The Electrochemical Reduction of Purine in Nonaqueous Solvents

Toshio Yao and Sōichirō Musha

Department of Applied Chemistry, College of Engineering, University of Osaka Prefecture,
Mozuumemachi, Sakai 591

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The electrochemical reduction of purine, which is the model compound for the reduction of the biologically-important purine bases, has been investigated in nonaqueous solvents (acetonitrile and DMF) by polarography, cyclic voltammetry, controlled potential electrolysis, and the ESR technique. In neutral solutions, purine is initially reduced in a one-electron, diffusion-controlled process at a fairly negative potential to produce an unstable radical anion, which then abstracts a proton from the parent purine to produce a free radical and an electro-inactive purine anion. The free radical is quickly deactivated by fast dimerization to form a purine dimer and by a further one-electron reduction to produce, ultimately, 1,6-dihydropurine. The addition of various acids to purine solutions results in the appearance of a new, drawn-out wave at a more positive potential than the original purine reduction wave, which is attributed to the two-electron reduction of *N*-protonated purine species to produce 1,6-dihydropurine.

In recent years, the electrochemical reduction and oxidation of biologically-important compounds have been investigated with much interest. It is important to fully understand the redox behavior of these in considering the metabolism and analytical determination of biologically-important compounds. Recent works[1-3] have suggested a possible parallelism between the modes of electrochemical behavior and the biological transformation, *e.g.*, the enzymatic oxidation. It is also important, however, to fully investigate the electrochemical reduction behavior of biologically-important molecules, especially regarding those factors that influence the electron transfer processes, such as, *e.g.*, the pH.

A few works[4-6] have been done on purine itself and various purine derivatives in aqueous solutions. However, these works are due to the reduction of prototype purines. Consequently, the electrochemical reduction of purine, which is the model compound for the reduction of the biologically-important purine series, has been investigated in aprotic solvents on the basis of the idea that the information obtained in an inert solvent would reflect, more directly, the properties of purine itself and not its solvent-solute interaction. Another reason for investigating the purine reduction mechanism in nonaqueous solvents is to understand the properties and behavior of the anion free radical expected in a proton-poor medium.

Experimental

Chemicals. The purine (Sigma chemical company) was recrystallized four times from toluene. The acetonitrile was purified essentially by the method of Coetzee *et al.*[7] The dimethylformamide (DMF) was purified by the method of Hatano.[8] The moisture in the purified acetonitrile and DMF was determined by Karl-Fischer titration to be 0.01—0.02%. Tetraethylammonium perchlorate (TEAP) was used as the supporting electrolyte. TEAP was prepared following Fujinaga *et al.*,[9] and was recrystallized five times from distilled water before drying at 60 °C *in vacuo*. All the other reagents were of an extra-pure grade.

Apparatus. The polarographic curves were recorded using a Yanagimoto P-8 type Polarograph. Cyclic voltammograms were obtained with a versatile solid-state instrument constructed in this laboratory following the design of Goolsby and Sawyer.[10] The electrolysis cell consists of three electrodes. The reference electrode was constructed by dipping a coil of silver wire into a solution of 0.1 M $AgClO_4$ in solvents (acetonitrile or DMF)–0.1 M TEAP contained in a glass tube with a medium-porosity glass frit and salt bridge at one end. DME was used for the polarography ($m^{2/3}t^{1/6}=3.14$ $mg^{2/3}s^{1/6}$ in a 0.1 M TEAP–acetonitrile medium at −2.2 V *vs.* $Ag/AgClO_4$), and a hanging-drop mercury electrode (HDME) was used for cyclic voltammetry as the working electrode. A platinum wire served as the counter electrode. The ESR spectra were recorded on a Hitachi MES-4001 ESR spectrometer, and the sample was electrolyzed in the cavity of the ESR spectrometer. The temperature control of the samples was done by means of a Hitachi M-4602 variable-temperature accessory.

Procedure. A freshly-prepared sample solution which contained 0.1 M TEAP as the supporting electrolyte was placed in a cell. The dissolved oxygen was removed by bubbling with highly-purified nitrogen gas saturated with solvent vapor for about 15 min, and polarograms were recorded with nitrogen passing over the solution. All the experiments were carried out at 25.0±0.1 °C.

Results and Discussion

Polarography. The polarographic data for purine in various solvents are shown in Table 1. Purine is reduced at a very negative potential in aprotic solvents. The initial purine reduction wave is quite similar in both acetonitrile and DMF, but in the case of DMF the second wave appears at a more negative potential. In an aqueous solution, it is known[6] that purine exhibits two pH-dependent waves when in an acidic solution but none when in a neutral or alkaline solution, and that the first wave of these is attributable to a two-electron, diffusion-controlled process. The diffusion-current constant corrected for the change in viscosity obtained in nonaqueous solvents is about 1/4 that of the first wave obtained in an aqueous solvent; therefore, purine is initially reduced in a 1/2-electron process in aprotic solvents.

The limiting current of purine increases linearly with the concentration below 2 mM, as is shown in Fig. 1. The effect of the height of the mercury reservoir is shown in Fig. 2. The limiting current of purine is approximately proportional to $h^{1/2}$. The temperature

TABLE 1. POLAROGRAPHIC DATA FOR PURINE REDUCTION IN VARIOUS SOLVENTS

Solvent	$E_{1/2}$, V	$E_{3/4}-E_{1/4}$ mV	I_d[d]	$\eta_{25°C}$	$I_d\eta^{1/2}$
CH_3CN	−2.16[a]	80	1.60	0.345	0.91
DMF	−2.38[b]	110	1.03	0.802	0.92
	−2.66[b]		0.22		0.20
H_2O	−0.691−0.083pH[c]	76	3.81	0.894	3.60
	−0.910−0.080pH[c]	89	5.67		5.36

a) V *vs.* Ag-AgClO$_4$ (0.1 M) electrode in CH_3CN. b) V *vs.* Ag-AgClO$_4$ (0.1 M) electrode in DMF. c) V *vs.* SCE (aqueous). d) Diffusion current constant.

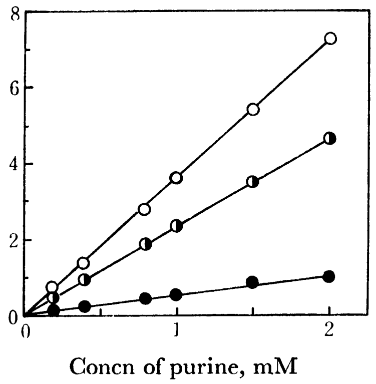


Fig. 1. Calibration curves of initial reduction waves of purine in acetonitrile (○) and DMF (◐: 1st wave, ●: 2nd wave).

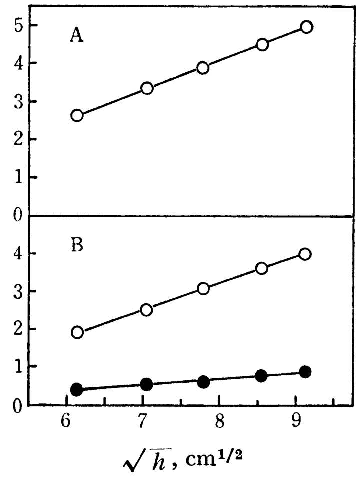


Fig. 2. Effect of the height of the mercury reservoir.
A: 1.0 mM purine in acetonitrile
B: 1.0 mM purine in DMF
(○: 1st wave, ●: 2nd wave)

coefficient of the limiting current of purine in acetonitrile is 1.2% per degree in the temperature range from 15 to 40 °C. These results show that the initial reduction wave of purine is diffusion-controlled in both acetonitrile and DMF.

Cyclic Voltammetry. The cyclic voltammograms are shown in Fig. 3. In the case of acetonitrile, purine first gives a cathodic peak corresponding to the polarographic wave, whose height is proportional to the concentration and the square root of the scan rate. However, on the return sweep, no anodic peak is seen until about −0.56 V (peak 3a), and a redox couple (peak 4a and 4c) on the positive-potential side of Peak 3a appears. In the case of DMF, a typical curve indicates a cathodic peak, followed by a reversible reduction step. However, the other voltammetric behavior for purine in DMF is similar to that in acetonitrile.

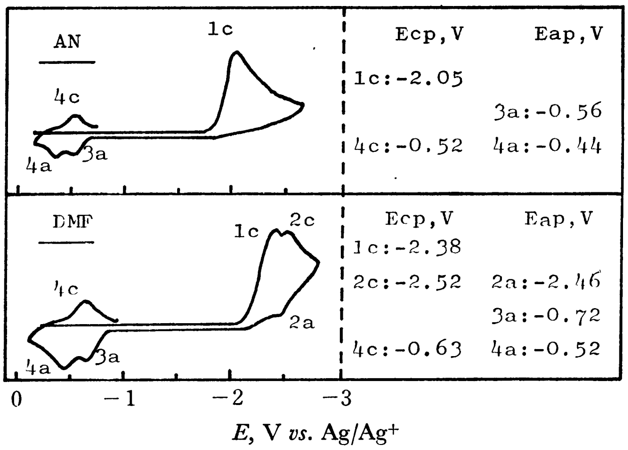


Fig. 3. Cyclic voltammogram of purine in aprotic solvents.
scan speed: 0.20 V/s

ESR Spectroscopy. The electrogenerated radical species of purine and the various biologically-important purine bases have not been detected[11] at room temperature. This is presumably because of the rapid dimerization of the radical anion of purines. There-

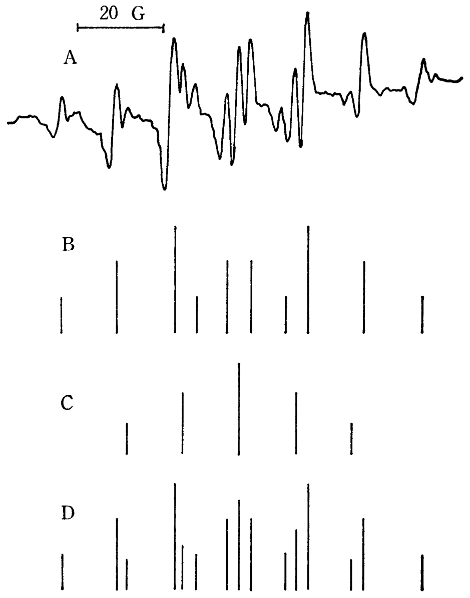
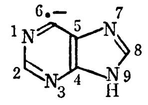


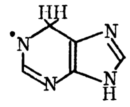
Fig. 4. A; Observed ESR spectrum obtained by controlled potential electrolysis at the potential of the first polarographic wave in DMF at −60 °C. B; calculated ESR spectrum of radical (1) with coupling constant A_H(6-Position) = 30.4 gauss and A_N(1 and 3-Position) = 12.7 gauss. C; calculated ESR spectrum of radical (2) with coupling constant A_N(1 and 3-Position) = 12.6 gauss. D; combined spectrum of B and C.

fore, the ESR technique at a low temperature was used to detect the electrogenerated radicals of purine. A typical ESR spectrum obtained by controlled potential electrolysis at the potential of the first polarographic wave in DMF at −60 °C is shown in Fig. 4A. The half-peak width of the hyperfine components is about 1.6 gauss, and the total width between the centers of the outermost observed lines is 41.0 gauss.

The results of the interpretation indicate that this spectrum consists of a combination of the hyperfine splitting of the two radical species shown below.



(1)



(2)

The spectrum (Fig. 4B) obtained by the use of Radical (1) is interpreted as deriving from the interaction of the unpaired electron with a proton (A_H=30.4 G) and an equivalent pair of the nitrogen nucleus (A_N=12.7 G). It may also be interpreted that the spectrum (Fig. 4C) obtained by the use of Radical (2) is attributable to the interaction of the unpaired electron with an equivalent pair of the nitrogen nucleus (A_N=12.6 G). The combined spectrum of B and C is shown in Fig. 4D; it is in good agreement with the observed ESR spectrum. The results of this ESR spectrum and the diffusion-current constant which corresponds to the 1/2-electron reduction wave indicate that purine is initially reduced in a one-electron process to produce an unstable radical anion, which then abstracts a proton from the parent purine to produce a free radical and an electro-inactive species.

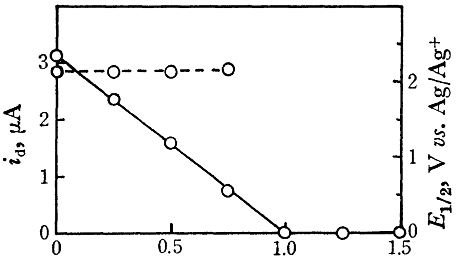
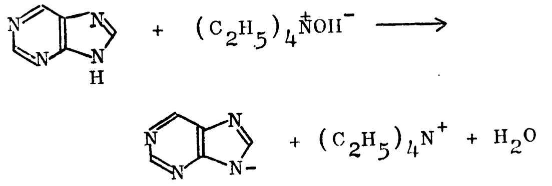


Fig. 5. Effect of strong base addition on polarograms of 1 mM purine in acetonitrile.
(——): i_d, (------): $E_{1/2}$

Effect of Base Addition. An acetonitrile solution of tetraethylammonium hydroxide (TEAH), which is a strong base in acetonitrile, was added to an acetonitrile solution of purine. The polarographic behavior of purine in the presence of TEAH is shown in Fig. 5. The presence of TEAH in the solution causes the wave-height of the original purine wave to decrease, approximately in proportion to the base concentration, and at a molar ratio of TEAH/purine ratio of about 1.0 the wave dissappears entirely. However, $E_{1/2}$ is constant in spite of the addition of TEAH. This behavior can be explained in terms of the production of an electro-inactive anion by a kind of acid-base reaction like that shown below:



Effect of the Acid Addition. The various acids were added to both acetonitrile and a DMF solution of purine, and their effects on the polarograms were studied. In general, the addition of strong acid produces a new, drawn-out wave at a more positive potential than the original purine reduction wave.

On the addition of monochloroacetic acid to an acetonitrile solution of purine, a new wave appears at −1.89 V. With an increase in the acid/purine ratio, the wave-height of the new wave, that is approximately constant, in $E_{1/2}$ increases, and that of the original wave decreases, as is shown in Fig. 6. At a molar ratio of acid/purine higher than about 2.2, the new

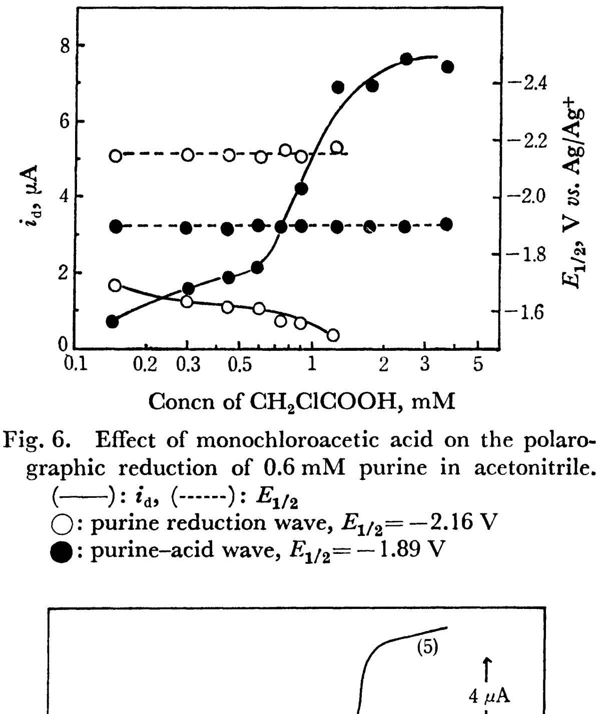


Fig. 6. Effect of monochloroacetic acid on the polarographic reduction of 0.6 mM purine in acetonitrile.
(——): i_d, (------): $E_{1/2}$
○: purine reduction wave, $E_{1/2}$= −2.16 V
●: purine–acid wave, $E_{1/2}$= −1.89 V

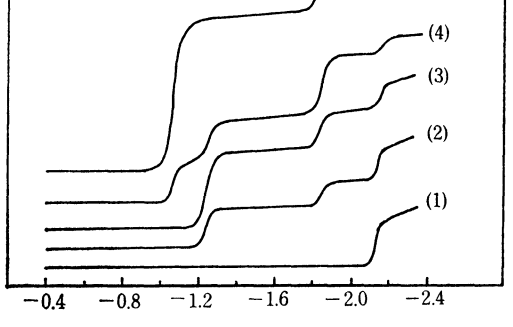
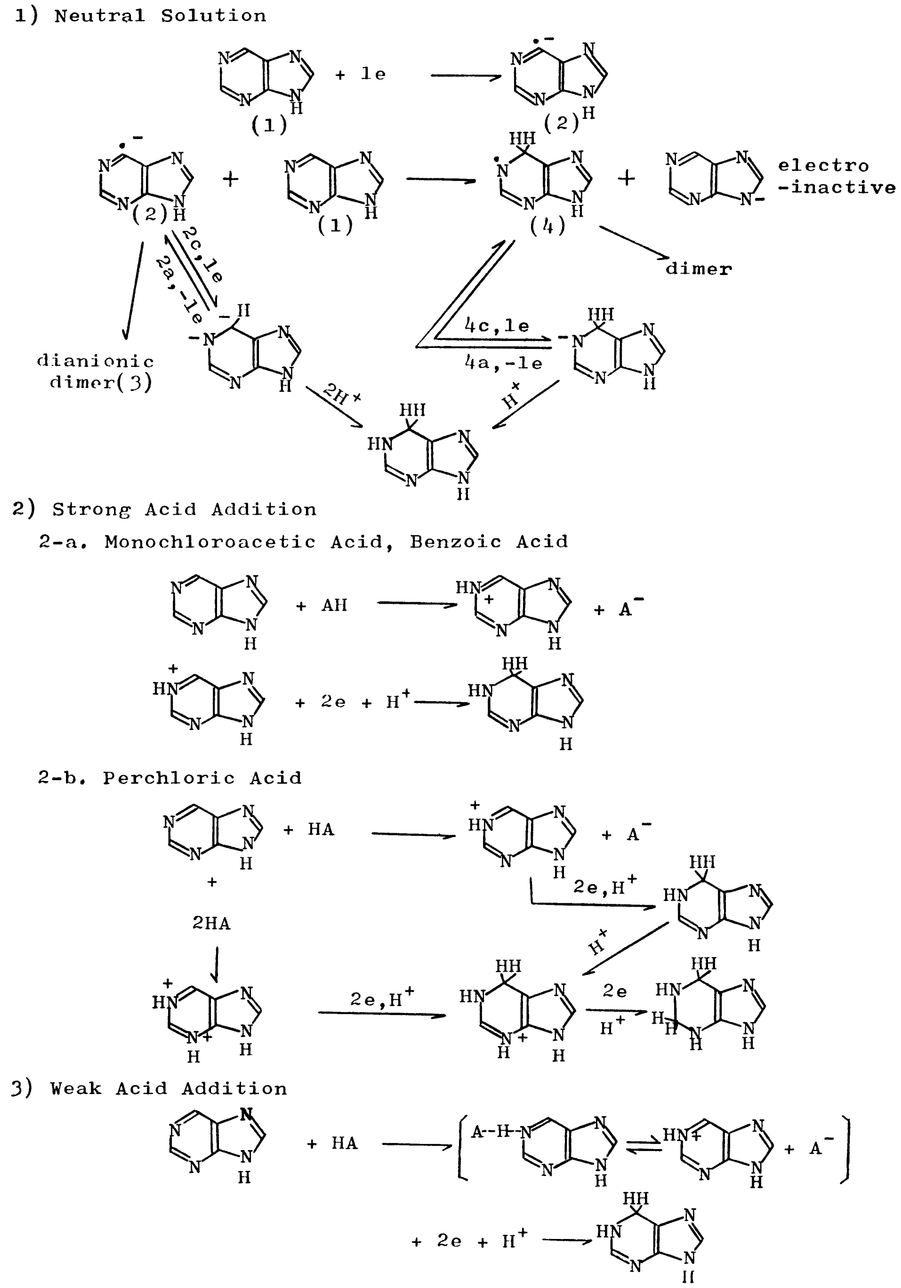


Fig. 7. Effect of $HClO_4$ concentration on polarograms of 0.6 mM purine in acetonitrile.
[$HClO_4$]/[purine] ratio: curve (1), 0; (2), 0.5; (3), 1.0; (4), 1.5; (5), 2.5

wave becomes constant, with a height that corresponds to that of the 2-electron reduction wave, and the original wave disappears. In the case of the addition of benzoic acid, the same behavior was observed.

The addition of a very strong acid, perchloric, results in the appearance of several waves at more positive potentials than the original purine wave, as is shown in Fig. 7. At a molar ratio of acid/purine lower than 1.0, two new waves appear at −1.24 and −1.84 V; their heights increase in proportion to the acid concentration. Perchloric acid present in a molar ratio of 1 : 1 decreases the height of the original wave to about 1/2 of the original value, and the wave at −1.24 V increases in height to nearly double the value of the original wave. A further increase in the perchloric acid concentration causes a new wave to appear at −1.07 V; its height increases until it is about 4 times the value of the original purine wave. However, the wave at −1.24 V and the original purine wave completely disappear at an acid/purine ratio of about 2.1, and the polarographic behavior is close to that observed for purine in unbuffered aqueous solutions. These results are consistent with a prior protonation of the purine molecule at one of the nitrogen atoms to give a species more easily reducible than the original purine wave. Consequently, the waves at −1.24 V and −1.07 V presumably contribute to the reduction of monoprotonated and diprotonated purine respectively. The presence of a weak acid, such as acetic acid and water, causes the half-wave potential of the original wave to shift to more positive values, also increasing its height until it is about 4 times that in dry acetonitrile. The polarographic behavior of purine in DMF in the presence of various acids is similar to that in



Scheme 1.

Table 2. Correlation of potential for the purine reduction wave with the strength of the added acid

Acid added	pK_a^{aq}	$E_{1/2}$, V vs. Ag/Ag$^+$	
		Acetonitrile	DMF
Perchloric acid	a)	-1.07, -1.24, -1.84	$-1.86+0.15$ log[Acid]
Monochloroacetic acid	2.8	-1.89	$-1.92+0.10$ log[Acid]
Benzoic acid	4.2	-2.01	$-2.02+0.10$ log[Acid]
Acetic acid	4.8	$-1.64+0.16$ log[Acid]	$-2.29+0.03$ log[Acid]
Water		$-2.11+0.09$ log[Acid]	

a) Perchloric acid is completely dissociated in both acetonitrile and water.

acetonitrile in the presence of acetic acid and water.

The half-wave potentials of the purine reduction waves produced by adding the various acids are indicated in Table 2 in relation to the strengths of the added acids. The potential of the acid-produced purine reduction is more positive the stronger the acid added. The nature of the purine-acid species formed on acid addition is most probably a hydrogen-bonded or an ion-pair complex.

Mechanism of Purine Reduction. The mechanism presumed for purine reduction in acetonitrile and DMF at the mercury electrode is summarized in Scheme 1. In a neutral nonaqueous solution, purine undergoes a one-electron reduction to produce an unstable anion radical (2), which then abstracts a proton from the parent purine to produce a free radical (4) and an electro-inactive purine anion. The redox couple (peak 2c and 2a) which appears when DMF is used as the solvent, presumably corresponds to the further reduction of a solvated anion radical stabilized by the solvation with DMF, because this solvation effect prevents the fast dimerization reaction from producing the purine dianionic dimer (peak 3a). This is apparent from the fact that the line intensity of the ESR spectrum in the case of acetonitrile is smaller than that in the case of DMF. The three peaks (3a, 4a, and 4c) which appear at the positive potential above -0.7 V presumably correspond to the anions disappearing on addition of the acids. The unstable purine free radical (4) undergoes a fast dimerization to produce a purine dimer, but a part of that is further reduced almost instantaneously to produce the 6-hydropurine anion (peak 4c). When monochloroacetic acid and benzoic acid are present, a preprotonation reaction can occur, which is reduced in a two-electron process to produce 1,6-dihydropurine.[6] When perchloric acid is present, monoprotonated and diprotonated purine species are initially reduced in a two-electron process; this is followed by a further two-electron reduction to produce 1,2,3,6-tetrahydropurine.[6] The addition of weak acids produces an acid-base equilibrium between purine and acids, which equilibrium is reduced in a two-electron process.

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