Electrochimica Acta, Vol. 26, No. 12, pp. 1755–1769, 1981.
Printed in Great Britain.

0013-4686/81/121755-15 $02.00/0
Pergamon Press Ltd.

ELECTROCHEMICAL REDUCTION OF 4-AMINOPYRIMIDINE IN AQUEOUS MEDIA

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(*Received* 12 *March* 1981)

Abstract—The redox and associated chemical behavior of 4-aminopyrimidine (4-AP) and its reduction products in buffered aqueous media have been examined by a variety of electrochemical techniques. In acidic medium (pH 0–2), 4-AP undergoes a two-step reduction: pH-dependent wave I corresponds to a three-electron (3*e*) process; wave II, which corresponds to a 1*e* transfer, is accompanied by catalytic hydrogen discharge. Between pH 2 and 7.5, only wave I is visible; its height diminishes above pH 4, becoming zero by pH 8. Macroscale electroreduction at potentials on the crests of waves I and II has been followed electrochemically and by ultraviolet spectrophotometry with isolation and identification of the wave I reduction products. The photochemical transformation of the reduction products on ultraviolet irradiation has been examined. The reduction of 4-AP is accompanied by isomerization, ring opening and/or deamination of the primary reduction products and of the resulting secondary chemical products.

INTRODUCTION

In recent years, the polarographic behavior has been extensively investigated of pyrimidines, purines, and their nucleosides, nucleotides and higher polymers ranging in complexity to the nucleic acids[1–5]. Much of this activity has involved cytosine (4-amino-2-hydroxypyrimidine) and adenine (6-aminopurine) (Fig. 1); these are the only two of the five commonly encountered nucleic acid bases which are polarographically reducible within the potential range available in aqueous media. The nonreducible bases are uracil, thymine, and guanine; the first two are polarographically reducible in dimethyl sulfoxide medium [6, 7].

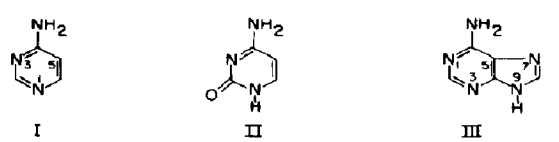
The electrochemical reduction of cytosine is best seen between pH 4 and 6[8, 9]. Protonation at N(3) is followed by two-electron (2*e*) reduction of the 3,4 N=C bond to form a carbanion which rapidly acquires a proton to form the dihydro derivative; the latter deaminates to form 2-hydroxypyrimidine, which—since it is more readily reduced than cytosine itself—is immediately reduced to a free radical which rapidly dimerizes; the overall process produces a single *dme* wave, which approaches 3*e* magnitude; the 3*e* magnitude is confirmed by coulometry.

The electrochemical reduction of adenine, best seen at and below pH 5, involves protonation at N(1) followed by 2*e* reductions (with further protonation) of the 1,6 N=C and 3,2 N=C bonds to form the tetrahydro derivative (seen as a single 4*e* *dme* polarographic wave); the latter compound deaminates (more slowly than cytosine) to regenerate the 1,6 N=C bond, which is reduced (2*e* process) as formed[10–12].

Since the pyrimidine 3,4 N=C bond is structurally similar to the purine 1,6 N=C bond, 4-aminopyrimidine (Fig. 1) is a model compound for both cytosine and adenine, and might provide a link for comparing the latter two compounds. Consequently, this paper describes a detailed investigation of the reduction of 4-aminopyrimidine (4-AP), whose electrochemical behavior in aqueous media up to now has been only scantily studied[3].

4-Aminopyrimidine was reported[13] to produce two polarographic waves at pH 1.2 ($E_{1/2} = -1.13$ and -1.23 V) with apparent n values of 2 and 1, and one wave between pH 2.3 and 6.8 (apparent n, based on the limiting current, decreases from 3 to 1; because of possible current contribution due to hydrogen ion reduction, such evaluation of n is equivocal). Two 4-AP derivatives have been polarographically examined; 4-amino-2,6-dimethylpyrimidine gives a single wave in acidic and slightly basic media, whose I_d between pH 2 and 5 corresponds to a 3*e* process[8]. The 2,5-dimethyl derivative is reported to undergo a 3*e* reduction[14].

It developed in this study of 4-AP that the instability of the primary electrolytic products, as well as that of the secondary products, results in a much more complicated reaction path than had been expected or had been previously reported for pyrimidines. The results amply illustrate the dangers, which may be inherent, in deducing an electrolytic reaction mechanism from polarographic curves alone.



I II III

Fig. 1. Formulae for (I) 4-aminopyrimidine, (II) cytosine (4-amino-2-hydroxypyrimidine), and (III) adenine (6-aminopurine).

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EXPERIMENTAL

Chemicals

4-Aminopyrimidine was synthesized[15]; its ultraviolet and infrared spectra agreed with the literature data.

Buffer solutions (0.5 M ionic strength) were prepared from reagent grade chemicals: (1) HCl–KCl (pH 0.4–2); (2) McIlvaine (pH 3–8); (3) acetate (pH 4–5). Nitrogen used for deoxygenating was purified and equilibrated by bubbling it successively through acidic V(II) kept over heavily amalgamated zinc, saturated CaO, and distilled water.

Apparatus

The potentiostat used was a multipurpose instrument, based on solid-state operational amplifiers; its construction, operation, and performance have been described[16]. For phase-selective *ac* polarography, a Princeton Applied Research Model 122 lock-in amplifier was incorporated. Automatic compensation for series resistance was achieved by positive feedback[17]. Current–voltage and current–time curves were recorded with a Moseley Model 7001A(S) *X–Y* recorder. Rapidly occurring events were recorded with a Tektronix Type 502 oscilloscope and C-12 camera system; the oscilloscope was also utilized as a general purpose monitoring device.

Polarographic and voltammetric measurements were made in a water-jacketed three-compartment cell, kept at 25.0 or 0.5°C; agar salt bridges were inserted on the counter and reference electrode sides of the medium porosity glass frits separating the compartments. The reference compartment contained a saturated calomel electrode (*sce*); the counter compartment contained a platinum mesh electrode immersed in saturated KCl solution. *Dme* capillaries had drop-times between 6 and 7 s at potentials of interest and an *m* value of 1.0 mg/s. A Metrohm E-140 microburet was used to generate the hanging mercury drop electrode (*hmde*) employed in cyclic voltammetry.

For coulometry, a mercury pool electrode (3 cm^2) was used in a water-jacketed cell (25 ml capacity), kept at 25°C and fitted with a Teflon cap for introduction of sample, salt bridges, and deaeration tubing. Reference and counter electrodes (thermostatted and identical with those used for voltammetry) were connected to the cell *via* saturated KCl salt bridges prepared from polyethylene tubes (2.5 mm i.d.) fitted at the cell end with 5 mm lengths of Vycor rod (Corning Code 7930 porous glass).

Ultraviolet spectra were recorded with Cary 14 and Zeiss VSU-2 spectrophotometers; pH was measured with a Beckman Model G pH meter. Potentials reported are *vs sce*.

Polarographic and voltammetric procedures

About 10 ml of test solution, prepared by diluting 4-AP stock solution with the desired buffer, was added to the cell, purged with N_2 for 15 min, and then examined electrochemically with N_2 passing over the solution. The background current, obtained on an identically treated buffer solution, was algebraically subtracted from the observed current.

A natural drop-time was employed for *dc* polarography; $E_{1/2}$ and i_d were determined graphically, utilizing the recorder trace. For *ac* polarography, a controlled 3 s drop-time and an applied alternating voltage of 50 Hz and 5 mV peak amplitude (3.54 mV *rms*) were used; the instantaneous *ac* current amplitude at the end of the drop-life was recorded.

Cyclic voltammetric data are reported for the first scan except for some indicated situations where subsequent scans were used to follow decomposition processes.

Coulometric procedures

An aliquot of buffer solution was deoxygenated in the cell for 20 min; Hg was then introduced and the solution electrolyzed at the potential to be employed for electrolysis until the current fell to near zero (0.01–0.03 mA). The potential was then shifted to −0.6 V; an aliquot of 4-AP stock solution was added; N_2 was vigorously bubbled through the solution until the current dropped to zero (*ca.* 5 min); electrolysis was begun at the desired potential under a N_2 blanket and was stopped when a constant current level (0.1–0.2 mA) was reached. Current was recorded as a function of time; coulombs passed during electrolysis were determined by measuring the current–time curve area with a Gelman Model 39231 compensating polar planimeter.

Immediately after each electrolysis, an aliquot of the electrolyzed solution was diluted and examined spectrophotometrically, using an identical concentration of buffer in the reference cell.

Photochemical procedure

Photochemical irradiation of electrolyzed solutions of 4-AP was made with a RPR lamp (3000 Å) having a photon intensity of 4×10^{17} quanta ml^{-1} min^{-1}. Solutions were also irradiated at 254 nm with a Philips TUV 6 W germicidal lamp, whose radiation was first passed through a 5 mm layer of 35% aqueous acetic acid to eliminate traces of radiation below 230 nm. The intensity incident on the 10 mm pathlength cuvette surface was estimated from the rate of photohydration of a 10^{-4} M aqueous uridine solution in the same cuvette, using the known quantum yield of 2.1×10^{-2} for this reaction. When the irradiated solution was to be chromatographed, a ten-fold higher concentration was irradiated in a 1 mm pathlength cuvette. Ascending chromotography was used with Whatman Nos 1 and 3 papers, and solvent systems of (a) water saturated *n*-butanol and (b) 2-propanol/concentrated ammonium hydroxide/water (7 : 1 : 2). Spots were located with a dark ultraviolet lamp and/or by spraying with 1 M alkali and then with *p*-dimethylaminobenzaldehyde.

RESULTS AND DISCUSSION

Polarographic waves and cyclic voltammetric peaks are designated by Roman numbers with addendum *a* indicating an anodic faradaic reaction and *c* indicating a cathodic reaction.

dc Polarography

Below pH 2, 4-AP exhibits two cathodic waves, which are of equal height at pH 0.4 (Table 1; Figs 2 and 3). The wave Ic height is constant between pH 0.4 and 3; its I_d corresponds to a faradaic n of about 3. The wave IIc height decreases to one-third that of wave I at pH 1.8 and is zero at pH 3. Above pH 4, wave Ic decreases in a manner characteristic of protonated depolarizers involved in an acid–base equilibrium and is zero at pH 7.5. Wave Ic is diffusion-controlled over the pH range (current varies linearly with the square root of mercury height (h) and has a temperature coefficient of 1.5–1.7 %/°C); wave IIc is independent of h at pH 0.4 and slightly linearly dependent at pH 1.8. The pH-dependencies of the half-wave potential ($E_{1/2}$) are as follows:

$$\text{Wave Ic}: E_{1/2} = -1.070 - 0.025\,\text{pH (pH 0.4–2)} \quad (1)$$

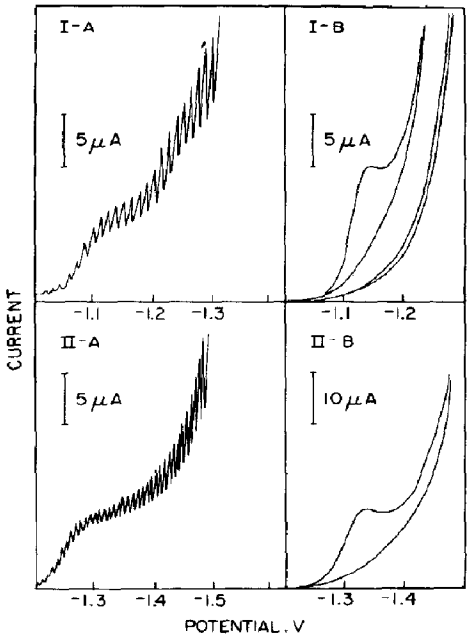

Fig. 2. *Dme* polarograms (A) and cyclic voltammograms (B) for 0.5 mM 4-aminopyrimidine at pH 0.4 (I) and 3.0 (II). Scan rate on cyclic voltammetry = 0.12 V s^{-1}; background scan is shown in I–B.

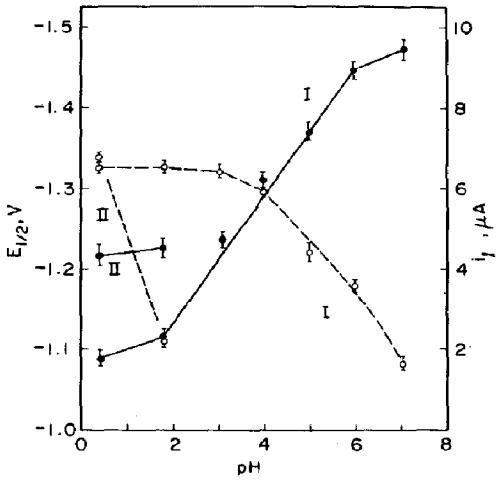

Fig. 3. Variation with pH of $E_{1/2}$ (solid line) and limiting current (maximum i_l; dashed line) of polarographic waves of 4-aminopyrimidine (0.5 m M) in aqueous buffered solution at 25°C. Successive polarographic waves are indicated by Roman number. Estimated maximum experimental error is indicated, *ie* 3% for currents and 20 mV for potentials.

Table 1. *dc* Polarographic behavior of 4-aminopyrimidine in aqueous media[a]

Buffer No.	pH	$E_{1/2}$(V)	Wave slope[b](mV)	i_d[c](μA)	I_d[d]
Wave I					
1	0.4[e]	−1.080	37	6.5	5.6
1	1.8	−1.115	50	6.5	5.7
2	3.1	−1.235	45	6.4	5.7
3	4.0	−1.310	35	5.9	5.3
3	5.0	−1.370	35	4.4	3.8
2	6.0	−1.445	40	3.6	3.0
2	7.1	−1.470	35	1.7	1.5
Et_4NCl		−1.43		1.7	1.5
Wave II					
1	0.4	−1.215	32	6.5	5.6
1	1.8	−1.225		2.2	1.8
Et_4NCl		−1.70		2.2	1.8

[a] Concentration of 4-aminopyrimidine = 0.5 mM; h = 65 cm; temperature = 25°C.

[b] Wave slope = $E_{1/4} - E_{3/4}$.

[c] Maximum current.

[d] $I_d = (6/7)\, i_d(\text{max})/Cm^{2/3}t^{1/6}$. An approximate value of the faradaic n can be obtained by dividing I_d by 2.

[e] The ionic strength of 0.4 M HCl was adjusted to 0.5 M by KCl addition; the pH was *ca.* 0.4.

$$E_{1/2} = -0.960 - 0.080\,\text{pH (pH 2–7)} \quad (2)$$

Wave IIc: $$E_{1/2} = -1.207 - 0.007\,\text{pH (pH 0.4–2)} \quad (3)$$

The wave Ic slope somewhat exceeds the *ca.* 30 mV theoretically expected for a reversible uncomplicated 2*e* process or for dimerization following a reversible electron transfer[18].

The presence of 4-AP shifts the background discharge positively from about 70 mV in acidic media to 200 mV at pH 7. This behavior is similar to that of adenine[19].

In 0.1 M Et_4NCl solution, 4-AP produces two waves of about equal height; $E_{1/2}$ and I_d for wave Ic are similar to those in pH 7 buffered solution.

ac Polarography

In-phase current component peaks I and II (Table 2) correspond to *dc* polarographic waves Ic and IIc, *eg* peak II is not seen at pH exceeding 2. With increasing pH, peak I shifts to more negative potential and decreases in height to vanish above pH 7. The low peak I current, *eg* Δi_s of 1.0 μA compared to 15 μA calculated for a 1*e* diffusion-controlled reversible process, could reflect a slow chemical equilibrium involving the reactant or a rapid equilibrium involving the product of the electron-transfer process. The sharper peaks and larger current magnitudes for peak I indicate that the reoxidation reaction is more prevalent for the first reduction process than for the second (peak II).

A weak depression from background in the quadrature current component (depression I), centered at about −0.45 V, appears over the pH range. Weak depression II appears at pH 1.8 following peak II, and increases in magnitude to pH 4 and shifts to more negative potential. At pH 6 and 7, depressions II and III are separated by peak III. It seems probable that the peak I reduction product or its rearrangement product is adsorbed at the solution/electrode interface at pH exceeding 2 and desorbs at pH exceeding 6 where desorption peak III appears.

Cyclic voltammetry

The cyclic voltammetric behavior of 4-AP (Table 3; Figure 2) is consistent with its *dc* and *ac* polarography. Between pH 0.4 and 2 only peak Ic appears at polarization or scan rate (v) less than 0.2 V/s, at v exceeding 1 V/s, peak IIc also appears. Above pH 2, only peak Ic is seen.

The peak Ic current function, $i_p/ACv^{1/2}$ (A, electrode area; C, depolarizer concentration) at slow v (*ca.* 37 at pH 4.0) is similar to that for cytosine (34 at pH 4.2), supporting the occurrence of a 3*e* process. The Ic current functions (Fig. 4) at pH 0.4–5 are relatively constant with increasing v up to 1 V/s, indicating diffusion control, but then increase with v, suggesting the presence of adsorption[9, 20]; the effect is more evident at pH 5 where the waveheight is decreasing; at pH 6, the function decreases slightly but linearly for v exceeding *ca.* 0.2 V/s. The peak IIc current function is constant with increasing v at pH 0.4 but decreases at pH 1.8. The appearance of peak IIc only at v exceeding 1 V/s indicates that the species producing it is relatively short-lived.

E_p for peak Ic becomes somewhat more negative with increasing v up to 1 V/s (25 mV at pH 0.4 and 6) but then increases only slightly up to 50 V/s (30 mV at pH 0.4 and 5); the pattern is generally similar to that seen for cytosine[9]. The pH-dependence of E_p at the v indicated is as follows:

Peak Ic: $$E_p = -1.10 - 0.070\,\text{pH}\ (v = 0.12\,\text{V/s}) \quad (4)$$

$$E_p = -1.13 - 0.070\,\text{pH}\ (v = 1\,\text{V/s}) \quad (5)$$

Peak IIc: $$E_p = -1.365 - 0.025\,\text{pH}\ (v = 20\,\text{V/s}). \quad (6)$$

No anodic peak complementary to peak Ic or IIc appears on the return sweep at pH 0.4 to 5, even at v = 200 V/s. However, at pH 6, a very small peak Ia (E_p = −1.44 V) appears at $v \geqslant 20$ V/s, which seems to be complementary to peak Ic (E_p = −1.52 V) and which increases in relative magnitude with increasing v; its appearance only at large v indicates the presence of a moderately rapid chemical reaction involving the charge–transfer reaction product[21]; the 80 mV difference in cathodic–anodic peak potentials is somewhat larger than the 60 mV expected for a reversible 1*e* redox couple. On the second and subsequent sweeps at pH 6, small complementary peak couple IIIc–IIIa appears at v = 50 V/s, whose height (as seen on successive sweeps) increases with time

Table 2. *ac* Polarographic behavior of 4-aminopyrimidine in aqueous media[a]

Buffer No.	pH	In-phase component		Quadrature component			
				Depressions[b]		Peaks	
		E_s (V)	Δi_s (μA)	E_{min} (V)	Δi_{min} (μA)	E_q (V)	Δi_q (μA)
1	0.4	I −1.165	0.9	None		I −1.19	0.30
		II −1.30	0.6			II −1.36	0.15
1	1.8	I −1.230	0.85	II −1.63	0.18	I −1.26	0.20
		II −1.36	0.23			II −1.41	0.04
2	3.1	−1.340	0.9	II −1.71	0.45	I −1.36	0.25
3	4.0	−1.410	1.0	II −1.76	0.65	I −1.44	0.22
3	5.0	−1.445	0.7	II −1.79	0.45	I −1.46	0.18
2	6.0	−1.500	0.4	II −1.81	0.13	I −1.69	0.08
				III −1.94	0.28	III −1.86	0.03
2	7.1	−1.530	0.2	II −1.83	0.10	I −1.7	0.06
				III −1.94	0.28	III −1.88	0.05

[a] Concentration of 4-aminopyrimidine = 0.5 mM. Temperature = 25° C.
[b] A weak depression from background current, centered at about −0.45 V, is seen at all pH.

Table 3. Cyclic voltammetric behavior of 4-aminopyrimidine in aqueous media[a]

pH	Scan Rate (V/s)	Peak Ic[b] E_p (V)	Peak Ic[b] i_p (μA)	Peak Ic[b] $i_p/ACv^{1/2}$	Peak IIc[b] E_p (V)	Peak IIc[b] i_p (μA)	Peak IIc[b] $i_p/ACv^{1/2}$
0.4	0.014	−1.13	4.4	41			
	0.12	−1.14	12.2	39			
	1.0	−1.15	36	40	−1.26	40	44
	20	−1.18	180	45	−1.34	190	47
	50	−1.18	340	53	−1.37	320	50
	100	−1.19	480	53	−1.40	400	44
1.8	0.014	−1.22	4.0	38			
	0.12	−1.23	12.1	39			
	1.0	−1.24	34	38			
	20	−1.26	180	45	−1.38	70	17
	50	−1.28	320	50	−1.41	150	24
	100	−1.28	450	50	−1.42	200	22
3.1	0.014	−1.30	4.0	38			
	0.12	−1.32	11.5	37			
	1.0	−1.33	38	42			
	20	−1.36	175	43			
	50	−1.38	320	50			
4.0	0.014	−1.35	4.0	38			
	0.12	−1.35	11.0	35			
	1.0	−1.36	36	40			
	20	−1.40	180	45			
	50	−1.42	340	53			
5.0	0.014	−1.42	2.9	27			
	0.12	−1.43	8.0	26			
	1.0	−1.44	25	29			
	20	−1.46	170	42			
	50	−1.46	290	46			
6.0	0.014	−1.48	2.5	23			
	0.12	−1.49	7.0	22			
	1.0	−1.50	12	13			
	20	−1.52	70	17			
	Ia[c]	−1.44	6	1.5			Peak IIIc[d]
	50	−1.52	90	14	−1.32	8.0	1
	Ia[c]	−1.44	15	2.4	IIIa −1.26	8.0	1
7.0	0.014	−1.53	1.0	9			
	0.12	−1.55	1.6	5			
	1.0	−1.56	2.2	2			Anodic peak
	20		None		−1.22	5.0	1

[a] Concentration of 4-aminopyrimidine = 0.5 mM. Temperature = 25° C. Area of *hmde* = 1.8 mm^2.
[b] The current function, $i_p/ACv^{1/2}$, has been calculated for A = 1.8 mm^2, C = 0.5 mM, and v in V/s.
[c] Anodic peak appears at higher scan rate and results in appearance of the peak IIIc–IIIa couple.
[d] Peaks IIIc and IIIa increase in magnitude with time (*cf* text).

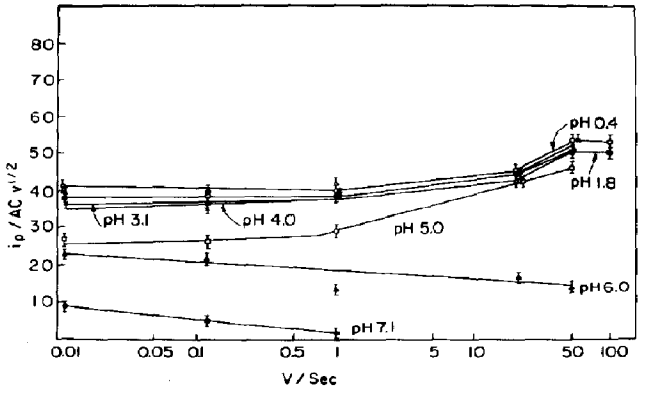

Fig. 4. Variation of current function, $i_p/ACv^{1/2}$, with scan rate, v, at a hmde for peak Ic of 4-aminopyrimidine at 25°C. Points are plotted with an indicated error limit of ±3% of the $i_p/ACv^{1/2}$ magnitude. pH: open circles, 0.4; solid squares, 1.8; open triangles, 3.1; crosses, 4.0; open squares, 5.0; solid triangles, 6.0; solid circles, 7.

(30–120 s) while that of peak Ia decreases, suggesting that the species producing peak Ia is transformed by a solution process into the species which produces peak IIIc; the 60 mV difference in the cathodic–anodic peak potentials is characteristic of a 1*e* reversible redox couple.

At pH 7.0, the height of peak Ic, which diminishes with the concentration of protonated 4-AP, is very small. On the other hand, the rate of transformation of the 3*e* reduction product of 4-AP into species producing peak pair IIIc–IIIa increases with pH, as can be observed by the enhanced rate of decrease of the absorption peak at 308 nm at neutral pH. At pH 7.0, only a small peak IIIa is seen at v of 20 V/s (whereas peaks Ic–Ia are too small to be measured); this is probably due to the longer lived species responsible for the pink color formed after exposure to air of a 4-AP solution reduced at the wave I potential.

Electrolysis at controlled potential

For three electrolyses at a potential on wave Ic at pH 0.4, n (mean and standard deviation) was 2.84 ± 0.07 (Table 4); the linear $\log i$ *vs* t plot indicates the reduction to be a relatively clean process. For electrolysis on wave IIc, n could not be estimated exactly due to interference by background discharge; the n of *ca.* 5 probably corresponds to 3 for wave Ic, 1 for wave IIc and 1 for background electrolysis, or to 3 for Ic and 2 for IIc. At pH 4.0, n was 2.69 ± 0.14 (three electrolyses). At pH 5.0, n was 2.0; the $\log i - t$ plot consisted of two linear segments, indicating the presence of a secondary process or processes.

To aid in product identification, *dc* polarograms were made of the electrolyzed solutions at the pH of electrolysis and at pH 7; no electroactivity was seen after electrolysis on a wave Ic plateau potential at pH 0.4. Ultraviolet absorption spectra were also recorded.

Unfortunately, identification of the electrolytic products is complicated by the fact that the reduction products of 4-AP are quite unstable. Thus, on catalytic reduction of 4-AP[22, 23], only degradation products of the primary reduction product were isolated or identified, *eg* NH_4Cl, an acrylamidinium salt and formaldehyde; the evidence was considered to favor initial formation of the 1,2,5,6-tetrahydro species. In general, catalytic nuclear reductions of pyrimidines give mixtures of products and the tetrahydropyrimidines generally degrade in acidic aqueous medium [24]. Thus, catalytic reduction of pyrimidine in acidic medium leads to the 1,4,5,6-tetrahydro derivative [23, 25, 26], which rapidly hydrolyzes.

1. *Ultraviolet absorption spectra*

Spectra recorded for the electrolyzed solutions are summarized in Table 5.

After electrolysis at pH 0.4 at a potential on the wave Ic plateau, the characteristic 4-AP absorption maximum at 246 nm had been replaced by maxima at 308 and 225 nm; a solution electrolyzed at a wave IIc potential did not exhibit any ultraviolet absorption maximum as would be expected for a 4*e* reduction of the 4-AP which would leave only one double bond in the ring system.

Electrolysis at pH 4.0 and 5.0 also produced solutions with maxima at 305 and 225 nm.

Although one λ_{max} at both pH 0.4 and 7.0 (308 nm) for the 4-AP solution electrolyzed at pH 0.4 differs slightly from that of 305 seen for 4-AP solutions electrolyzed at pH 4 and 5 (Table 5), the difference is unlikely to be significant and it is safe to conclude that the relative constancy of the ratios of the apparent molar absorptivities at the long and short wavelengths, *ie* 305 or 308 and 225 nm, respectively, at both pH 0.4 (likely to be a protonated species) and 7.0 (likely to be largely a neutral species) of 4-AP solutions electrolyzed at pH 0.4, 4.0 and 5.0 (Table 5) strongly supports the same final products or products being produced on electrolysis with the added implication that the variation in magnitude of the apparent ε among the reduction products is a measure of the variation in relative amount of one particular final product and/or in the product ratio.

It is helpful to compare the spectral data for 4-AP and its reduction products with the spectral data recorded by Wierzchowski and Shugar[27] for 2,6-dimethyl-4-aminopyrimidine and its isomeric rearrangement product, 2-amino-3-cyanopent-2-en-4-imine, produced on ultraviolet irradiation (Table 6), *eg* in respect to ε ratio and λ_{max}.

Table 4. Coulometric estimation of the number of electrons involved in the controlled electrode potential reduction of 4-aminopyrimidine in aqueous media[a]

pH	Electrolysis potential (V)	Quantity of 4-AP (μmol)	Current passed[c] (mC)	n
0.4	−1.1	1.9	521	2.84
		1.86	498	2.77
		6.0	1,678	2.90
	−1.27[b]	6.0	2,975	5.14
4.0	−1.40	6.0	1,628	2.81
		6.0	1,576	2.72
	−1.45	4.5	1,100	2.53
5.0	−1.45	6.0	1,163	2.01

[a] Concentration of 4-aminopyrimidine = 1 mM. Temperature = 25°C. Background solution was preelectrolyzed.

[b] This potential is on the wave II limiting current pleateau; in all other cases, the potential was on the wave I plateau.

[c] Values obtained by subtracting the background current from the total current passed during the electrolysis.

Table 5. Ultraviolet absorption data for 4-aminopyrimidine and its electrolytic reduction products in aqueous solution

Solution examined[a]	pH[b]	λ_{max} (nm)	ε[c]	ε ratio[d]
4-Aminopyrimidine[e]	0.4	246	18500	
($pK_a = 5.7$)	7.0	268	5000	0.28
		233	18000	
E.P. (wave I; pH 0.4)[f]	0.4	308	3300	1.83
		225	1800	
	7.0	308	3600	0.95
		225	3800	
E.P. (wave I; pH 4.0)[f]	0.4	305	4500	2.25
		225	2000	
	7.0	305	4600	0.89
		225	5200	
E.P. (wave I; pH 5.0)[f]	0.4	305	6500	2.32
		225	2800	
	7.0	305	4800	0.92
		225	5200	

[a] E.P. = product after electrolysis of 4-aminopyrimidine at a potential on the crest of the wave noted at the pH given.

[b] After electrolysis, the aliquot examined was diluted to the pH indicated, at which the spectrum was taken.

[c] In the case of electrolyzed solutions, ε is based on the original 4-AP concentration.

[d] Where two absorption maxima are recorded, the ratio of ε at the longer wavelength to ε at the shorter wavelength is given.

[e] Literature data for ultraviolet absorption spectrum of 4-aminopyrimidine[33]: at pH 0 (cationic form), $\lambda_{max} = 246$ nm ($\varepsilon = 18600$); at pH 13 (neutral form), $\lambda_{max} = 268$ nm ($\varepsilon = 5200$) and 233 nm ($\varepsilon = 18200$) (ε ratio = 0.29).

[f] It is helpful to compare the data for the electrolysis products with those reported[27] for 2 amino-3-cyano-pent-2-en-4-imine: Table 6.

Solutions electrolyzed at pH 0.4 turned pink after 2 h exposure to air; the half-life of the product at room temperature, based on decrease in the 308 nm peak, is about 70 h. When the pH of the electrolyzed solution was adjusted to pH 3 or greater, the solution turned pink immediately on exposure to air and the decrease in the 308 nm peak was much faster, increasing with pH. Solutions electrolyzed at pH 0.4 but stored at 0°C under a nitrogen atmosphere were stable over a period of one to two weeks.

Solutions electrolyzed at pH 5 and stored at 0°C under a nitrogen atmosphere are stable, showing only a small decrease in absorbance at 305 nm after two weeks. After 72 h at room temperature, about 30% of the product kept at pH 0.4 had decomposed and about 4% of that kept at pH 7.

2. *Photochemical irradiation of electrolyzed solutions*

Photochemical transformations were attempted in order to see if the 4-AP wave I reduction product

Table 6. Ultraviolet absorption data for 2,6-dimethyl-4-aminopyrimidine (I) and 2-amino-3-cyanopent-2-en-4-imine (II)[a]

	Absorption maxima (nm) (ε)		
		Protonated form	
Compound	Neutral form at pH 8.15[c]	at pH 5.5[d]	at pH 3[e]
I[b]	265 (4700)	248–50 (13100)	
($pK_a = 6.9$)	234 (10200)		
ε ratio	0.46		
II	292 (11800)	301 (20500)[f]	302 (22000)
($pK_a = 7.0$)	225 (13200)	225–6	228 (7700)
ε ratio	0.89		2.86

[a] Data taken from ref.[27], to whose text and figures citations in the following footnotes refer.

[b] First protonation site in I is N(3); second protonation site is most likely an amino nitrogen (*cf* footnote on page 386). For the doubly protonated form of I: $pK_a = -0.14$ (p. 388); $\lambda_{max} = 247$ nm (*cf* Fig. 5).

[c] Data estimated from Fig. 4.

[e] Data estimated from Fig. 3.

[f] ε is based on a quantitative conversion of 68% of monoprotonated I to monoprotonated II (*cf* Fig. 4 of ref.[27]).

is photochemically oxidized to the parent compound as are the dimeric reduction products of 2-hydroxypyrimidine[28] and its derivatives[29, 30], as well to evaluate the photochemical stability of the electrolytic products of 4-AP.

Photochemical exposure in the presence of metallic mercury was examined because the presence of the mercury significantly increased the efficiency of the photochemical transformation. Unfortunately, lack of time prevented exploration of the mechanism of action due to the mercury.

On irradiation (λ_{max} = 300 nm) at pH 0.4 of a 4-AP solution previously electrolyzed at pH 5.0, the 305 nm maximum slowly decreases, shifts to shorter wavelength, and finally disappears (Table 7); if the solution is in contact with mercury, a maximum appears at 235 nm as the 305 nm maximum disappears. Exposure at pH 0.4 of an electrolyzed (at pH 5) 4-AP solution to air in the presence of mercury also results in disappearance of the 305 nm maximum, and appearance of the 235 nm maximum (ε = 20 000) and of a *dc* polarographic wave ($E_{1/2}$ = −1.13 V), whose height about equals the sum of the two 4-AP waves. Thus, in presence of mercury, ultraviolet irradiation and oxidation by air cause similar transformation of the reduction product(s) of 4-AP. However, it is evident that the photochemical product formed at pH 0.4, while close to 4-AP in several physical properties, is not identical to it, *eg* $E_{1/2}$ = −1.08 V and λ_{max} = 246 nm (log ε = 4.27) for 4-AP, but −1.13 V and 235 nm (4.30) for the photochemical product.

Irradiation at pH 0.5 at 254 or 300 nm in presence of a mercury drop of a 4-AP solution previously electrolyzed at pH 4.0 immediately after electrolysis resulted in decrease of the 308 nm peak and simultaneous growth of an absorption peak at 245 nm with all intermediate absorption curves passing through an isosbestic point indicating formation of a single ultraviolet-absorbing photochemical product (Fig. 5). Ultraviolet spectrophotometry and polarography confirmed that the product is 4-AP, as did paper chromatography using for comparison an authentic sample of 4-AP (R_f on Whatman No. 1 paper is 0.57 in neutral solvent and 0.76 in alkaline solvent); no other ultraviolet absorbing spots were seen on chromatography but spraying revealed yellow traces presumably due to hydrolytic decomposition.

If the electrolyzed solution is allowed to stand for an hour before irradiation in presence of a mercury drop, the photochemical transformation proceeded more slowly and only an absorption maximum at 236 nm resulted; it is apparent that the final electrolysis

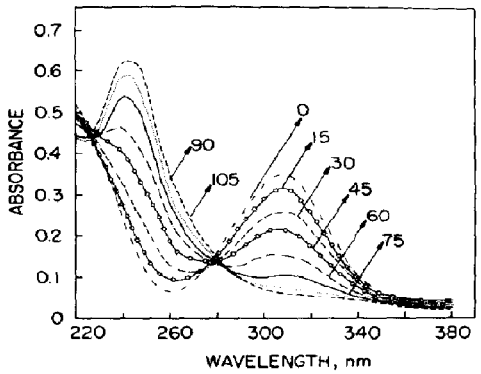

Fig. 5. Photochemical transformation at pH 0.4 on irradiation at 254 nm of the wave I reduction product of 4-aminopyrimidine in the presence of mercury. Product prepared by complete electrolysis of 2 mM 4-AP at pH 4.0 (0.2 M Britton–Robinson buffer) and subsequent 2.5-fold dilution with 0.4 M HCl. Numbers besides each curve correspond to time of irradiation in minutes. (In comparing Fig. 5 and Table 5, it should be noted that an acetate buffer and the Cary spectrophotometer were used in obtaining the Table 5 data; the Zeiss instrument, which is much less sensitive than the Cary in the 200–220 nm region, was used to get the Fig. 5 data.)

Table 7. Transformation of electrolysis product of 4-aminopyrimidine[a] on ultraviolet irradiation

pH	Hg presence[d]	Irradiation[b] period min	Ultraviolet spectra of solutions[c]			
			Before irradiation		After irradiation	
			λ_{max}	ε	λ_{max}	ε
0.4	No	25	305	6500	NAM[e]	
			225	2800		
0.4	Yes	25	305	6500	235	20000
			225	2800		
7.0	No	25	305	4800	300	3600[f]
			225	5200	225	4000[f]
7.0	Yes	15	305	4800	300	3600[f]
			225	5200	225	4000[f]

[a] Solutions were originally electrolyzed at pH 5.0.

[b] Solutions were irradiated with a RPR 3000 Å lamp with a photon intensity of 4×10^{17} quanta/ml/min.

[c] ε values for the reduced solutions were calculated assuming a concentration of the reduction product equal to that of the original 4-aminopyrimidine before electrolysis. Values for dimers should be about twice as great.

[d] Solution of electrolysis product was irradiated in presence or absence of mercury drop.

[e] NAM—no absorption maximum observed.

[f] Ratio of ε after irradiation to ε before irradiation equals 0.75 to 0.77.

product had undergone a transformation during the hour interval.

Irradiation at pH 7.0 of a 4-AP solution previously electrolyzed at pH 5.0 does not result in photochemical transformation but only in hydrolytic decomposition (Table 7). This behavior would support the view that only the protonated reduction product(s) is photochemically active. On chromatography on Whatman No. 3 paper with neutral solvent, the product absorbing at 225 and 308 nm gave a spot of $R_f = 0.26$; with alkaline solvent, only a spot absorbing very faintly at 260 and 320 nm was found ($R_f = 0.8$), which indicated further decomposition.

Spraying with *p*-dimethylaminobenzaldehyde revealed non-ultraviolet absorbing spots ($R_f = 0.25$ in neutral solvent and 0.79 in alkaline solvent), which became red on exposure to air, as well as traces of yellow decomposition products.

3. *Chemical examination of electrolyzed solutions*

The Nessler test for NH_3[31] was negative for unreduced 4-AP solutions and for 4-AP solutions electrolyzed at pH 0.4, but was positive for 4-AP solutions electrolyzed at pH 4 and 5, indicating that deamination of the electrolytic product should be considered as a follow-up reaction at the latter pH. The tetraphenylborate test for NH_3, amines, alkaloids and basic nitrogen compounds in general (as well as for potassium)[32] was negative for unreduced 4-AP and for 4-AP electrolyzed at pH 0.4; 4-AP solutions electrolyzed at pH 4 and 5 gave about twice as much precipitate as would be expected for one amine group, suggesting that, in addition to ammonia produced by deamination (*cf.* Nessler test), a second amine group may have been generated by ring opening. Such ring opening is known to occur with tetrahydropyrimidines[33] and on adenine reduction[10].

The solution electrolyzed at pH 4.0 gave a negative test for diazotizable amine with Bratton–Marshall reagent[34] and for formaldehyde, which is a possible product of ring cleavage, with chromotropic acid.

Treatment of 4-AP solutions electrolyzed at pH 0.5 and 4 with *p*-dimethylaminobenzaldehyde (with NaOH) produced a bright red color, indicating degradation of the pyrimidine ring[35]; 4-AP itself was unaffected. While the 306 nm peak of a 4-AP solution electrolyzed at pH 4.0 decreased by only 8% in 30 min, addition of hydroxylamine resulted in its almost total disappearance in 10 min, suggesting that the electrolytic or derived product(s) contains an aldehyde group.

In summary, hydrolysis of reduced 4-AP is more rapid at higher pH. At pH 4, for example, ring opening occurs; ammonia is probably formed, but formaldehyde and a diazotizable amine are not formed (compare to behavior of reduced purine and adenine[10]).

4. *Isolation of reduction products*

On evaporation under vacuum (13 mmHg) of a pH 0.4 solution of 4-AP electrolyzed at a wave Ic potential, white crystals precipitated, which, on dissolution in pH 7.0 solution, only absorbed slightly at 235 nm and which is unstable even at low temperature, *eg* it turned black on the surface after a few hours of storage in a refrigerator both on exposure to air and under a nitrogen atmosphere. The black residue, obtained on evaporation of the solution remaining after filtering off the white crystals, gave on dissolution in pH 7.0 solution absorption maxima at 305 and 225 nm, similar to the 4-AP solutions on completion of electrolysis, including ε ratio (Table 5); this material is relatively stable in solution, *eg* about 10% decrease in absorption after 7 days in a refrigerator.

The presence of two products—one with appreciable absorption at 225 and 305–308 nm and the other with appreciable absorption only at 235 nm—could account for the variation in the apparent numerical values of the molar absorptivities (ε) (Table 5) for solutions electrolyzed at different times and at different pH values even though the original solutions were of the same concentration and the ε ratios for absorption at 308 (or 305) and 225 nm show the same pattern in each instance.

REACTION PATH

As has been noted, definitive positive identification of the primary electrolytic products of 4-AP with subsequent rigorous formulation of a reaction path, is excluded by the instability of apparently both primary electrolytic products and secondary chemical products, which instability can manifest itself, *inter alia*, in molecular rearrangement and ring degradation. However, comparison of the electrochemical behavior of 4-AP with those of related compounds, when combined with chemical, spectrophotometric and electrochemical behavior of electrolyzed solutions of 4-AP, does allow knowledgeable surmizes to be made concerning the probable primary electrolytic products and their subsequent alteration.

Wave Ic characteristics

The principal 4-AP wave Ic is seen in aqueous solution between pH 0 and 7. Comparative $E_{1/2}$ and faradaic n data for 4-AP and related compounds (Table 8) are informative, *eg* some support for common mechanistic factors being involved is provided by the pH-dependencies of 0.08 ± 0.01 V for $E_{1/2}$ for most multielectron waves and for at least some initial 1*e* waves. Calculated and experimental $E_{1/2}$ values for pyrimidine and 4-AP are summarized in Table 9 and Fig. 6.

Addition of an amino group at C(4) in pyrimidine [or at the equivalent C(6) site in purine] makes reduction of the initially reduced pyrimidine 3,4 N=C bond (or equivalent purine 1,6 N=C bond) more difficult by 0.3 V or more. Consequently, 4-AP wave I occurs at more negative potential then pyrimidine wave III, which involves 2*e* reduction of the 3,4 N=C bond.

The importance of deamination in the configuration –NH–CH(NH_2)–, at the 3,4 position, in regenerating the reducible 3,4 N=C bond, which would be reduced as formed (*ece* process), can be estimated by considering the relative contribution of deamination in the

Table 8. Comparative reduction patterns in aqueous media for 4-aminopyrimidine and related compounds[a]

Compound	pH range		pH-dependence of $E_{1/2}$[b](V)	n	$E_{1/2}$ at pH 4.2 (V)[c]
Pyrimidine	0.5–5	I	−0.576–0.105 pH	1	−1.02
	3–5	II	−1.142–0.011 pH	1	−1.19
	5–8	III	−0.680–0.089 pH	2	(−1.05)
	7–8	IV	−1.600–0.005 pH	2	
	9–13	V	−0.805–0.079 pH	4	
4-Aminopyrimidine	0.4–2.0	I	−1.070–0.025 pH	3	(−1.18)
	2.0–7.5	I	−0.960–0.080 pH	3	−1.30
	0.4–2.0	II	−1.207–0.007 pH	1	(−1.24)
2-Aminopyrimidine	2–2		−0.685–0.049 pH	1	
	4–7	I	−0.425–0.121 pH	1	−0.93
	4–7	II	−1.360–0.004 pH	1	−1.38
	7–9	III	−0.680–0.090 pH	2	
4-Amino-2,6-dimethyl-pyrimidine	2–8		−1.130–0.073 pH	3 or 4	−1.44
4-Amino-2,5-dimethyl-pyrimidine	1–8		−1.06 −0.076 pH	3	−1.38
2-Hydroxypyrimidine	2–9		−0.530–0.078 pH	1	−0.86
Cytosine (4-amino-2-hydroxypyrimidine)	4–6		−1.125–0.073 pH	3	−1.44
Purine	1–6	I	−0.697–0.083 pH	2	−1.05
	1–6	II	−0.902–0.080 pH	2	−1.24
Adenine	1–6		−0.975–0.090 pH	4	−1.35

[a] Data for 4-aminopyrimidine are based on Table I; sources of the data for the other compounds are taken from tables in ref. 3.

[b] Roman numbers refer to the sequence of waves.

[c] Values in parentheses have been calculated by the adjacent equations even though the wave in question is not seen at pH 4.2.

electrochemical reduction of cytosine and adenine. In reduced adenine, deamination is relatively slow (rate constant is less than 1 s^{-1} at pH 4)[11]; accordingly, the *dme* wave shows a faradaic n of 4, whereas an n of 6 is found on the longer time scale of exhaustive controlled potential electrolysis (*cpe*). In cytosine, the hydroxyl substituent on C(2), which is involved in a tautomeric shift (Fig. 1), has a profound effect, *eg* the initial 1*e* 3,4 N=C pyrimidine reduction in 2-hydroxypyrimidine occurs energetically more easily (Table 8) and dimerization of the free radical produced is greatly accelerated; the 2-hydroxy substitutent may also accelerate deamination in the 3,4 $-NH-CH(NH_2)-$ configuration[8]. Thus, cytosine is more difficult to reduce initially than pyrimidine; at pH 5, $E_{1/2}$ is −1.49 V for cytosine and −1.13 V for pyrimidine wave III (2*e* 3,4 N=C bond reduction). However, deamination in cytosine, whose 3,4 N=C bond has been reduced, is moderately rapid (rate constant is 10 s^{-1} at pH 4)[9]; consequently, deamination is sufficiently rapid for 1*e* reduction of the deamination product (2-hydroxypyrimidine) to contribute significantly to the observed faradaic *dme* current, *eg* I_d is equivalent to n between 2 and 3, and apparently increases with pH in the pH range of 4–6 where the wave is clear[8, 9]; n on *cpe* approaches 3.

Based on the behavior of adenine and cytosine, deamination of 3,4-dihydro-4-aminopyrimidine—the expected product of an initial 2*e* reduction of 4-AP—to produce pyrimidine would likely be less rapid than in reduced cytosine. Since 4-AP is reduced at more negative potential than that required to reduce the

Table 9. Experimental and calculated half-wave potential data for pyrimidine and 4-aminopyrimidine

Compound		$E_{1/2}$(V) at pH indicated[a]				
		pH 0.4	pH 2.0	pH 4.2	pH 5.0	pH 7.0
Pyrimidine	I	−0.62	−0.79	−1.02	(−1.10)	(−1.31)
	II	(−1.15)	(−1.16)	−1.19	(−1.20)	(−1.22)
	III		(−0.86)	(−1.05)	−1.13	−1.30
	IV				(−1.63)	−1.64
	V					(−1.36)
4-Amino-pyrimidine	I	−1.08	−1.12	−1.30	−1.37	−1.47
	II	−1.22	−1.23			

[a] Parentheses around a potential indicates that wave does not appear at pH in question. The enclosed calculated $E_{1/2}$ values are derived from the $E_{1/2}$-pH equations given in Table 8. Roman numbers refer to the wave sequence.

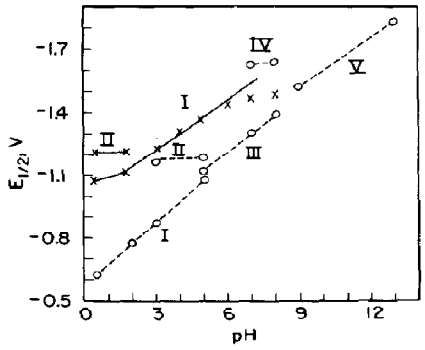

Fig. 6. Variation with pH of $E_{1/2}$ for the polarographic waves of 4-aminopyrimidine (0.5 mM) (solid line and crosses) and pyrimidine (dashed line and circles) in aqueous buffered solutions. Pyrimidine data are taken from ref.[8]. Successive polarographic waves are indicated by Roman numbers.

pyrimidine 3,4 N=C bond, the pyrimidine would be reduced as formed, resulting in a second 2*e* reduction to 3,4-dihydropyrimidine and an eventual 4*e* reduction of each original 4-AP molecule. However, two pieces of evidence are contrary to this argument. In the range of pH 0.4–4.0, *n* for the 4-AP reduction, as determined coulometrically, approaches 3 (Table 4) and the *dme* I_d magnitudes (Table 1) are as expected for *n* of 3; at pH 5, the coulometric and *dme* *n* are 2. Second, the product of electrolysis at pH 4.0 absorbs at both pH 0.4 and 7.0 at 225 nm ($\log \varepsilon = 3.30$ and 3.72) and 305 nm ($\log \varepsilon = 3.65$ and 3.66) (Table 5) whereas 3,4-dihydropyrimidine at pH 3.7 and 5.8 absorbs at 284 and 285 nm ($\log \varepsilon = 3.04$ and 3.08)[8]. The fact that λ_{max} for 4-AP electrolyzed even at pH 5 remains at 305 nm supports a slow and incomplete deamination; on the other hand, cytosine, electrolyzed at pH 4.5, has the same ultraviolet absorption spectrum as reduced 2-hydroxypyrimidine[8,36].

Alternative approaches for a 3*e* process must, obviously, provide for deactivation of a free radical, probably by dimerization, and must, in all likelihood, choose between a 2*e* process (which may involve two almost simultaneous 1*e* processes), followed by a 1*e* process or the opposite sequence. The concordance between *cpe* coulometry and *dme* polarography at pH 5 on an *n* of 2 favors a 2*e* process followed by a 1*e* process with the latter not making an appreciable contribution above pH 4, perhaps as a result of a preceding chemical reaction, which produces the reactant for the 1*e* process, not occurring sufficiently rapidly at pH 5 even on the *cpe* time scale. These hypotheses have some support in the slight decrease in coulometric and *dme* *n* at pH 4 as compared to lower pH. There is the further consideration that the *cpe* log *i vs t* plot is linear at pH 0.4 but consists of two linear segments, indicative of a mother–daughter reaction, at pH 4 and 5.

The general agreement below pH 4 of the coulometric and *dme* *n* values at 3 would indicate that deamination to produce a species reducible at the applied potential either occurs sufficiently rapidly to be essentially complete on the *dme* time scale or does not occur sufficiently rapidly to contribute to the total current flow on the time scale of *cpe*. The data support the latter alternative.

The presence of a moderately rapid chemical reaction, coupled to the charge–transfer process, is indicated by the *ac* polarographic and cyclic voltammetric data, and is evident at pH 6 where a complementary anodic peak is seen at high *v* on cyclic voltammetry.

The presence of a preprotonation reaction due to only protonated 4-AP being electrochemically reducible in aqueous media, is indicated by the pH-dependence of $E_{1/2}$ and, even more, by the decrease in *dme* faradaic current with increasing pH in a pattern characteristic of an acid dissociation coupled to a polarographic reduction. The participation of protons in the potential-determining step in reduction of organic substances seems generally to proceed with a preprotonation step; the simultaneous addition of a proton and an electron is scarcely possible for such processes, *eg* ref.[37]. The protonation rate, if it is rapid enough, does not influence the wave-height, which in this case is controlled by diffusion, but does shift the $E_{1/2}$[37]. The pK_a of 4-AP is 5.7, but it does take up a second proton in strongly acid medium, *eg* pH 0–2[33].

1. *Kinetics of coupled chemical reactions*

For a diffusion-controlled, reversible electron-transfer process with no associated following chemical reaction (reversible or irreversible), a plot of the cyclic voltammetric current function, $i_p/ACv^{1/2}$ *vs* *v* is a horizontal straight line. Such a plot for cytosine[9] shows the current function decreasing at high *v* to a limiting value, which is about 90% of that calculated for a reversible 2*e* reduction; from this plot, k_f for the following chemical reaction was estimated to be $10\ s^{-1}$ and was assigned to deamination of 3,4-dihydro-4-amino-2-hydroxypyrimidine.

From the data 4-AP at pH 6 where the plot of $i_p/ACv^{1/2}$ *vs* *v* is apparently decreasing to a limiting value (Fig. 4), k_f is estimated to be about $2\ s^{-1}$, indicating a moderately slow chemical reaction, *eg* the value of $1\ s^{-1}$ or less estimated for adenine[11].

If the moderately slow follow-up reaction of the previous paragraph is actually due to deamination, it may be possible to estimate a rate constant for an accompanying more rapid follow-up reaction, *eg* free radical dimerization or carbanion protonation, through the cyclic voltammetric i_a/i_c ratio. Unfortunately, there are a number of problems in using this ratio for the apparently complementary peak pair seen at pH 6 at large *v* (Table 3).

At pH 6, the peak Ic current function, $i_p/ACv^{1/2}$, has decreased markedly from the presumed 3*e* level at lower pH, *eg* at $v = 50$ V/s, it is barely at the level characteristic of a 1*e* reaction. It is uncertain how much of this decrease can be ascribed to (a) an initial 1*e* reaction being seen due to a chemical step intervening between it and a subsequent 2*e* reaction being outrun, (b) the out-running of a chemical step between an intial 2*e* reaction and a subsequent 1*e* reaction coupled with incomplete reduction in the initial step, and (c) an overall multielectron process in which only a fraction of the available 4-AP is reduced. The *dme* I_d at pH 6 corresponds to an *n* of about 1.5, which is in line with

the decrease in I_d starting at pH 4 (Table 1; Fig. 3).

If i_c at pH 6 for v of 20 and 50 V/s is assumed to result from 1*e* reduction to a free radical or radical anion whose oxidation (or that due to a very rapidly produced rearrangement product) is reflected in i_a, the rate constant for an intervening dimerization, k_2, can be approximated from the i_a/i_c ratio for a reversible charge–transfer followed by dimerization[38]. Unfortunately, the latter reference does not include data for peak ratios as low as the 0.086 and 0.167 calculated from the Table 3 data. However, a lower limit for k_2 can be estimated by using the lowest ratio of 0.436 given in Fig. 4 of ref.[38], which corresponds to $\log(k_2 C_0^* \tau) = 1.00$. For a 5×10^{-4} M 4-AP solution and a switching time (τ) of less than 6 ms, k_2 would exceed $3 \times 10^6\ M^{-1}s^{-1}$. Such a rapid dimerization would be in accord with that of the pyrimidine radical anion in acetonitrile ($8 \times 10^5 M^{-1}s^{-1}$)[39] and the exceedingly rapid dimerizations of pyrimidine and 2-hydroxypyrimidine free radicals in aqueous media[8, 40].

2. *Origin of cyclic voltammetric peaks*

Peaks Ic and IIc originate from the same reduction processes which produce *dme* waves Ic and IIc. Peak Ia, which first appears at pH 6, can be ascribed to oxidation of a free radical species produced on 1*e* or 3*e* reduction of 4-AP. It is unlikely to be due to oxidation of a hydrodimer, which is normally oxidized only at much more positive potentials. The appearance of this anodic process only at pH 6 may be related to the apparent desorption of the wave Ic product at pH 6 (*cf. ac* polarographic data); lack of adsorption of a hydrodimer might sufficiently slow down the rate of free radical dimerization so that oxidation of a fraction of the radical can be observed.

The 80 mV difference in peak potentials Ic–Ia would seem to indicate a redox couple; a 60 mV difference is expected for a reversible 1*e* couple. Formation of a free radical species in peak Ic at pH 6 ($E_p = -1.44$ V) is supported by the observation of $E_p = -1.38$ V for the 1*e* reduction of 4-AP in DMSO at a 1:1 ratio of 4-AP:$HClO_4$[41, 42]. Peak IIIc at -1.32 V appears only after the emergence of peak Ia and, accordingly, must be assigned to reduction of a species which is formed as a result of chemical transformation of the peak Ia product.

If deamination is assumed to occur, the free radical formed at pH 6 by 1*e* or 3*e* reduction of 4-AP (peak Ic) would probably be oxidized (peak Ia) in near neutral solution to form a dihydropyrimidine, which, after deamination, is converted to pyrimidine. The peak couple IIIc–IIIa at -1.32 and -1.26 V could then be due to 1*e* reduction of pyrimidine and oxidation of the resulting free radical. The peak IIIc potential is reasonably close to the -1.27 V observed for pyrimidine peak IIIc at pH 6[19]; however, a corresponding anodic peak has never been observed for pyrimidine at pH 6 even at high scan rate[19, 39, 40]. Furthermore, the deamination rate is rather slow at pH 6. Consequently, it is necessary to conclude that peak couple IIIc–IIIa is due to a species formed by rearrangement which likely involves ring fission. In this connection, it was considered that a chemical reaction other than dimerization might be involved in the disappearance of the pyrimidine radical anion formed on 1*e* reduction in acetonitrile[39].

Wave IIc characteristics

It is difficult to describe the innate characteristics of the electrolytic process or processes involved in the production of wave IIc. Its occurrence only in rather acidic solution (pH < 2) and its decrease in magnitude with increasing pH support involvement of a protonated species, but the relatively slight variation in $E_{1/2}$ with pH [equation (3)] indicates that any protonation–deprotonation reaction is insufficiently coupled to the electron-transfer reaction to affect the latter's energetics; this may be due to saturation protonation of the reacting species since, even at pH 2, the proton activity is over an order or magnitude greater than that of 4-AP or derived species. Cyclic voltammetry indicates that IIc is due to a short-lived species, *eg* a free radical which dimerizes in preference to being reduced.

The apparent n values for IIc, based on *dme* I_d, is *ca.* 3 at pH 0.4 and 1 at pH 1.8 (Table 1) and, based on *cpe* coulometry, is *ca.* 2 at pH 0.4 (Table 4); unfortunately, the possible presence of current resulting from catalytic hydrogen ion reduction connected with the wave I reduction product introduces an uncertainty in the n data. The catalytic character of wave IIc is confirmed by its $E_{1/2}$ dependence on the ionic strength; on increase of ionic strength from 0.5 to 3.0 M at pH 0.4, wave IIc shifts negatively and is finally screened by background discharge whereas wave Ic is unaltered at both pH 0.4 and 6.0 by ionic strength increase. The negative shift of a wave with increasing depolarizer concentration and increasing ionic strength is usually associated with a catalytic hydrogen wave. However, the temperature and h dependencies of wave IIc indicate predominantly diffusion control. Such behavior is also seen with adenine in aqueous buffered media[10], where catalytic hydrogen discharge is involved.

Nature of the reduction products

Two products have been isolated from the macroscale electrolysis of 4-AP, one of which does not absorb in the ultraviolet whereas the other absorbs strongly in the 225 and 305–310 nm regions. The following discussion is concerned only with the latter product, which is essentially the same within the pH range of 0.4–5 as indicated by the constancy of the spectrum and of the ratio of ε_{305} to ε_{225} both at pH 0.4, where the protonated form is being observed, and at pH 7, where the neutral form of the product or a derived species is probably present (Table 5). This product is ascribed to hydrodimers formed following 1*e* reduction of 4-AP.

At pH 6 and 7, the product seems to undergo ring fission, probably as a result of rearrangement with hydrolysis and oxidation being other possible factors. This is suggested by several considerations, *eg* chemical examination of the reduced solution, appearance of an anodic peak on cyclic voltammetry and nonconversion to 4-AP on irradiation.

Deamination of the product does occur at pH 4 and 5, but not at pH 0.4. However, the failure of solutions

electrolyzed even at pH 5 to show appreciable absorption at 285 nm, which is characteristic for solutions of reduced pyrimidine[8], indicates that deamination proceeds rather slowly and does not result in products involving appreciable contributions to the faradaic current or to the ultraviolet spectrum. Apparently in solution whose pH approaches or exceeds pK_a, slow deamination of the electrolysis product occurs as shown by positive Nessler tests. However, since dimerization is far more rapid than deamination, the product initially produced in such solutions is probably similar to that produced in more acidic media.

Formaldehyde and a diazotizable amine, which are possible products of ring fission, are not produced; however, an aldehyde group may be present. The presence of the latter and of two tetraphenylborate reactive nitrogens per original 4-AP molecule are supportive of ring opening by hydrolysis at the 1,2 N–C bond. Such ring opening reactions have been frequently encountered with pyrimidines[43] and purines[44]. Ring opening can also occur as a result of ring–chain tautomerization.

An important property of reduction product II is its sensitivity to photodissociation to monomer following irradiation at 254 or 350 nm. This transformation is accompanied by decomposition as indicated by paper chromatography. Comparison of the apparent ε of *ca.* 9000 for dimeric reduction product II (λ_{max} = 308 nm) after complete irradiation with ε of 20000 for 4-AP (λ_{max} = 296 nm) indicates that less than 50% of product II has been transformed into monomer.

1. *Dimer structure*

The initial 1*e* nucleophilic attack on the pyrimidine ring results in reduction of the 3,4 N=C bond and formation of the 4,4′ dimer (or, preferably, hydrodimer) by the free radical produced[3, 8].

Since reduced pyrimidines are more basic than the unreduced species[45] and since the hydrodimers produced in the present study are generally protonated, protonation may be at least partially responsible for the shift to longer wavelength on ultraviolet absorption (Table 5). A similar shift is seen for the cationic form of 2,2′-bipyridyl[46], *ie* λ_{max} are 233 and 278 nm for the neutral form and 242 and 302 for the protonated form; the ε ratio for the long wavelength form to short wavelength form for the protonated species is 2.18, similar to the ratio for the reduced 4-AP forms (Table 5).

Molecular models confirm the relatively rigid structure of the 4,4′ hydrodimers of 4-AP resulting from steric hindrance by the amino groups. The resulting close to planar configuration of the two rings can influence the ultraviolet spectrum. The 1*e* reduction product of 4-AP in DMSO also absorbs at 300 nm [41].

The fact that in neutral medium, where the hydrodimers should be unprotonated, they have the same ultraviolet spectra but are not converted to the parent 4-AP on photometric irradiation, supports a rearrangement of the dimers on loss of protonation.

Reduction of the 1,2 N=C bond, following reduction of the 3,4 N=C bond, can result in a free radical which forms a 2,2′ dimer.

Mechanistic pattern

After allowance is made for reduction product alteration and catalytic hydrogen discharge, the reaction scheme outlined in Figures 7 and 8 seems to fit best the available experimental data for the elec-

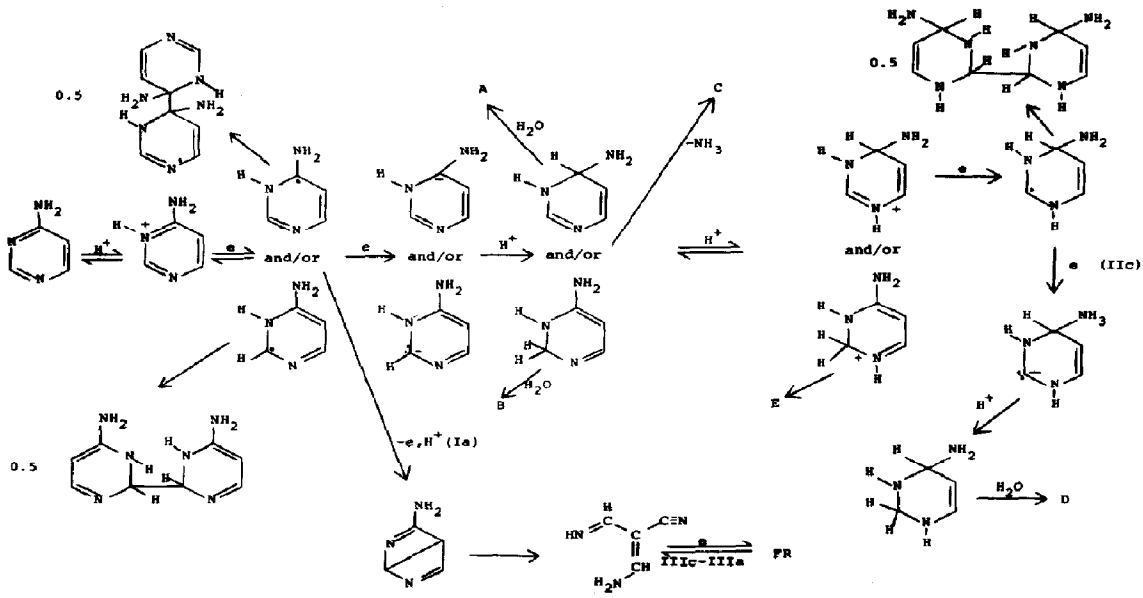

Fig. 7. Proposed reaction pathway for the electrochemical reduction of 4-aminopyrimidine in aqueous media. Some of the species shown can exist in resonant, tautomeric and/or isomeric forms, as well as in protonated and deprotonated states. Upper case letters A through E refer to the reaction path continuations in Fig. 8. FR indicates a free radical.

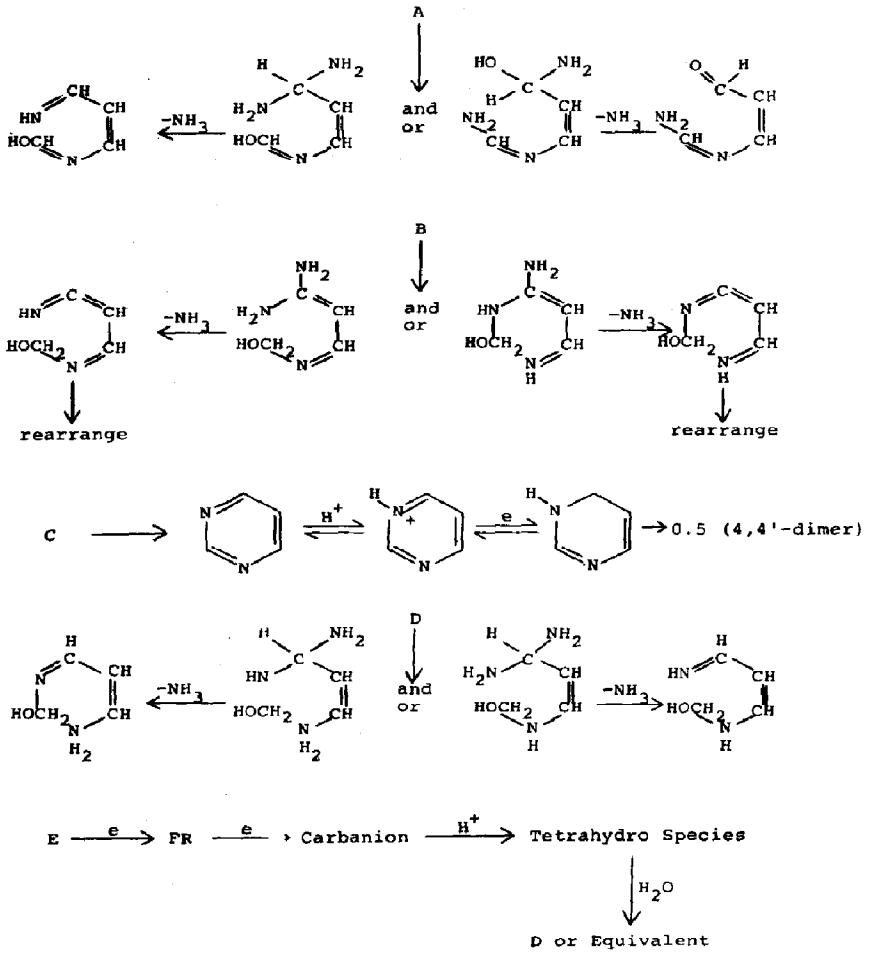

Fig. 8. Proposed reaction pathway for the electrochemical reduction of 4-aminopyrimidine in aqueous media: continuations of path branches in Fig. 7 as indicated by upper case letters A through E.

trochemical reduction of 4-AP as well as previously well-established patterns for the electrochemical reduction of pyrimidines and purines[3–5].

Protonation of 4-AP occurs at N(3)[47]; this has been confirmed by theoretical calculations[48]. However, the most recent nmr studies[49] on 4-AP derivatives have indicated that 94% of the 6-methyl derivative is protonated at N(1). Consequently, the presence among the reduction and decomposition products of 4-AP of a compound similar to the 2,6-dimethyl-4-aminopyrimidine photochemical transformation product, may indicate that, under the conditions used in the present study, a small fraction of the 4-AP is protonated at N(1).

The 4-AP itself is reducible only in the protonated state; consequently, the initial potential-controlling 1*e* reduction (peak Ic) is preceded by a reversible protonation. The free radical produced can (a) dimerize (probably largely to the 4,4′ species), (b) be reduced by a second electron addition to the 3,4 N=C bond to the carbanion since the potential necessary for the first electron transfer is sufficiently negative to cause further electron transfer and/or (c), above pH 6, be oxidized (peak Ia).

If the proton activity is sufficiently large, *eg* pH $\leqslant$ 3, the carbanion path is favored since the carbanion is rapidly protonated to the dihydropyrimidine, which is, in turn, equally rapidly protonated with the resulting positive species being reduced, as formed, to a free radical which rapidly dimerizes, probably predominantly to the 2,2′ species, since the third electron would effectively reduce the 1,2 N=C bond.

At very high proton activity, *eg* pH $\leqslant$ 2, the free radical resulting from the overall 3*e* addition can, prior to dimerization, be reduced at more negative potential (wave IIc) to a carbanion which is rapidly protonated to form the tetrahydropyrimidine (1,2,3,4 species?). The latter species hydrolyzes, opening the ring, probably at the 1,2 position. The reduction involving the fourth electron addition is accompanied by catalytic hydrogen discharge.

At pH 4 and above, the dihydro compound resulting from 2*e* reduction can deaminate at least to some extent to regenerate the most readily reduced pyri-

midine 3,4 N=C bond with the resulting species, after protonation, being reduced (1*e* process) to a free radical which dimerizes, probably to the 4,4' species. At the same time, the dihydropyrimidine can hydrolyze with consequent ring opening; a variety of products are possible with, for example, an aldehyde being formed due to deamination. The relatively slowness of deamination of the dihydroaminopyrimidine as compared to its hydrolysis is indicated by lack of appreciable absorption at 285 nm as would arise from the presence of dihydropyrimidine or the corresponding hydrodimer[8].

Oxidation at pH 5 and above of the free radical produced on the initial 1*e* reaction does not seem to regenerate 4-AP *per se* but an isomeric transitory species which undergoes ring opening to species which can form a reversible redox couple (peak IIIc–IIIa).

At least some of the various reduction products can undergo hydrolytic, rearrangement and/or oxidation reactions at differing rates, *eg* photolytic irradiation regenerates 4-AP only on freshly electrolyzed solutions.

Acknowledgments—The authors thank the National Science Foundation, which helped support the work described, and Dr Tamotsu Wasa, who synthesized the 4-aminopyrimidine and made the preliminary experiments on that compound. Supplementary studies were supported by the Polish Ministry of Science, Technology and Higher Education, in connection with which B.C. thanks Mr Powel Przybora for technical assistance.

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