[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MICHIGAN, ANN ARBOR, MICH.]

Electrochemical Reduction of Pyrimidine, Cytosine and Related Compounds: Polarography and Macroscale Electrolysis

BY DAVID L. SMITH AND PHILIP J. ELVING

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The electrochemical reduction of pyrimidine and certain derivatives has been investigated over the normal *p*H range by polarography, coulometry and macroscale electrolysis. Reduction products were examined chemically, polarographically and spectrophotometrically. Pyrimidine is first reduced at the 3,4-position in a 1*e* process to a free radical, which may dimerize or be reduced (1*e* process) to 3,4-dihydropyrimidine; the latter can be further reduced (2*e* process) to tetrahydropyrimidine. The 2-amino- and 2-amino-4-methylpyrimidines are reduced in two 1*e* steps, first to a free radical and then to the 3,4-dihydro derivative. 4-Amino-2,6-dimethylpyrimidine gives a single 4*e* wave, which likely involves 2*e* reduction to the 3,4-dihydro compound followed by deamination to 2,6-dimethylpyrimidine, which is reduced (2*e* process) to 2,6-dimethyl-3,4-dihydropyrimidine. Cytosine (4-amino-2-hydroxypyrimidine) undergoes a 3*e* reduction, which probably involves 2*e* reduction of the 3,4-N=C bond, deamination to form 2-hydroxypyrimidine and 1*e* reduction of the latter to a free radical which dimerizes. 2-Hydroxypyrimidine itself shows only 1*e* reduction. The dihydro and tetrahydro products are unstable in aqueous solution; pseudo-first-order rate of disappearance is shown by the two compounds examined. In general, 4-aminopyrimidines having the 3,4-position hydrogenated deaminate readily to generate the reducible 3,4-N=C bond.

The polarography of pyrimidine (I of Fig. 1) and its derivatives has been studied by a number of investigators.[1-5] While the experimental data obtained seem generally good, the interpretations made need to be more firmly supported. In particular, pyrimidine has not been adequately studied in basic solution and the relationships of the current magnitudes for the different compounds over the *p*H range have not been sufficiently correlated.

The most comprehensive study is that of Cavalieri and Lowy,[4] who examined polarographically between *p*H 1.2 and 6.8 pyrimidine and twenty-five derivatives, nine of which were reducible. The reduction was suggested as due to the presence of the N=C—C=C system, *i.e.*, a hydroxy substituent at the 4-position[6] must be in the enol form, having the N=C double bond in the ring; the stability to reduction of isocytosine and other molecules containing the N—CO—C=C system was ascribed to their failure to enolize. It was further suggested that a hydroxy group at the 2-position must be in the keto form for reduction to occur. The mechanism was largely based on the assumption of the first step being a reversible 1*e* process, which was assumed to be indicated by the slope of the familiar log $[i/(i_d - i)]$ *vs.* E plot. Actually, the reciprocal slopes varied as much as 15 mv. from the theoretical 59 mv. expected for a 1*e* reversible reaction at 25°; furthermore, the diffusion current constants in all cases but two indicate that more than one electron is involved in the faradaic process.

Inspection of the data of Cavalieri and Lowy[4] reveals that the behavior of their reducible compounds seems to divide them into two classes. Those substituted in the 4-position generally gave considerably higher currents and were more difficult to reduce by at least 0.2 or 0.3 v. than the compounds which were unsubstituted at that position. Significantly, all of the compounds unreducible within the available experimental potential range were substituted in the 4-position.

The present more extensive experimental investigation of pyrimidine and certain selected derivatives, which included not only polarography but also electrolysis and coulometry at controlled potential at large, *i.e.*, macroscale, mercury electrodes, with chemical and spectrophotometric examination of the electrolytically reduced solutions, shows the reduction processes to be more complicated than assumed by previous investigators.

Experimental

Pyrimidines from the following sources were used: cytosine and thymine from Nutritional Biochemicals Corp.; pyrimidine and isocytosine from Mann Research Laboratories, Inc.; 2-hydroxypyrimidine from K & K Laboratories; 2-amino-4-methylpyrimidine from Dougherty Chemicals; 4-amino-2,6-dimethylpyrimidine from Fluka AG; and 2-aminopyrimidine (practical grade) from Eastman Organic Chemicals (the latter compound was recrystallized once from water and twice from hot benzene). Elemental and spectrophotometric analysis and chromatographic assay showed the compounds to be of sufficient purity for polarographic study, *i.e.*, the purities exceeded 98–99% and the polarographic waves for each compound failed to indicate any electroactive impurity. Aqueous stock solutions of the polarographically-active pyrimidines were stable, as evidenced by the constancy of their reduction currents with time.

Buffer solutions (Table V) were prepared from analytical reagent grade chemicals. The nitrogen used for deoxygenating was purified and equilibrated by bubbling it successively through alkaline pyrogallol solution, sulfuric acid and distilled water.

The apparatus and procedures used in polarography, macroscale electrolysis including coulometry, spectrophotometry and product treatment were the same as previously described[7] with the following exceptions: (a) only the silver coulometer was used; (b) Triton X-100 was not used in any runs; (c) residual currents in macroscale electrolysis were generally less than 1 ma.; and (d) m values, mg./sec., for the capillaries used were (A) 1.85 at 40 cm. of mercury, (B) 2.18 (40 cm.) and 3.23 (60 cm.), and (C) 1.85 (40 cm.) and 2.82 (60 cm.). The macroscale electrolyses were done

(1) Y. Asahi, *Yakugaku Zasshi*, **80**, 1222 (1960).

(2) E. Palecek, *Naturwissenschaften*, **45**, 186 (1958).

(3) D. Hamer, D. M. Waldron and D. L. Woodhouse, *Arch. Biochem. and Biophys.*, **47**, 272 (1953).

(4) L. F. Cavalieri and B. A. Lowy, *Arch. Biochem. and Biophys.*, **35**, 83 (1952).

(5) K. Sugino, K. Shirai, T. Sekine and K. Ado, *J. Electrochem. Soc.*, **104**, 667 (1957).

(6) Since the pyrimidine nucleus is symmetrical about a plane including C-2 and C-5, alternative numbering systems are possible. The nomenclature used in the references cited has been changed when necessary to conform to the system used in this paper (*cf. Chemical Abstracts*). The hydroxy and amino derivatives are named without regard to keto–enol and amino–imino tautomerism, *i.e.*, the nomenclature does not reflect the actual state of the molecule.

(7) D. L. Smith and P. J. Elving, *J. Am. Chem. Soc.*, **84**, 1412 (1962).

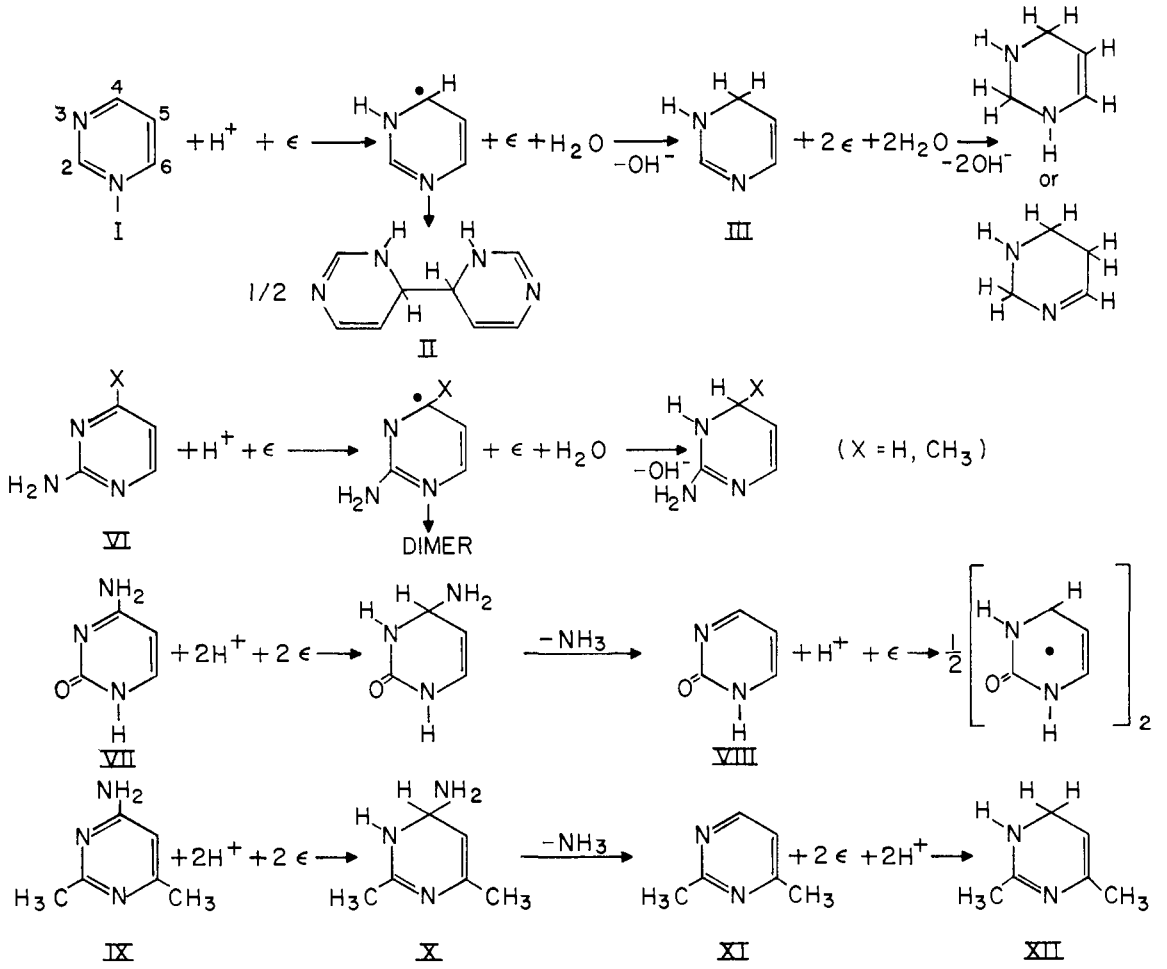

Fig. 1.—Interpretation of the electrochemical and chemical behavior observed for pyrimidine (I), the 2-aminopyrimidines (VI), cytosine (VII), 2-hydroxypyrimidine (VIII) and 4-amino-2,6-dimethylpyrimidine (IX). Protonation, dissociation and other acid–base and keto–enol equilibria are not shown.

at a stirred mercury electrode, whose quiescent area was 24 cm.^{2}.

Presentation of Data.—For convenience in reviewing the experimental behavior of the pyrimidines, the variation of $E_{1/2}$ and diffusion current constant, I, with pH are presented in Fig. 2, and the data on the variation of $E_{1/2}$ with pH are summarized in Table I, the data on polarographic wave slopes in Table II, the data on coulometric n values in Table III, and the data on the ultraviolet absorption maxima of the compounds and their products on macroscale electrolysis in Table IV. (Although wave slopes must be interpreted rather cautiously, they are useful in comparing electrode processes.) The dependences upon drop-time (mercury height) and temperature of the current for the various waves observed indicate all of the waves to be essentially diffusion controlled.

Pyrimidine: Polarography.—Pyrimidine (Fig. 2A) gives a single polarographic wave in highly acidic solution (wave I); a second wave (wave II) of approximately equal height appears at *ca.* pH 3, below which it is masked by the background discharge. Wave I is pH dependent (Table I). Since wave II is essentially independent of pH, the two waves merge near pH 5 to form pH-dependent wave III, whose diffusion current constant about equals the sum of those of waves I and II. Near pH 7.2 wave IV appears; this wave is about equal in height to wave III and essentially pH independent. Since wave III is pH dependent, it ultimately merges with wave IV near pH 9.2 to form pH-dependent wave V, whose height is about twice that of wave III.

The data in acidic solution agree closely with those of Cavalieri and Lowy,[4] who reported "ill-defined waves" for

TABLE I

LINEAR $E_{1/2}$ *vs.* pH RELATIONSHIPS FOR PYRIMIDINE AND DERIVATIVES

Compound	Wave	Approximate pH range	$E_{1/2}$[a]
Pyrimidine	I	0.5–5	−0.576 − 0.105 pH
	II	3–5	−1.142 − .011 pH
	III	5–8	−0.680 − .089 pH
	IV	7–8	−1.600 − .005 pH
	V	9–13	−0.805 − .079 pH
2-Amino-	I	2–3	−0.685 − .049 pH
		4–7	−0.425 − .121 pH
	II	4–7	−1.360 − .004 pH
	III	7–9	−0.680 − .090 pH
2-Amino-4-methyl-	I	2–4	−0.770 − .063 pH
		4–7	−0.550 − .113 pH
	II	5–7	−1.424 − .008 pH
	III	7–9	−0.745 − .094 pH
2-Hydroxy-		2–9	−0.530 − .078 pH
4-Amino-2-hydroxy- (cytosine)		4–6	−1.125 − .073 pH
4-Amino-2,6-dimethyl-		2–8	−1.130 − .073 pH

[a] The equations given indicate the $E_{1/2}$, when extrapolated to zero pH, and the variation of $E_{1/2}$ with pH, *e.g.*, the $E_{1/2}$ of pyrimidine at pH 3, is −0.891 volt.

TABLE II

SLOPES OF THE POLAROGRAPHIC WAVES OF PYRIMIDINES AT 25°

Pyrimidine	*p*H range		Slope = $0.056/(E_{1/4} - E_{3/4})$ [e]
(Pyrimidine)	0.5–5	I	1.3 ± 0.1
	3–5	II	*ca.* 0.6
	5.2–6.8	III	0.6 → 1.4
2-Amino-	2–5[a]	I	1.1 ± 0.1
	5[a]	II	0.9
	7.8–8.0[b]	III	0.9
2-Amino-4-methyl-	2–5[a]	I	0.95 ± 0.05
	8.0[b]	III	0.7
	9.2[c]	III	1.6
2-Hydroxy-	1.7–8.0		1.0 ± 0.1
	9.2, 12.4		1.4
2-Hydroxy-4-amino- (cytosine)	3.7–5.7		0.9[d]
4-Amino-2,6-dimethyl-	2–7		1.0 → 1.4

[a] Slopes of waves I and II above *p*H 5 have little significance due to the proximity of the two waves. [b] McIlvaine buffer. [c] Ammonia buffer. [d] Same values given by a plot of log $[i/(i_d - i)]$ *vs.* *E*. [e] The slopes calculated from this expression should equal n_a, in those cases where the electrode reactions are "reversible." The Roman numerals refer to the waves discussed in the text.

pyrimidine at *p*H 9.2, but did not give data or perform coulometry on any of the waves.

Pyrimidine: Macroscale Electrolysis.—Coulometric *n*-values for all of the waves, determined by large-scale controlled-potential electrolysis (Table III), are directly proportional to the diffusion current constants and equal in magnitude to the values which would be predicted from then, *i.e.*, *n* is 1, 1, 2, 2 and 4 for waves I through V, consecutively. After electrolysis at *p*H 3.7 at a potential corresponding to the crest of wave I, wave II is also eliminated, indicating that the wave I product, which gives wave II under polarographic conditions, is very reactive. This fact along with the 1*e* nature of wave I strongly supports a free-radical mechanism.

Upon complete electrolysis at a potential on the crest of wave III at *p*H 8, wave IV remains unchanged in potential; the wave III product decomposes on standing, but its rate of decomposition cannot be determined by following the rate of decrease of wave IV since the latter is soon obscured by the hydrogen discharge. Apparently, one or more of the decomposition products catalyze hydrogen evolution. The wave I and III products slowly turn yellow on standing even in the absence of oxygen; the wave V product (*p*H 9.2) does not become colored on standing.

Solutions of the wave I and II products, as well as pyrimidine itself, give negative chromotropic acid[8] tests for formaldehyde and Nessler tests for ammonia. A positive formaldehyde test is obtained with the wave V product.

The pyrimidine ultraviolet absorption maxima are eliminated upon electrolysis (Table IV, nos. 1, 2 and 3). The products obtained upon electrolysis at the crests of wave I at *p*H 3.7 and of wave II at *p*H 5.7 absorb; that obtained upon electrolysis at the wave IV crest (*p*H 8) has no absorption maximum. Since these products are unstable, *i.e.*, the ultraviolet absorbance slowly decreases, the reported log a_M values are slightly lower than their true values. This is also true for the wave I and II products of 2-amino- and 2-amino-4-methylpyrimidine (*vide infra*).

2-Aminopyrimidine: Polarography.—The three waves observed for 2-aminopyrimidine (Fig. 2B) correspond in general behavior to those observed for pyrimidine in acidic solution. Wave I is *p*H dependent; wave II is essentially *p*H independent; they merge near *p*H 7.2 to form *p*H-dependent wave III. Below *ca.* *p*H 3.6, wave II is masked by the background discharge. Waves corresponding to pyrimidine waves IV and V are not observed in basic solution.

The $E_{1/2}$–*p*H relation for wave I consists of two linear segments (Table I); the inflection at *p*H 3.6 corresponds closely to the pK_a of 2-aminopyrimidine, 3.54.[9,10,11]

(8) P. W. West and B. Sen, *Z. anal. Chem.*, **153**, 177 (1956).

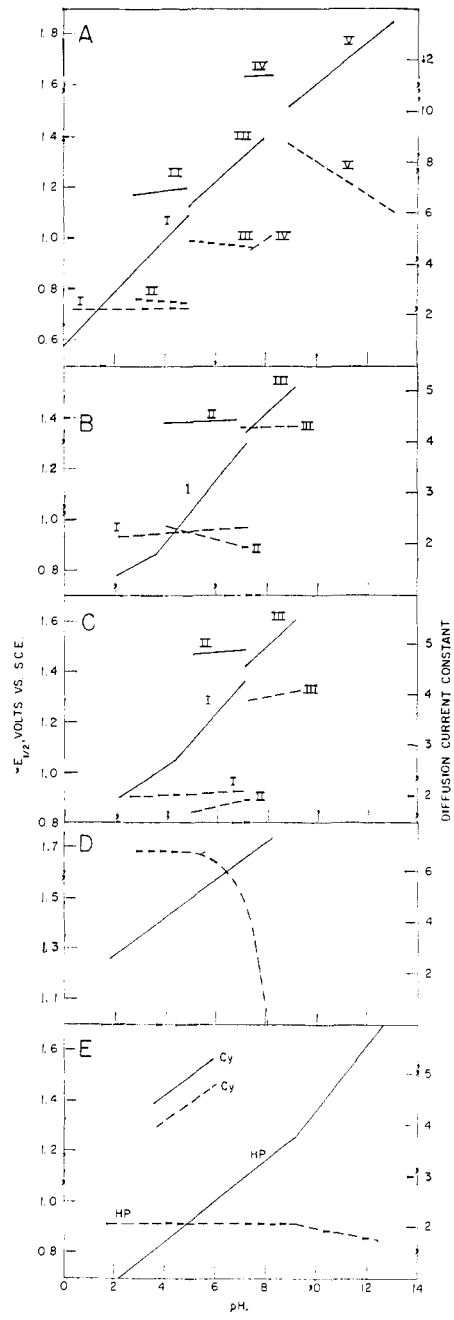

Fig. 2.—Variation of diffusion current constants (dashed lines) and half-wave potentials (solid lines) with *p*H: (A) pyrimidine; (B) 2-aminopyrimidine; (C) 2-amino-4-methylpyrimidine; (D) 4-amino-2,6-dimethylpyrimidine; (E) cytosine (Cy) and 2-hydroxypyrimidine (HP).

2-Aminopyrimidine: Macroscale Electrolysis.—Electrolysis at *p*H 3.7 at a potential on the wave I crest gave an *n* of 0.97 and eliminated wave II, which is fairly well-defined at this *p*H, indicating that the wave I product, which gives wave II under polarographic conditions, is very reactive. This behavior is identical to that observed upon electrolysis of pyrimidine wave I. Electrolysis at *p*H 5.7 on the wave II plateau gave an *n* of 2.07 for the two waves. Consequently, waves I and II are both 1*e* processes.

(9) A. Albert, D. J. Brown and G. Cheesman, *J. Chem. Soc.*, 474 (1951).

(10) A. Albert, R. Goldacre and J. Phillips, *ibid.*, 2240 (1948).

(11) M. P. V. Boarland and J. F. W. McOmie, *ibid.*, 3716 (1952).

TABLE III

COULOMETRIC DETERMINATION OF THE NUMBER OF ELECTRONS INVOLVED IN THE REDUCTION OF PYRIMIDINE AND DERIVATIVES[a]

Concn., mM	Buffer no.	μ, M	pH	Mmoles electrolyzed	$-E_{max}$, v.	n
			Pyrimidine			
			Wave I			
4.06	1	0.25	0.4	0.406	0.87	0.95
4.06	1	0.25	0.4	0.406	0.87	0.93
					Av.	0.94
			Wave II			
3.19	3	0.25	3.7	0.319	1.02	1.01
2.49	3	0.25	3.7	0.249	1.02	1.14
					Av.	1.08
			Wave III			
4.97	3	0.25	5.7	0.497	1.28	1.72
3.32	3	.25	5.7	.332	1.28	2.31
1.66	3	.25	5.7	.166	1.28	1.98
					Av.	2.00
			Wave V			
1.37	5	0.25	9.2	0.137	1.48	3.99
1.37	5	0.25	9.2	0.137	1.48	3.86
					Av.	3.93
			2-Aminopyrimidine			
			Wave I			
2.50	3	0.25	3.7	0.250	1.00	0.97
			Waves I + II			
1.25	3	0.25	5.7	0.125	1.45	2.07
			2-Amino-4-methylpyrimidine			
			Wave I			
1.25	3	0.25	4.7	0.125	1.20	1.19
			Wave III			
1.25	2	0.25	8.0	0.125	1.65	2.14
			2-Hydroxypyrimidine			
1.41	1	0.33	1.3	0.211	0.80	1.04
1.41	3	.17	4.7	.211	1.00	1.10
2.50	5	.25	9.2	.250	1.35	1.06
			Cytosine[b]			
1.25	3	0.25	4.7	0.125	1.45	3.25, 2.72, 3.06
1.25	3	.25	4.7	.125	1.45	3.18, 2.45, 2.75
1.25	3	.25	4.7	.125	1.45	3.26, 2.29, 3.01
1.00	3	.20	4.7	.125	1.45	3.75, 3.23, 3.45
1.25	3	.25	4.7	.125	1.45	3.27, 2.73, 2.95
1.25	3	.25	4.7	.125	1.45	3.43, 2.62, 3.07
					Av.	3.36, 2.67, 3.05

[a] Values of n are corrected for the background electrolyte current. [b] The first value of n in each set is not corrected for background current; the second is corrected for the current blank in the presence of the final concentration of product; the third value is corrected for the average background electrolyte current in the absence of product. The actual n-value, consequently, is between the second and third value since the product concentration is built up during the electrolysis.

The ultraviolet absorption maximum at 299 mμ (Table IV) is eliminated upon electrolysis at the wave I crest and a shoulder appears on the end absorption at *ca.* 250 mμ (log a_M = 3.34 at *p*H 3.8). The product of wave I electrolysis shows strong absorption below 230 mμ, whose λ_{max} and log a_M values could not be determined accurately in *p*H 3.8 acetate buffer. A maximum at 253 mμ also appears upon electrolysis at *p*H 5.7 on the plateau of wave II.

The wave I product gives a weakly positive chromotropic acid test for formaldehyde. Since the intensity of the color produced increases upon standing, the formaldehyde is very likely produced under the conditions of the test, *i.e.*, concentrated sulfuric acid is used. The wave II product gives a negative formaldehyde test. The wave I and II products give negative Nessler tests for ammonia; although a very light yellow precipitate was obtained, its nature and color did not compare favorably with the orange precipitate obtained with an equivalent concentration of NH_4Cl. Since many amines give yellow colors and precipitate with Nessler reagent,[12,13,14] this faintly yellow precipitate was considered to be a negative test for ammonia.

2-Amino-4-methylpyrimidine: Polarography.—The three waves observed (Fig. 2C) correspond to those of 2-aminopyrimidine in general behavior, *i.e.*, *p*H-dependent wave I, *p*H-independent wave II and merger of the two waves to form *p*H-dependent wave III. The diffusion current constants of the three waves are essentially identical to those of the corresponding waves of pyrimidine and 2-aminopyrimidine.

The $E_{1/2}$–*p*H relation for wave I consists of two linear segments (Table I); the inflection at *p*H 4.3 corresponds closely to the pK_a of 2-amino-4-methylpyrimidine, 4.15.[10]

2-Amino-4-methylpyrimidine: Macroscale Electrolysis.—Electrolysis at *p*H 4.7 at a potential on the wave I crest gave an n of 1.19; both waves are eliminated, *i.e.*, the wave I product is very reactive. Electrolysis at *p*H 8.0 at a potential on the wave III crest gave an n of 2.14.

The ultraviolet maxima are eliminated upon electrolysis; the absorption of the products obtained upon electrolysis at the crest of wave I or II resembles that for the product of electrolysis of 2-aminopyrimidine (Table IV).

Negative tests for ammonia and formaldehyde were obtained immediately after completion of electrolysis at the crest of both waves I and II.

2-Hydroxypyrimidine: Polarography.—2-Hydroxypyrimidine gives a single, linearly *p*H-dependent wave (Table I) over the entire *p*H range (Fig. 2E), the magnitude of whose diffusion current constant, which is remarkably constant from *p*H 1.7 to 12.4, indicates a 1*e* reduction.

2-Hydroxypyrimidine: Macroscale Electrolysis.—Electrolyses of 2-hydroxypyrimidine in both acidic and basic solution show the reduction to be a 1*e* process (Table III). The new absorption maximum which appears at 246 mμ (Table IV) decreases with time even in the absence of oxygen; a plot of the log absorbance *vs.* time yields a straight line, indicating that a first-order or, more likely, a pseudo-first-order reaction is occurring. This reaction, which is probably hydrolysis of the 1*e* reduction product of 2-hydroxypyrimidine, has a pseudo-first-order rate constant of 4.2×10^{-4} min.$^{-1}$ at 25° in 0.125 M acetate buffer of pH 4.7.

A polarogram of a non-buffered 0.5 M NaCl solution of 2-hydroxypyrimidine hydrochloride shows two waves; wave I corresponds to compound reduction and wave II to hydrogen ion reduction. After incomplete electrolysis of 1.41 mM 2-hydroxypyrimidine hydrochloride at a potential on the wave I plateau, wave I was reduced 65% and wave II 76%; the silver coulometer indicated that 0.71 Faraday had passed per mole of compound, *i.e.*, 71% reduction based upon the 1*e* nature of the process. In three such experiments the average ratio of the reduction in height of the compound wave to that of the hydrogen wave was 0.89 ± 0.03, *i.e.*, one hydrogen ion is consumed per molecule of 2-hydroxypyrimidine reduced; this observation is also supported by the *p*H change during electrolysis and by the magnitude of the shift of wave I to more negative potential during electrolysis.

The reduced 2-hydroxypyrimidine solution, whether buffered or not, gives no precipitate with tetraphenyl borate and a negative Nessler test, showing that ammonia is definitely not produced during electrolysis. A negative formaldehyde test is also obtained.

(12) M. J. Taras, in D. F. Boltz, "Colorimetric Determination of Nonmetals," Interscience Publishers, Inc., New York, N. Y., 1958, p. 85.

(13) A. Maschka, M. Stein and W. Trauer, *Monatsh. Chem.*, **84**, 1071 (1953).

(14) D. Sugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

TABLE IV

ULTRAVIOLET ABSORPTION DATA FOR PYRIMIDINES AND THEIR ELECTROLYTIC REDUCTION PRODUCTS IN AQUEOUS SOLUTIONS

No.	Substance[a]	Observed pH	Observed Maximum[b] λ, mμ	Observed Maximum[b] log a_M	Literature pH	Literature Maximum λ, mμ	Literature Maximum log a_M
1	Pyrimidine	6.2	243	3.43	7[1]	243	3.5
			238	3.40			
			270	2.55			
2	EP: no. 1 wave I, pH 3.7	3.7	284	3.04			
3	EP: no. 1 wave II, pH 5.7	5.8	285	3.08			
4	2-Aminopyrimidine	3.8	299	3.54	3.0[11]	300	3.58
		6.2	224	4.21	7.0[11]	225	4.04
			291	3.60		292	3.51
5	EP: no. 4 wave II, pH 5.7	5.8	253	3.33			
6	2-Amino-4-methylpyrimidine	6.2	291	3.59	6.8[13]	290	3.59
			225	4.08			
7	EP: no. 6 wave I, pH 4.7	4.7	252	3.19			
8	EP: no. 6 wave II, pH 8.0	8.0	252	3.08			
9	2-Hydroxypyrimidine	4.2	299	3.68	6.1[11]	299	3.66
10	EP: no. 9, pH 4.7	4.7	246	3.28			
11	2-Hydroxy-4-aminopyrimidine (cytosine)	5.2	272	3.84	4.4[14]	272	3.86
					5.0[14]	269	3.82
12	EP: no. 11, pH 4.7	4.7	246	3.23			
13	4-Amino-2,6-dimethylpyrimidine	5.2	247	4.12			
14	EP: no. 13, pH 4.7	4.7	288	3.10			

[a] EP = electrolysis product of compound indicated after electrolysis at crest of wave noted at pH given. [b] Log a_M values for the reduced solutions were calculated assuming a concentration of the reduction product equal to that of the original pyrimidine before electrolysis.

TABLE V

BUFFER AND BACKGROUND ELECTROLYTE SOLUTIONS

Buffer no.	pH	Composition
1	0.4– 2.9	KCl or NaCl + HCl
2	2.2– 8.0	$Na_2HPO_4 \cdot 7H_2O$, citric acid monohydrate + KCl
3	3.7– 5.7	NaOAc + HOAc
4	6.1– 7.0	KCl
5	8.5– 9.2	$NH_4Cl + NH_3$
6	11.0–13.2	KCl + NaOH

Cytosine: Polarography.—Cytosine (2-hydroxy-4-aminopyrimidine) does not give a polarographic wave between pH 1.2 and 2.9 (chloride buffer). A fairly well-defined wave, which lies close to the background discharge, is observed in pH 3.7 to 5.7 acetate buffer; with increasing pH, $E_{1/2}$ becomes more negative (Table I) and the diffusion current constant increases slightly (Fig. 2D).

In McIlvaine buffer the cytosine wave is very poorly defined, but a definite inflection is observed on the background discharge between pH 4 and 8; this wave is very difficult to measure; first-derivative polarography was of no assistance in resolving it.

Cytosine: Macroscale Electrolysis.—Electrolysis in pH 4.7 acetate buffer shows the cytosine reduction to be a *3e* process (Table III). The average background current in the presence of 1.25 m*M* reduced cytosine was 1.53 ma. at −1.45 v., compared to 0.63 ma. for the background electrolyte alone. Consequently, the coulometric results were corrected for both background currents; both sets of results confirm the *3e* nature of the process. (The background current on completion of the cytosine reduction was determined separately for each electrolysis.)

The ultraviolet absorption maximum, which appears at 246 mμ upon electrolysis, decreases with the passage of time even in the absence of oxygen, yielding a linear log absorbance *vs.* time plot; the pseudo-first-order rate constant, calculated from this plot, is 4.3×10^{-4} min.$^{-1}$ at 25° in 0.125 *M* acetate buffer of pH 4.7, which is identical to that obtained for the 2-hydroxypyrimidine reduction product.

The reduced cytosine solution gives a positive Nessler test for ammonia and a precipitate with tetraphenyl borate (TPB), whereas the same concentration of cytosine treated identically (stirred over mercury in coulometer cell), but not electrolyzed, does not. The weight of TPB precipitate, assuming it to be NH_4TPB, corresponded to one NH_3 per cytosine molecule. The reduced cytosine solutions gave a negative formaldehyde test with chromotropic acid, as did cytosine itself.

4-Amino-2,6-dimethylpyrimidine: Polarography.—4-Amino-2,6-dimethylpyrimidine gives a single wave in acidic and slightly basic solution; its diffusion current constant is 6.8 ± 0.1 between pH 2 and 5 but decreases to zero with increasing pH (Fig. 2D). A *4e* reduction is indicated on comparing the value of 6.8 to the diffusion current constant (*ca.* 4.8) for the *3e* cytosine reduction. The linear pH dependence of $E_{1/2}$ is identical to that observed for cytosine (Table I).

4-Amino-2,6-dimethylpyrimidine: Macroscale Electrolysis. After reduction by controlled potential electrolysis, a new ultraviolet absorption maximum appears at 288 mμ (Table IV) and the solution gives a positive Nessler test for ammonia and a negative formaldehyde test.

Isocytosine.—Isocytosine (2-amino-4-hydroxypyrimidine) does not give a wave in any of the buffers listed in Table V.

Thymine.—Thymine (2,4-dihydroxy-5-methylpyrimidine) does not give a wave in any of the Table V buffers or in 0.05 *M* tetramethylammonium bromide solution. Since thymine is resistant to chemical reduction with zinc and HCl,[3] it is not surprising that it cannot be reduced under normal polarographic conditions.

Mechanism of Pyrimidine Reduction

Effect of Substituents.—Before considering the evidence for the proposed mechanisms of electrochemical reduction (Fig. 1), the effects of substituents on the reduction will be summarized. The ease of reduction of a pyrimidine generally decreases with the number of added amino and hydroxy substituents, whose effect seems to involve saturation of the ring by means of tautomeric shifts, thereby removing possible reduction sites, *i.e.*, double bonds, in the ring. None of the previously polarographically studied[4] pyrimidines which are tri- and tetrasubstituted with tautomeric groups are reducible; the 2,4-disubstituted compounds are also either non-reducible or difficult to reduce. Pyrimidines having a 4-amino or 4-hydroxy substituent are more difficult to reduce than

the corresponding 2-substituted compounds; a hydroxy-substituted compound is somewhat more difficult to reduce than an amino-substituted one, *e.g.*, at *p*H 2.3 $E_{1/2}$ values for 2-hydroxy- and 2-aminopyrimidine are −0.83 and −0.79 v., while the values[4] for the corresponding 4-substituted compounds are −1.25 and −1.23 v. (the difference between the latter compounds increases at higher *p*H). The greater stability of the 4-hydroxy-substituted compounds has also been observed in the purine series[7] and probably accounts for the difference in the ease of reduction of cytosine and isocytosine. Hamer, *et al.*,[3] conclude on the basis of thymine and uracil being resistant to zinc reduction that the presence of oxygen at the 4-position confers some stability to the ring system. Ultraviolet absorption[15] indicates that all 2- and 4-hydroxypyrimidines are largely in the keto form. The keto form of the 4-hydroxy compound removes the N=C—C=C system from the ring and replaces it with an O=C—C=C system, whereas, if the equilibrium favors the imino form of the 4-amino compound, the former system is retained.

As mentioned in the Introduction, pyrimidines, substituted in the 4-position, generally give higher currents than those substituted in the 2-position.

Pyrimidine.—The data do not indicate electrochemical reversibility for any of the pyrimidine waves observed. Since the pK_a of pyrimidine is 1.30[10], a neutral species undergoes reduction over most of the *p*H range investigated; discontinuity in $E_{1/2}$ and I-values is not observed near the pK_a.

Reduction of pyrimidine involves hydrogenation of the nucleus, *i.e.*, the 2*e* wave III (I + II) and 4*e* wave V (III + IV) processes represent the formation of dihydro- and tetrahydropyrimidine, respectively. Although molecular-orbital calculations[16] predict the order of reactivities of the positions to nucleophilic attack to be 2 > 4 > 5 (4 and 6 are identical positions), association of the wave I reduction step with the 3,4-double bond (the 1,6-double bond of the alternative Kekulé structure) is indicated by the fact that substituents added to the pyrimidine ring at the 4-position decrease the ease of reduction, whereas addition of the corresponding substituents at the 2-position have little effect, *i.e.*, a substituent added to the pyrimidine ring at the reduction site should be expected to affect more greatly the ease of reduction than one added to a position which is not involved in the reduction. Association of the wave I process with the 4-position is also supported by the following: (a) N,N′-diphenylacetamidine, which contains the 1,2,5,6-system of pyrimidine, is not reduced polarographically,[17]; (b) the first 2*e* reduction step of purine involves the 1,6-double bond[7]; and (c) the data of Cavalieri and Lowy.[4]

The wave I and II processes each definitely involve one electron. The observation that wave II is eliminated upon controlled-potential electrolysis of wave I supports a free-radical mechanism: *i.e.*, the free-radical produced, since the potential is not sufficient for its further reduction to a carbanion, immediately dimerizes (it may, of course, react with non-reduced pyrimidine, water or some other solution component).

The ultraviolet spectra of the solutions (Table IV) obtained after electrolysis at potentials on the crests of waves I and II are essentially identical in λ_{max} and a_M (calculated on pyrimidine reacted), suggesting the formation of a dimer in the wave I process, which has the same type of conjugation as the wave II (or III) product. If a_M for the wave I product were calculated on the basis of the molecular weight of the dimer, it would be about one-half that of the wave II product based on the molecular weight of dihydropyrimidine. The two double bonds in the dihydropyrimidine produced are probably conjugated; this is indicated from λ_{max} and from the consideration that an isolated C=C or C=N system would be more difficult to reduce than is indicated by the half-wave potentials of waves IV and V. Based on the above evidence, the wave I dimer and the wave II (or III) product are probably 4,4′-bis-(3,4-dihydro)-pyrimidine and 3,4-dihydropyrimidine, respectively (II and III of Fig. 1).

The location of the isolated double bond in tetrahydropyrimidine cannot be stated with certainty, and, in fact, may have little meaning since the product is unstable under the alkaline conditions of its formation. The wave IV reduction involves either "1,2-addition" to the 1,2-(N=C) or 5,6-(C=C) double bond or "1,4-addition" to this system as a whole. Since tetrahydropyrimidine prepared by catalytic hydrogenation in acidic solution[18,19] gave a monobenzoylated derivative, it was assumed to contain C=N unsaturation.[18] There is no reason to postulate that the electrolytic product is identical to that obtained under the conditions of catalytic hydrogenation. Actually, pyrimidine is not catalytically hydrogenated in the alkaline solutions,[18,19] in which electrolytic reduction does occur. The second 2*e* step in the reduction of purine[7] involves the 2,3-double bond (equivalent to the 1,2-double bond of pyrimidine). However, the wave IV 2*e* reduction of pyrimidine differs from the second 2*e* step of purine in being essentially *p*H independent; in addition, the ethylene double bond in purine may be stabilized by the tendency to retain the stable configuration of the imidazole ring.

Although the available evidence does not eliminate the possibility of the 5,6-double bond reduction, the latter is considered unlikely since even conjugated ethylene bonds are difficult to reduce under polarographic conditions[20]; consequently, the tetrahydropyrimidine produced during the wave IV and V processes probably results either from "1,2-addition" to the 1,2-double bond or "1,4-addition" to the 1,2,5,6-system, producing 1,2,3,4- or 2,3,4,5-tetrahydropyrimidine, respectively (IV or V of Fig. 1).

2-Amino- and 2-Amino-4-methylpyrimidine.—In acidic solution the 2-aminopyrimidines (VI of

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Fig. 1) show the same general behavior pattern as pyrimidine; *i.e.*, waves I and II are each 1*e* process, wave II is eliminated upon controlled-potential electrolysis of wave I, and the ultraviolet spectra obtained after electrolysis at the crests of waves I and II are essentially identical. Similar to pyrimidine, the first 1*e* step then involves the formation of a free radical, which probably dimerizes when the potential is not sufficient for its further reduction. At potentials corresponding to wave II, a carbanion is formed, which reacts with water to form the corresponding dihydropyrimidine.

The 2-amino substituted pyrimidines do not undergo the second 2*e* reduction, which in the case of pyrimidine very likely involves the 1,2-double bond. This is in agreement with the observation that an amino group decreases the ease of reduction when substituted at the reduction site.

The wave I slopes of both compounds (Table II) agree closely with the theoretical value for a 1*e* reversible reaction. The $E_{1/2}$–*p*H plots (Table I) show inflections very close to the corresponding pK_a values; the slope of each segment is in general agreement to the 0.059 and 0.118 v. expected for one and two hydrogen ions, respectively, taking part in a 1*e* reversible reduction; *i.e.*, at *p*H below the pK_a, where the compound is largely protonated, the $E_{1/2}$–*p*H relationship indicates that only one additional hydrogen ion is involved in the reduction process, whereas above the pK_a two hydrogen ions are involved.

The dihydro derivatives, as well as the dimers formed by the free radicals produced in the 1*e* reduction of both compounds, are unstable as evidenced by the gradual decrease in absorbance with time. Sugino, *et al.*,[5] who electrolyzed 2-aminopyrimidine at mercury and lead cathodes, also found the dihydro derivative to be unstable; however, they reported that catalytic hydrogenation of the latter gave a stable tetrahydro derivative.

2-Hydroxypyrimidine.—A single 1*e* reduction wave is observed for 2-hydroxypyrimidine (VIII of Fig. 1) over the normal *p*H range; the available potential is apparently insufficient to reduce the free-radical produced. Such stability must result from the presence of the hydroxy group, which may be in keto–enol equilibria with both nitrogens of the ring.

Cytosine.—The ultraviolet λ_{max} values of the reduction products of cytosine and 2-hydroxypyrimidine are identical with the cytosine product having apparently the smaller a_M value (Table II). However, both products are unstable and the cytosine electrolysis takes longer than that of 2-hydroxypyrimidine; the a_M values are virtually identical when corrected for the elapsed time. The identity of the spectra, along with the fact that ammonia is produced during the cytosine reduction, constitutes good evidence for the two products being identical. Further evidence is provided by the hydrolysis (or decomposition) rate of the two products; the pseudo-first-order rate constants are identical within experimental error.

The formation of identical products requires the elimination of ammonia during the cytosine reduction (VII of Fig. 1), which likely involves a 2*e* reduction at the 3,4-double bond followed by deamination at this position resulting in the formation of 2-hydroxypyrimidine, which immediately undergoes a 1*e* reduction. This accounts for the over-all 3-electron nature of the process.

4-Amino-2,6-dimethylpyrimidine.—The ease of reduction of this compound (IX of Fig. 1) and its $E_{1/2}$ dependency on *p*H are almost identical to those observed for cytosine, which illustrates that a hydroxy substituent at the 2-position has little effect upon the ease of reduction. This compound undergoes a 4*e* reduction in acidic solution, the first step being 2*e* reduction of the 3,4-double bond; since ammonia is formed during electrolysis, the resulting dihydropyrimidine (X) very likely deaminates to 2,6-dimethylpyrimidine (XI), which then undergoes a further 2*e* reduction at the potential of its formation to 2,6-dimethyl-3,4-dihydropyrimidine (XII). The ultraviolet spectrum of the solution obtained upon electrolysis of 4-amino-2,6-dimethylpyrimidine is very similar to that of the solution from the 2*e* reduction of pyrimidine (Table IV, nos. 14 and 3), which supports the deamination mechanism.

If the deamination mechanism for cytosine and 4-amino-2,6-dimethylpyrimidine is correct, reduction of 4-aminopyrimidine between *p*H 3 and 7 should involve four electrons and result in the same product as obtained from the reduction of pyrimidine.

The reduction of 4-amino-2,5-dimethylpyrimidine is a 3*e* process (diffusion current constant = 4.8) resulting in the formation of ammonia[1]; this further supports a deamination mechanism in the case of the 4-amino compounds. Although the reduction was attributed to a "proton adduct" of the compound, it appears more likely that a 2*e* reduction takes place at the 3,4-position followed by deamination. The resulting 2,5-dimethylpyrimidine then probably undergoes a 1*e* reduction at the potential of its formation.

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