near zero is generally indicative of an adsorption-controlled process[15]). At lower concentrations (*e.g.*, 0.6 m*M*), the prewave merges with the normal wave ($E_{1/2} = -1.89$ V) to form a single well defined wave. Table I *I* values are for low concentrations when the prewave does not appear; in the presence of a prewave, *I* is for the sum of the two limiting currents. The slightly higher *I* (2.72) for 6-methylpurine is presumably due to protonation by residual proton donor in the medium.[16] The wave slopes ($E_{1/4} - E_{3/4}$) vary with purine concentration, *e.g.*, 110–140 mV for the merged pre- and normal waves and about 66 mV for the main wave when it is separated from the prewave.

The 1e electrochemical reduction processes seen for the purines in nonaqueous media are in marked contrast to their previously described 2e and 4e reductions in aqueous media. The 1e reduction product in nonaqueous media is a free radical,[17] which is in agreement with the initial 1e reduction of pyrimidine in AN.[10] The free radical can be deactivated by at least two pathways: protonation and dimerization. The reactivity of the purine free radicals toward proton donors has been studied by addition of water, and benzoic, chloroacetic, and perchloric acids; the acid strength of these compounds in solvents such as DMF increases in the order given.[18]

Protonation by Water. Water is reported to act as a proton donor in the electrochemical reduction in nonaqueous media of various types of organic molecules[18–20] including pyrimidine.[10,21] The problem of removing the last traces of water from such solvents as DMF and AN is well recognized; in the present experiments, water is estimated not to exceed 0.5 m*M*.

The i_l (sum of prewave and normal wave) of purine (0.76 m*M*) in DMF increases on addition of water to reach a limiting value of 1.6 times the original wave height at a molar water–purine ratio of 719; the normal wave shifts positively and merges with the prewave at $E_{1/2} = -1.76$ V. Similar current enhancement and positive $E_{1/2}$ shift are observed for the 6-substituted purines. The pyrimidine wave height in AN is also increased by water addition[10] to a maximum value (1.7 times the original i_l) at a water–pyrimidine ratio of 666.

The i_l enhancement can be explained as due to abstraction of a proton by the free radical to form the protonated radical; since the latter has a higher electron affinity than the parent base, it is further reduced, resulting in an ECE (electron transfer–chemical reaction–electron transfer) process. The rate of the intermediate chemical reaction in the ECE process for purine and adenine can be calculated, using the theory developed by Nicholson, *et al.*,[22] to be 1.0 sec^{-1} in the presence of excess water (mole ratio of water to substrate > 800).

The corresponding rate constant for protonation by water of the pyrimidine free radical in AN is 7 sec^{-1}. The more rapid rate is as expected for a more highly reactive monocyclic compound. It is interesting to note that the rate of protonation of the pyrimidine carbanion in water is an order of magnitude faster than that for the purine carbanion.[4]

Protonation by Benzoic Acid. Addition of benzoic acid enhances i_l for the purines and shifts $E_{1/2}$ toward more positive values (Figure 1; Table II). At molar acid–purine ratios of 3.5 to 4.0, i_l levels off at a magnitude of about four times that observed in the absence of proton donor. On the basis of *I* values, the number of electrons involved in the reduction is estimated to be four for all of the purines.

Aromatic hydrocarbons and other aromatic molecules, whose electrochemical reduction in nonaqueous

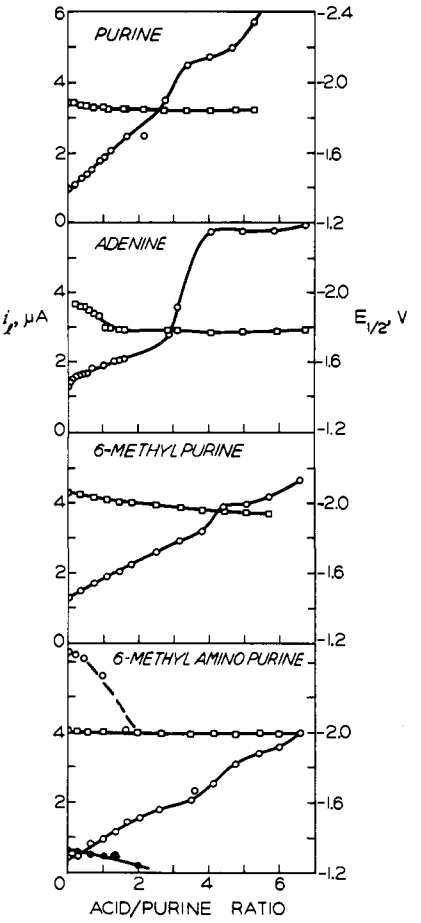

Figure 1. Effect of benzoic acid addition on the polarographic reduction of purine and 6-substituted purines in DMF; squares, $E_{1/2}$; circles, limiting current; concentrations, 0.54 m*M* purine, 0.40 m*M* adenine, 0.34 m*M* 6-methylpurine, 0.36 m*M* 6-methylaminopurine.

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Table II. Purine Half-Wave Potentials in DMF Containing Acids

Compd	pK_a[a]	$-E_{1/2}$,[b] V (None)	Benzoic acid Wave I	Chloroacetic acid Wave I	Perchloric acid Wave I	Perchloric acid Wave II
Purine	2.30	1.89	1.85	1.70	1.03	1.51
6-Methylpurine	2.50	2.06	1.94	1.83	1.21	1.69
6-Aminopurine (adenine)	4.12	2.41	1.77	1.79	1.76	NW[c]
6-Methylaminopurine	4.15	2.46	2.07	1.83	1.77	NW

[a] Aqueous pK_a values. [b] Potentials are measured *vs.* aqueous saturated calomel electrode and are for limiting values when excess acid designated is present. [c] NW = no wave.

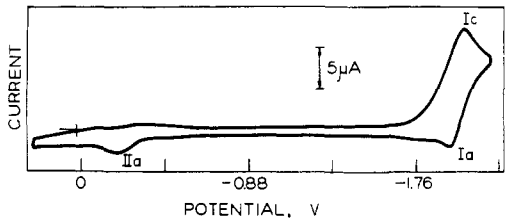

Figure 2. Cyclic voltammogram of 0.64 m*M* purine in acetonitrile. Scan rate = 0.15 V/sec. Peaks identified in the text.

media proceeds through a free radical, show i_l enhancement on benzoic acid addition, which reaches a maximum of two times the original i_l and which is explained as due to an ECE process.[18] The final products are dihydro compounds which are not further reduced at the potential of their formation. Reduction of dihydro compounds at the latter potential in a 2e process in the presence of excess of proton donor has been observed for a few heterocyclic molecules, *i.e.*, 2,5-diphenyloxazole, 2-(4-fluorophenyl)-5-phenyloxazole, and 2-(3-methoxyphenyl)-5-phenyloxazole.[23] Comparison of these results with those for the purines suggests that an initial ECE process generates the dihydro compounds, which are further reduced at the potential of their formation in a second ECE process. Although the evidence for the latter mechanism is convincing on the basis of *n* values and the molar ratios at which maximum limiting currents are obtained, conclusive demonstration of dihydropurines being reduced at the potential of their formation could be provided by examining the dihydro compounds themselves. Unfortunately, attempts to prepare the dihydro derivatives of the 6-substituted purines by chemical methods have proved futile.[24] However, comparison of the results obtained on the purines with a stronger acid ($HClO_4$) used as proton donor with those for aqueous medium strongly favor the dihydropurines being reduced at the potential of their formation (*cf.* subsequent discussion).

Protonation by Chloroacetic Acid. The presence of chloroacetic acid in the medium (solvent, DMF) increases the purine limiting currents in much the same way as benzoic acid. $E_{1/2}$ values are shifted positively (Table II).

Protonation by Perchloric Acid. In the presence of perchloric acid, the behavioral patterns of purines can be divided into two categories (Table II). Purine and 6-methylpurine show two well defined waves at a molar acid–purine ratio of 2.2; the height of each wave is 1.8 times that in the absence of $HClO_4$. 6-Methoxy-, 6-amino-, 6-methylamino-, and 6-dimethylaminopurine are characterized by a single wave at an acid–purine ratio of 4.2, whose height is 3.6–3.8 times that of the original purine wave.

Since the purine waves are shifted positively in the presence of acids with the magnitude of the shift being generally proportional to the strength of the acid added (Table II), the reductions involved are due either to protonated purines or to acid–substrate complexes similar to those observed with pyrimidine.[10] The appearance of a two-wave pattern for purine and 6-methylpurine involving 2e reductions and of a single 4e reduction wave for the other 6-substituted purines is compatible with the wave patterns observed in aqueous media.[6]

Free Radical Dimerization. The purine free radicals are sensitive to dimerization. Purine (0.24 m*M*) exhibits on cyclic voltammetry a well defined cathodic peak Ic (Figure 2; $E_{pc} = -2.03$ V) and, on potential scan reversal, an anodic peak ($E_{pa} = -1.97$ V). The 60-mV difference in $E_{pa} - E_{pc}$ is indicative of a reversible 1e process (the theoretically expected difference is $60/n$ mV for a reversible *ne* transfer process[25a]). With increasing concentration, E_{pc} shifts positively about 20 mV for a tenfold change and the ratio of anodic, i_a, to cathodic, i_c, peak currents decreases (Table III). These

Table III. Cyclic Voltammetry of Purine (Effect of Concentration on the Ratio of Anodic to Cathodic Currents[a])

Purine concn, m*M*	Peak I $-E_c$, V	Peak I $-E_a$, V	Peak I i_a/i_c
0.24	2.03	1.97	0.56
0.44	2.03	1.97	0.53
0.60	2.03	1.97	0.36
1.00	2.02	1.97	0.20
2.00	2.01	1.97	0.10

[a] Solvent: acetonitrile (0.1 *M* n-Bu_4NClO_4). Scan rate: 0.15 V/sec. Electrode: hanging mercury drop of 0.022 cm^2 area. Ratios of i_a/i_c measured by Nicholson's method.

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$$E_{pc} = E_c^\circ + \frac{RT}{3nF}\ln\frac{(4.88\pi 3D_R)}{2D_O} + \frac{RT}{3nF}\ln\frac{a}{k_d C}$$

where E_{pc} is the cathodic peak potential, E_c° is the formal potential, $a = nFv/RT$ where v is the scan rate, k_d is the dimerization rate constant, and C is the bulk concentration of the electroactive species. Even if k_d is small, concentration dependence for E_{pc} will be observed. In the present study, the peak potential measurements at different concentrations were made at slow scan rates of 0.10 to 0.15 V/sec when the reversible anodic peak current is much smaller than the cathodic peak current.

results suggest reversible 1e transfer followed by second-order dimerization. Nicholson, *et al.*,[25b] have shown theoretically that, for the case of

$$O + ne \rightleftarrows R \quad (1)$$

$$2R \longrightarrow R_2 \quad (2)$$

E_{pc} would shift about $20/n$ mV positively for a tenfold concentration increase[25c] and i_a/i_c would decrease with increasing concentration.

At higher purine concentrations (>1.5 m*M*), a new cathodic peak ($E_{pc} = -1.86$ V) appears, which corresponds to the dme prewave.

Besides the anodic peak at −1.97 V, purine shows an anodic peak IIa ($E_{pa} = 0.04$ V). With increasing scan rate, v, the 0.04-V peak shifts positively and decreases in magnitude, while the −1.97-V peak magnitude increases. The 0.04-V peak is attributed to oxidation of the dimer (*cf.* section on controlled potential electrolysis).

In the concentration range of 0.2 to 5.0 m*M*, the 6-substituted purines have the general cyclic voltammographic pattern of cathodic prepeak and normal peak shown in Figure 3; the peak potentials depend on the substituent at C(6). For adenine, prepeak $E_{pc} = -2.0$ V and normal peak $E_{pc} = -2.55$ V. The normal peak shows a complementary reversible anodic peak only at high scan rates, *e.g.*, $v > 5$ V/sec. A complementary reversible anodic peak is seen for the prepeak at $E_{pa} = -1.94$ V (Figure 3), whose current magnitude is dependent on the switching potential or the time for which a potential past the cathodic peak is held; data for a typical sweep–hold–sweep experiment are given in Table IV. The prepeak anodic current increases with increasing duration of electrolysis and levels off at large enough times.

Table IV. Increase in Free Radical Concentration with Time of Electrolysis of Adenine[a]

Holding time, sec	$-E_{pa}$, V	i_{pa}, μA
10	1.94	3.5
20	1.94	5.0
30	1.94	6.0
40	1.94	8.0
50	1.94	9.5
60	1.94	9.5

[a] Solvent: DMF, adenine concentration: 1.89 m*M*. Experiment: the potential on cyclic voltammetry is held at −2.20 V for the time indicated after normal sweeping at a rate of 0.2 V/sec and is then reversed and swept to more positive potential at the same sweep rate. The value of i_{pc} at this sweep rate is 2.0 μA.

Similar to the purine behavioral pattern, an anodic peak appears at around 0 V for the 6-substituted purines; this is attributed to oxidation of the dimer. The 6-substituted purines also show a complementary reversible anodic peak at −1.94 to −1.97 V. Repetitive cycling between 0 and −2.7 V does not produce cathodic or anodic peaks in addition to those already discussed.

1. Effect of Proton Donor on Cyclic Voltammetric Peaks. The presence of excess proton donor (benzoic, chloroacetic, or perchloric acid) removes the anodic peak around 0 V. In the case of water, the peak is almost absent at low scan rates, but it is seen at higher scan rates.

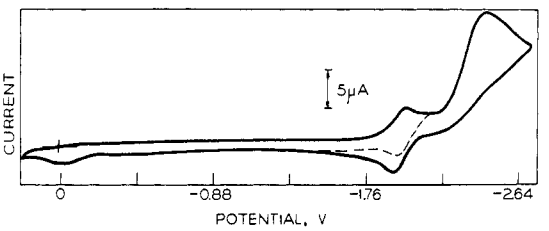


Figure 3. Cyclic voltammogram of 1.20 m*M* adenine in acetonitrile. Scan rate = 0.15 V/sec.

A positive shift in E_{pc} of 180 mV with chloroacetic acid and 120 mV with benzoic acid was observed for purine. The 6-substituted purines showed similar E_{pc} shifts. Also associated with acid addition is a sevenfold enhancement in i_p at a molar acid–purine ratio of approximately 3.5 (an enhancement of eight is expected for a reversible transition from a 1e to a 4e process; the enhancement for an irreversible process depends on the mechanism in a complicated manner).

Complementary peak Ia is absent in the presence of excess benzoic or chloroacetic acid at moderate v (0.1–0.2 V/sec); it is observed at higher v, *e.g.*, 10 V/sec. Purine exhibits two cathodic peaks ($E_{pc} = -1.21$ and −1.71 V) in the presence of perchloric acid; a complementary reversible anodic peak is seen for neither. The 6-substituted purines in the presence of $HClO_4$ showed only a single cathodic peak, whose height corresponds to a 4e reduction.

2. Rate of Dimerization. The rates of dimerization of the purine free radicals were calculated from the peak current ratio method,[25b] which involves correlating the ratio of i_{pa}/i_{pc} with the kinetic parameter, $C\tau k_d$, where C is the bulk concentration of the electroactive species, τ is the switching time (time required to shift the potential from $E_{1/2}$ to switching potential E), and k_d is the dimerization rate constant. Due to presence of the adsorption prepeak, the estimated rate constants (Table V) are considered to be accurate only to ±20%. In these measurements, switching times in the range of 30 to 300 msec were employed. The dimerization rate constants for the purine free radicals are less than that of the pyrimidine free radical in AN (6×10^5 l. mol^{-1} sec^{-1}),[10] since, as previously noted, a faster rate would be expected for the more highly reactive monocyclic compound. The order of magnitude difference is also seen in the rates of radical anion protonation by water and of carbanion protonation mentioned earlier.

Table V. Dimerization Rates of Purine Free Radicals at 25°

Compd	Solvent	k_d, l. mol^{-1} sec^{-1}
Purine	DMF	2.3×10^4
	AN	3.4×10^3
	DMSO	2.0×10^4
6-Aminopurine (adenine)	DMF	9.7×10^4
6-Methylpurine	DMF	1.1×10^4
6-Methylaminopurine	DMF	5.0×10^4
6-Methoxypurine	DMF	3.2×10^4

It is difficult to propose an explanation for the low value of k_d for purine in AN (Table V). Since the dimerization reaction is nondiffusion controlled, one would not expect viscosity differences between AN, DMF, and DMSO to cause changes in the dimerization

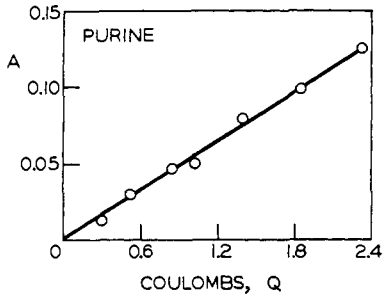


Figure 4. Variation of spectrophotometric absorbance (A) at 300 nm due to the reduction product formed during controlled potential electrolytic reduction of 2.06 mM adenine (0.04 mmol) in DMF as a function of quantity of coulombs (Q) passed.

rate constant. If the dielectric constant of the medium were important, the double sphere model predicts that k_d would be inversely proportional to the solvent dielectric constant; however, the values are 36 for AN, 37 for DMF, and 47 for DMSO.

Nature of the Prewave. The prewaves seen in polarography and cyclic voltammetry have been extensively investigated.[26] Due to the lower free energy state of the adsorbed product molecule, the process

$$O + ne \rightleftarrows R_{ads} \quad (3)$$

where R_{ads} refers to the adsorbed radical anion, occurs at more positive potential than the process

$$O + ne \rightleftarrows R_{sol} \quad (4)$$

where R_{sol} refers to the dissolved radical anion. The prewaves observed in the present study have been examined by dme polarography, cyclic voltammetry, and potential-step electrolysis. The low i_l temperature coefficient is characteristic of prewave processes which are limited by the electrode area (reaction 3). Among cyclic voltammetric diagnostic criteria for the prewave process[27] are the positive potential shift with increasing concentration of electroactive species, the nonlinear dependence of i_{pc} on concentration, and the relative variation with v of the adsorption peak current (that actually observed) to the diffusion peak current (corresponding to reaction 4).

Cyclic voltammetric data obtained for the purines show that the prewave is due to process 3.

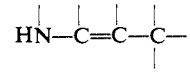
Potential-step electrolysis (chronocoulometry) involves switching the working electrode from an initial potential, *e.g.*, 0 V, to a potential on the limiting current region for reaction 3; under these conditions, a plot of Q (coulombs passed during the electrolysis) *vs.* $\tau^{1/2}$ (time of electrolysis) will be linear and will have an intercept higher than Q_{dl} (coulombs used in charging the double layer). The results are in qualitative agreement with those expected for the prewave case of reaction 3; however, no rigorous, *i.e.*, quantitative, treatment has been developed for this situation due to the involvement of the rate of adsorption of R_{ads}.[28]

Controlled Potential Electrolysis. Controlled electrode potential electrolysis at a mercury pool electrode was used to verify the nature of the products of electrochemical reduction.[29] Typically, a 2 mM purine solution in DMF was electrolyzed at a potential of −2.0 V with samples of the test solution being periodically removed for spectrophotometric examination. The maximum at 266 nm (molar absorptivity, ϵ, 7600), shown by the original purine solution, was absent in the completely electrolyzed solution which had a maximum around 300 nm; ϵ for the product, based on the 300-nm absorbance, is 3660. The correlation between the 300-nm absorbance and the electrons (coulombs) consumed during electrolysis is shown in Figure 4. The faradaic n was 1.

The completely electrolyzed solutions showed a well defined diffusion-controlled anodic polarographic wave ($E_{1/2}$ = −0.06 V) with a small prewave at its foot. On cyclic voltammetry, a well defined broad anodic peak (E_{pa} = 0.04 V) appeared. Exhaustive electrolytic oxidation of the reduced solution at 0.20 V regenerated the original purine wave (99%) with the consumption of the same number of coulombs as the original reductive electrolysis.

Electrolyses of the 6-substituted purines gave similar results, *e.g.*, 1e reductions and consumption of the same number of coulombs on reversal oxidation as on the reductive electrolysis.

3. Identification of Electrolysis Products. Positive identification of the bonding site in the dimers cannot be made at present. From the electrochemical and ultraviolet absorption patterns, which are marked by oxidation of the dimer at about 0 V and a maximum around 300 nm, the products of 1e electrochemical reduction appear to be similar for all of the purines. Chromophores of the type



have an absorption maximum around 296 nm.[30a] Replacement of >C= by —N= in an aromatic nucleus changes the absorption maximum very little,[30b] which indicates 2,2′, 6,6′, or 8,8′ dimer formation.

The dimerization sites of free radicals are dependent on the positions of greatest free valence in the molecule.[31] Examination of the free valences for purine at different carbon atoms, *i.e.*, 0.402 at C(2), 0.456 at C(6), and 0.443 at C(8), suggests the 6 position as the most favored site. In the case of adenine, the 8 position is a likely site since the 6 position has a free valence of 0.13, whereas positions 2 and 8 carry free valences close to that of purine. A similar situation prevails for the other 6-substituted compounds.

Mechanistic Pattern

Purine and the 6-substituted purines studied undergo 1e reduction in nonaqueous media to form the corresponding anionic free radical

$$R + e \rightleftarrows R^- \quad (5)$$

where R represents a purine and R^- is a negatively

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(29) A. J. Bard and K. S. V. Santhanam in "Electroanalytical Chemistry," Vol. 4, A. J. Bard, Ed., Marcel Dekker, New York, N. Y., 1970, pp 215–315.
(30) (a) R. H. Burton and N. O. Kaplan, *Arch. Biochem. Biophys.*, **101**, 150 (1963); (b) A. Alberts, "Heterocyclic Chemistry," Oxford University Press, New York, N. Y., 1968, p 264.
(31) B. Pullman and A. Pullman, *Proc. Nat. Acad. Sci. U. S.*, **45**, 136 (1959).

charged free radical. Free radical formation during the electrolysis of purines is amply demonstrated by the electrochemical data and by the observation of esr signals.[17]

The purine free radicals dimerize, forming initially anionic dimers which are rapidly protonated to give the neutral dimer

$$2R^- \longrightarrow R_2^{2-} \quad (6)$$

$$R_2^{2-} + 2H^+ \longrightarrow R_2H_2 \quad (7)$$

(Possible proton sources include residual traces of water and traces of acidic impurities and the solvent itself.) The dimer, R_2H_2, is oxidized at potentials around 0 V (cyclic voltammetric peak IIa) to regenerate the parent purine.

Identification of R_2H_2 as the ultimate product is based on absorption spectra data and paper chromatography. The fact that addition of protons does not shift the dimer oxidation peak would exclude R_2H^- being the ultimate product.

The reaction scheme outlined for purines in nonaqueous media is similar to that proposed for the electrochemical reduction of pyrimidine and other azabenzenes[16] but is in marked contrast to the behavior of purines in aqueous media where free radical formation is not observed due to prior protonation and initial multiple electron (2e or 4e) reduction. The general mechanistic pattern in aqueous media[4-6] can be represented as

$$R + H^+ \longrightarrow RH^+ \quad (8)$$

$$RH^+ + 2e + H^+ \longrightarrow RH_2 \quad (9)$$

$$RH_2 + 2e + 2H^+ \longrightarrow RH_4 \quad (10)$$

where RH_2 and RH_4 represent the dihydro- and tetrahydropurines. Purine and 6-methylpurine exhibit two separate 2e waves due to RH_2 having a higher electron density at the 3,2 N=C bond than protonated RH^+ at the 1,6 N=C bond, which makes the 3,2 N=C reduction more difficult. However, when the presence of electron-releasing groups such as amino, methylamino, or methoxy on C(6) causes the initial reduction of the 1,6 N=C bond to be more difficult than that of the 3,2 N=C bond in the 1,6-dihydropurine species, both reactions 9 and 10 occur at the same potential.

That the differences in the mechanistic patterns in aqueous and nonaqueous media arise due to low proton activity of the nonaqueous media is demonstrated by the protonation studies described where either two 2e or one 4e reduction wave is observed in the presence of proton donors. Thus, the present studies have conclusively shown that neutral purines are reducible but that the potentials at which they are reduced are beyond realization in aqueous media of suitable pH due to prior reduction of hydrogen ion, background electrolyte cation, or water itself. This is probably the reason that the purine bases in double stranded polynucleotides could not be electrochemically reduced.[12] Studies of double stranded polynucleotides in nonaqueous solvents could provide information on the reducibility of polynucleotides. However, one cannot rule out the possibility that the hydrogen bonding present in the double stranded configuration will stabilize the bases sufficiently as to prevent their reduction within the available potential range. The mutual polynucleotide hydrogen bonding in nonaqueous solution would be expected to be more stable than in aqueous solution due to the decreased tendency for hydrogen bonding with the solvent.

Sequence of Steps in Adenine Reduction. In respect to the two proposed sequences for the total electrochemical reduction of adenine, *i.e.*, 2e reduction followed by deamination and subsequent 4e reduction[14] and 4e reduction followed by deamination and subsequent 2e reduction,[5-7,9] the results obtained for adenine in nonaqueous media in the presence of added acid show that a 4e reduction is the favored initial step in proton-containing media such as aqueous solution of pH less than 5 or 6. The 4e reduction is explicable on the basis of 1,2-dihydroadenine being reducible at the potential necessary to initiate the reduction of protonated adenine and of deamination of the reduced adenine species being a relatively slow process on the time scale of dme polarography and cyclic voltammetry.

Effect of Substituent on $E_{1/2}$. Substitution in the 6 position of purine with electron-releasing groups decreases the ease of reducibility, *i.e.*, $E_{1/2}$ shifts toward more negative potential in the order indicated for the following substituents: H, CH_3, OCH_3, $(CH_3)_2N$, H_2N, and CH_3NH. This is consistent with the order of electron-releasing groups, *e.g.*, methyl < methoxy < alkylamino,[32] which increases the electron density at the neighboring carbon atoms due to inductive effect. The order of $E_{1/2}$ of 6-substituted purines in aqueous medium at pH 2.5 and 4.0 consistently gave similar results.[6] An important aspect of this trend appears to be that the electron-releasing groups act in the same fashion in the uncharged (neutral) and positively charged (protonated) purines.

The rates of dimerization of the free radical anions (Table V) roughly increase in the same order as the decreasing ease of reduction of the parent compounds.

Chemical Reducibility. The relative ease of electrochemical reduction of purines in both nonaqueous and aqueous media is in contrast to the difficulty of chemical reduction, using 5% Pd–charcoal catalyst at room temperature,[24] in which the protonated purine is reduced to 1,2-dihydropurine while adenine and the other 6-substituted purines resist such reduction. Since reduction with Pd–charcoal catalyst proceeds through atomic hydrogen,[24] molecules with greater free valence or smaller localization energy should be favored.[33] The free valences of purine and purine cation at the 6 position (calculated on the basis of HMO energies) are 0.456 and 0.494, while it is 0.139 for adenine cation. Thus, the purine cation is reduced more easily than the adenine cation. Electrochemical reduction involves electron addition to the neutral or charged molecule (in aqueous medium below pH 5 or 6, purines are largely present as protonated species), which is governed by the electron affinity of the molecule. Electrolysis thus provides a good method for reducing the purines.

Experimental Section

Polarographic and spectrophotometric examination of purines, obtained from the sources indicated, indicated their purity to be adequate: purine, K & K; 6-methoxy-, 6-amino-, and 6-methylaminopurine, Aldrich; 6-methylpurine, Sigma; and 6-dimethylaminopurine, Mann.

(32) P. Zuman, "Substituent Effects in Organic Polarography," Plenum Press, New York, N. Y., 1967, pp 46–47.

(33) T. Nakajima and B. Pullman, *J. Amer. Chem. Soc.*, 81, 3876 (1959).

Electrochemical studies were conducted in a previously described vacuum line and cell,[3] to which solvent was added by distillation. After a freeze–pump–thaw cycle, the solvent was melted and released to atmospheric pressure by means of dried nitrogen gas. For protonation studies, the acid dissolved in the appropriate solvent was added and nitrogen gas was bubbled through the solution for at least 15 min before making electrochemical measurements.

Potentials were measured against an aqueous saturated calomel electrode (sce), which was separated from the working electrode compartment by a sintered glass disk and agar gel. The relative difference in liquid junction potentials between DMF, AN, and DMSO can be related to $E_{1/2}$ for Rb(I) reduction *vs.* sce: −1.94 V in AN and DMF; −1.95 V in DMSO; −2.13 V in water.[34]

Ultraviolet spectra were recorded using 1-cm silica cells, a Beckman Model DB spectrophotometer, and a strip-chart recorder.

Sources and purification of other chemicals, apparatus, and electrochemical procedures have been described.[3] The background electrolyte was 0.1 *M* tetra-*n*-butylammonium perchlorate (Matheson, dried).

The following discussion of specific aspects of the behavior of some of the purines investigated is intended to amplify and supplement previous statements and the data presented in the figures and tables.

Purine. Since purine exhibits an adsorption prewave, which is strongly dependent on purine concentration, most measurements were made at low concentration (<0.6 m*M*) where adsorption effects are small. The polarographic diffusion current, i_d, for purine varies linearly with concentration in the region below 1.5 m*M*, where only one wave is observed. When the prewave appears, the sum of the two wave currents is in line with the current seen at low concentration.

Controlled potential electrolysis of 0.8 to 2.1 m*M* purine at −2.0 to −2.2 V in DMF gave faradaic *n* values of 0.94 to 1.10 and replacement of the purine absorption maximum at 266 nm by a broad maximum in the 300-nm region. The original purine absorption maximum returned after controlled potential oxidation of the reduced solution at 0.2 to 0.4 V.

Chromatographic examination of the solution before and after electrolysis gave R_f values of 0.76 for purine and 0.54 for its reduction product. The following procedure was used. Four or five drops of the solution is spotted on a 5.5 in. Whatman No. 1 sheet with base line about 0.75 in. from the bottom. The paper is slowly introduced into the cyclindrical developing jar containing developing solution and is allowed to remain for 3 to 4 hr at about 24°. The paper is then air dried and the spots are visually examined on illumination with ultraviolet light. Cut portions of the spots showed ultraviolet absorption maxima at nearly the same wavelengths as described for solutions. The developing solution was prepared by dissolving 77 g of ammonium acetate in 750 ml of H_2O, adjusting the pH to 7.5 with NH_3, and diluting to 1 l., and then mixing 300 ml of the latter solution with 700 ml of 95% ethanol.

Adenine. Adenine showed both polarographic prewave and normal wave over the concentration range of 0.8 to 2.0 m*M* in DMF; the sum of the two currents is proportional to concentration.

The cyclic voltammetric prepeak (E_p = −2.0 V) showed a complementary reversible anodic peak in DMF at sweep rates as low as 0.041 V/sec. The normal peak showed a complementary reversible anodic peak at sweep rates of 10 to 25 V/sec. Due to the stirring effect,[19] the anodic peak is not a single smooth peak but consists of several peaks grouped around the reported E_{pa}. At lower concentration (0.2 m*M*), the magnitude of the stirring effect is smaller.

During controlled potential electrolysis at −2.60 V in DMF, the electrolysis current decayed smoothly with time and the solution remained colorless. The faradaic *n* value was 1.0. The adenine absorption maximum at 263 nm was replaced by one at 300 nm. Reversal oxidation at 0.20 V regenerated the original adenine absorption and cyclic voltammetric peaks.

Chromatographic examination of the solution before and after electrolysis, using the previously described procedure, gave R_f values of 0.80 and 0.56.

6-Methylpurine. Controlled potential electrolysis in DMF at −2.55 V consumed coulombs equivalent to an *n* of 1.02. The electrolysis current decayed smoothly to the background value. Cyclic voltammetry of the electrolyzed solution showed an anodic peak at 0.02 V with an associated sharp prepeak (presumably due to adsorption) at −0.20 V. The ultraviolet spectra of the solution before and after electrolysis showed maxima at 242 and 328 nm, respectively. Oxidation of the reduced solution at 0.20 V consumed 0.75 coulomb compared to 0.86 coulomb for the original electrolysis.

The other 6-substituted purines had behavior patterns similar to those described for 6-aminopurine (adenine) and 6-methylpurine.

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Effect of Charge Redistribution on Ion–Molecule Reaction Rates

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Abstract: The application of the charge redistribution model to ion–molecule reaction systems of general interest is discussed. The effect of charge redistribution on the reaction rates of 25 exothermic ion–molecule reactions is evaluated. A strong correlation is shown to exist between reactions involving the central atoms of the reactants and the electronegativities of those atoms. It is observed that reactions in which a large charge redistribution would have to take place occur with much lower probability than reactions where the charge redistribution would be small.

Since 1950 the field of ion–molecule reactions has been one of the most extensively studied in all of chemistry. However, it is well recognized that experiment has generally outstripped theory in this field.[1] There have been several theories set forth which do explain various aspects of ion–molecule reactions. The Gioumousis–Stevenson (G–S) treatment[2] permits one to calculate the total interaction cross section using an ion-

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