JOURNAL
OF THE AMERICAN CHEMICAL SOCIETY

VOLUME 95, NUMBER 4 FEBRUARY 21, 1973

Cytosine Nucleoside–Nucleotide Series. Electrochemical Study of Reduction Mechanism, Association, and Adsorption

James W. Webb, Borek Janik, and Philip J. Elving*

Contribution from the University of Michigan, Ann Arbor, Michigan 48104. *Received February 26, 1972*

Abstract: Similarities and differences in the mechanisms for electrochemical reduction of the series of cytosine, cytidine, CMP, and CpC have been considered in terms of their structure, including kinetics of intervening chemical reactions, association in solution, and adsorption at the charge-transfer interface and association in the adsorbed state. The basic reduction pattern is as follows (rate constants for cytosine are in parentheses): rapid protonation at N(3) to form the electroactive species, two-electron reduction of the 3,4 N=C double bond to form a carbanion, protonation of the latter (5×10^4 sec^{-1}), deamination to regenerate the 3,4 N=C bond (10 sec^{-1}), and protonation and one-electron reduction at the latter site to form a free radical which dimerizes. The corresponding rate constants for CMP are 5×10^4 sec^{-1} for protonation of the carbanion and 3 sec^{-1} for deamination. CpC does not deaminate under the experimental conditions. Cytidine and CMP adsorb at the interface more strongly than cytosine, indicating the influence of the ribose and ribosophosphate groups; CpC is very strongly adsorbed, suggesting the presence of more adsorption sites and possible ring interactions when it is in the stacked configuration. Adsorbed cytosine, cytidine, and CMP do not associate at 1 m*M* solution concentration; CpC associates even below 0.05 m*M*. CpU and CpG associate more strongly than CpC. The principal mode of association is probably that of vertical overlapping or stacking of bases.

We are currently concerned with the electrochemical investigation of the behavior of nucleic acid components in solution and at the electron-transfer interface. The solution behavior includes (a) the "shape" of the compounds as reflected in structure, conformation, and association including stacking, (b) association between different compounds, and (c) orientation of the compound as it approaches the solution–electrode interface. The behavior at the interface involves (a) adsorption of the original and product (oxidized or reduced) forms of the different species, (b) association in the adsorbed state, and (c) chemical reactions of reactants, intermediates, and products preceding, accompanying, and following electron transfer, *e.g.*, protonation and free radical reactions.

Of the nucleic acid components, there have been numerous investigations of the adenine series, while little work has been done on cytosine derivatives although they are also polarographically reducible.[1] Contrasting views as to the mechanism for the electrochemical reduction of cytosine have been presented.[2–4] There is agreement that the reduction of cytosine proceeds *via* a two-electron (2e) reduction of cytosine, deamination of the resulting product to produce 2-hydroxypyrimidine, and immediate reduction of the latter, which is more readily reducible electrochemically than cytosine itself. The disagreement is to whether 2-hydroxypyrimidine undergoes a 2e reduction[3] or a one-electron (1e) reduction.[4]

The claim of an overall 4e process was based on comparison of polarographic wave-heights for cytosine and compounds of known probable faradaic *n* values, but not for 2-hydroxypyrimidine; no diffusion current constant (*I*) or coulometric *n* data were presented. Macroscale electrolyses of cytosine were made at pH 7.0 and 6.8, where the cytosine wave is very poorly defined. The fact that a dimer could not be found, as judged from the absence of a 1,2-diol group in the electrolysis product, may be due to dimerization in positions other than 4,4′ (the 4,4′ dimer is prerequisite for the 1,2-diol formation).

The bases for the claim[4] of an overall 3e process were (a) coulometric *n* values of 3 for cytosine on electrolysis at pH 4.7, where cytosine gives a fairly well defined wave, and of 1 for 2-hydroxypyrimidine on

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

(2) B. Czochralska and D. Shugar, private communication.

(3) B. Janik and E. Paleček, *Arch. Biochem. Biophys.*, **105**, 225 (1964).

(4) D. L. Smith and P. J. Elving, *J. Amer. Chem. Soc.*, **84**, 2741 (1962).

Table I. Summary of Dc Polarography of Cytosine and Its Nucleosides

Compd	pH	Medium[a]	μ, M	Compd concn, mM	Temp, °C	Wave	$-E_{1/2}$, V	I
Cytosine	4–6[b]	Ac	0.25–0.5	0.5–1.2	25		1.125 + 0.073 pH	4.0–4.8
	4.2	Ac	0.5	0.97	25		1.44	6.3
	4.2	Ac	0.5	0.97	0		1.45	4.4
Cytidine	5.0	Mc	0.13	0.1	0		1.38	4.1
	4.1	Ac	0.5	1.00	25		1.38	6.3
CMP	5.0	Mc	0.13	0.1	0		1.46	3.2
	4.1	Ac	0.5	0.99	25		1.37	4.6
				0.49	0		1.37	3.0
CpC	5.0	Mc	0.13	0.018–0.89	0	I	1.30[c]	5.5–0.4
				0.053–0.89		II	1.39 to 1.49	1.6–5.9
				0.36–0.71		III	1.61	1.3–1.7
	2.5–5.5	Mc	0.5	0.05	25	I	1.040 + 0.041 pH	10[d]
	2.5–5.9	Mc				II	1.085 + 0.056 pH	
	5.9–8.1	Mc + Ca				II	0.800 + 0.104 pH	
	8.1–10.6[e]	Ca				II	1.225 + 0.051 pH	
CpU	4.5	Mc	0.13	0.094	0		1.32	2.8

[a] Ac = acetate buffer; Ch = chloride buffer; Mc = McIlvaine buffer; Ca = carbonate buffer. [b] Reference 4. [c] Average value for all concentrations. [d] Sum of waves I and II in pH 2.5 McIlvaine buffer. [e] $E_{1/2}$ in pH 9.1 ammonium buffer is 65 mV more positive.

electrolysis in both acidic and basic media, (b) I values, and (c) the related facts that pyrimidine itself initially undergoes a 1e reduction to form a free radical which dimerizes, that the three pyrimidines, other than 2-hydroxypyrimidine, investigated,[4] which have only H or CH_3 on the C(4), give initial 1e waves, and that 2-hydroxypyrimidine does not give a second 1e wave to complete reduction of the 3,4 N=C bond.

Recently, Czochralska and Shugar[2] found the electrochemical reduction of 2-hydroxypyrimidine at pH 4.5 and 7.0 to be a 1e process, which gives a nearly quantitative yield of dimer (identified as the 6,6′ species).

The present authors consider that the 3e mechanism for reduction of cytosine, leading to dimer formation, best represents the known facts.

The present investigation of cytosine, cytidine, cytidine monophosphate (CMP), and selected cytosine dinucleoside monophosphates utilized a variety of electrochemical techniques including direct current (dc) polarography, cyclic voltammetry, total and phase-selective alternating current (ac) polarography, and coulometry at controlled electrode potential. These approaches can provide reliable data concerning various aspects of solution behavior and the electron-transfer process, including intermediate species and reactions.

Reduction Mechanisms

The electrochemical behavior of each of the cytosine species supports the hypothesis that reduction of the cytosine moieties occurs by a similar mechanism for all of the species; *e.g.*, except for CpC above pH 7, the limiting current–pH variations are of the same type for the series.[3] In addition, $E_{1/2}$ becomes more negative with increasing pH at a rate which is between 0.07 and 0.08 V/pH for cytosine and cytidine[3,4] and between 0.10 and 0.11 V/pH for deoxycytidylic acid[3] and for CpC (between pH 4 and 8) (Table I). Experimental results obtained in the present investigation are summarized in the subsequent Experimental Section. Typical dc and ac polarograms are given in Figure 1.

Electroactive Form. The presence of the protonated form as the polarographically reducible species is supported by the above-mentioned pH relationships, *i.e.*, the decrease of the limiting current at a pH somewhat greater than the acidic dissociation constant (pK_a) for each cytosine compound. Such a current decrease and the kinetic character of the current in the pH range where it begins to disappear, *i.e.*, protonation as the rate-limiting step, are characteristic of the polarographic reduction of the acid form of a conjugate acid–base system.[5] The complicated variation of the CpC reduction current with pH (Figure 2) is subsequently discussed (*cf.* Reaction Path).

The presence of the protonated form as the electroactive species is in agreement with the proton magnetic resonance and ultraviolet absorption spectra, *e.g.*, ref 1 and 6 to 8, which indicate that the protonated cytosine species is predominant in acidic aqueous solution; any unprotonated cytosine would be rapidly converted to the more readily reducible protonated species as the latter is removed by reduction. The evidence for reduction of other pyrimidines and purines[1] also favors preprotonation in acidic solution and lack of a chemical step intervening between the initial two successive electron additions.

Based on studies of cytosine and of purines, the N(3) position in the cytosine moiety is the most likely protonation site as well as the initial reduction site.[1,4,6–8]

Identification and Kinetics of Accompanying Chemical Reactions. Electrochemical reduction of the cytosine derivatives is an overall irreversible process.[9] The irreversibility is due to a chemical reaction associated with the electron-transfer process as subsequently discussed. Because of the greater availability of data on cytosine and minimal interference from adsorption and association phenomena in the case of this compound, the following discussion on the kinetics of chemical reactions associated with the electron-transfer process will be centered around cytosine itself.

(5) P. J. Elving, *Pure Appl. Chem.*, 7, 423 (1963).
(6) A. Wróbel, A. Rabczenko, and D. Shugar, *Acta Biochim. Pol.*, 17, 339 (1970); W. C. Johnson, P. M. Vipond, and J. C. Girod, *Biopolymers*, 10, 923 (1971).
(7) J. E. O'Reilly and P. J. Elving, *J. Electroanal. Chem.*, 21, 169 (1969).
(8) B. M. Izatt, J. H. Christensen, and H. Rytting, *Chem. Rev.*, 71, 439 (1971).
(9) For example, ac in-phase peak heights are less than 5% of those expected for a reversible, diffusion-controlled process between 50 and 1000 Hz.

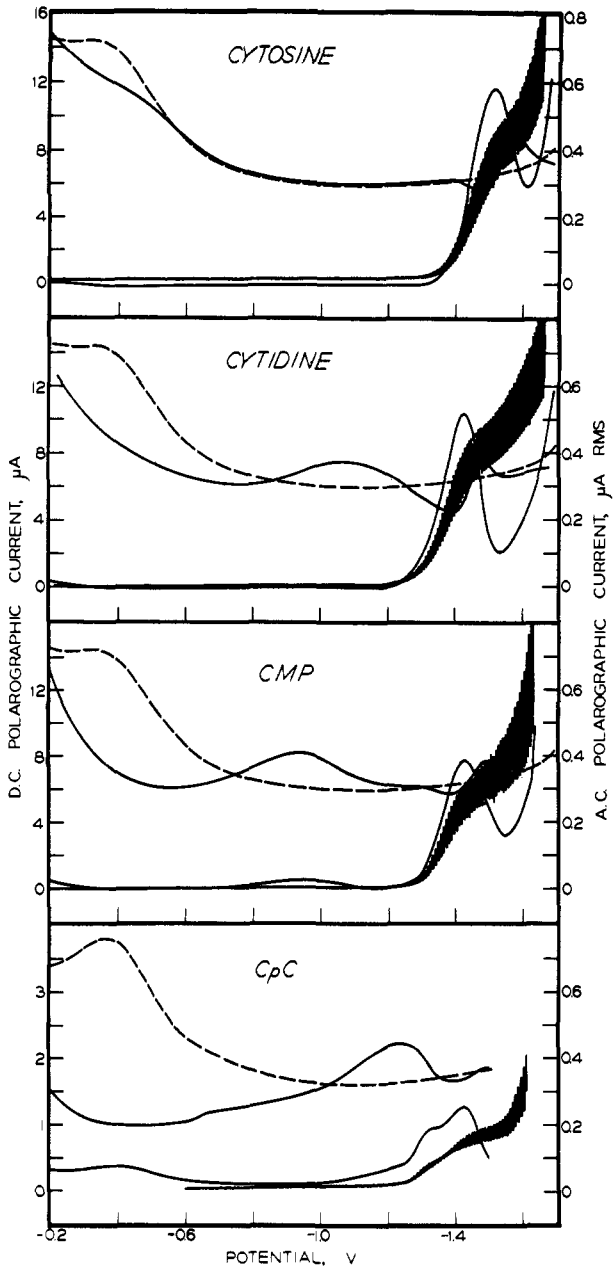

Figure 1. Dc and ac polarograms for cytosine, cytidine, CMP, and CpC. The upper pair of curves in each panel represents the quadrature ac component (dashed line is for background solution alone); the lower single line is for the in-phase ac component. Conditions are 1 m*M* cytosine derivative, pH 4.2 acetate buffer, 25° and 50 Hz, except for CpC, where the conditions are 0.09 m*M*, pH 5.0 McIlvaine buffer and 0.5°. The sensitivity of the in-phase current scale for CpC is four times that shown; *i.e.*, a scale reading of 0.2 μA corresponds to an actual current of 0.05 μA.

Smith and Elving[4] proposed that cytosine undergoes a 3e reduction in pH 3.7 to 5.7 acetate buffer involving 2e reduction of the 3,4 N=C bond, deamination of the product to 2-hydroxypyrimidine (2-HP) and 1e reduction of the latter to a free radical which, under experimental conditions, dimerizes before it can be further reduced. This process can be recognized as a chemical reaction intervening between two electron-transfer steps, which is usually referred to as an ECE mechanism where E corresponds to an electron-transfer reaction and C to a chemical reaction. In general terms, the reaction can be written as follows.

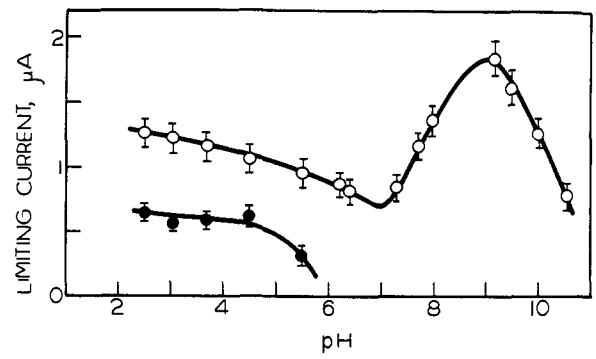

Figure 2. Variation with pH of limiting current of dme waves of 0.058 m*M* CpC in chloride, McIlvaine and carbonate buffers at 25°: normal wave (sum of normal wave and prewave when latter appears), open circles; prewave, solid circles. Estimated maximum experimental error is indicated.

$$C + H^+ \rightleftharpoons O \quad (1)$$

$$O + 2e \overset{k_{s,h}}{\rightleftharpoons} R \quad (2)$$

$$R \overset{k_f}{\longrightarrow} R' \quad (3)$$

$$R' + e \overset{k_{s,h}}{\rightleftharpoons} Z \quad (4)$$

where C represents cytosine, O the electroactive species, R the initial reduction product, R′ the chemically altered form of R, and Z the final reduction product; $k_{s,h}$ is the standard formal heterogeneous rate constant for the charge-transfer steps, and k_f is the rate constant for the homogeneous chemical transformation of R to R′. The rate of the preliminary protonation reaction (eq 1) is very rapid and does not affect the equilibria of eq 2–4.

The theory of cyclic voltammetry for ECE mechanisms has been derived and verified by Nicholson and Shain.[10] If k_f is sufficiently larger than the scan rate (v in V/sec), the peak current (i_p) observed will be due to the first electron-transfer process plus a fraction of the second process depending upon the values of k_f and v. When k_f is small compared to v, *i.e.*, the time scale of the experiment is too short for the chemical reaction to occur to an appreciable extent, i_p is just due to the initial electron-transfer process. A plot of $i_p/ACv^{1/2}$ *vs.* v, where A is the electrode area and C is the concentration, results in a horizontal straight line for a diffusion-controlled reversible electron-transfer process with no associated chemical reaction. Such a plot for cytosine (Figure 3) shows the ratio decreasing to a limiting value at high scan rates, which is over 90% of that calculated for a reversible 2e reduction; this is strongly suggestive of the electron transfer being close to reversible. From the curve of Figure 3, k_f is estimated[11] to be 10 sec^{-1} (this k_f is subsequently identified as k_d).

The magnitude of k_f can also be determined by ac in-phase polarography. Faradaic currents lower than those expected for a diffusion-controlled reversible process can generally be attributed to the intervening

(10) R. S. Nicholson and I. Shain, *Anal. Chem.*, 36, 706 (1964); 37, 197 (1965).

(11) The reaction is assumed to be first order on the basis of the subsequent discussion and by analogy to the behavior of purine and pyrimidine.[14] The calculations are based on the cathodic peak produced on the initial scan.[10]

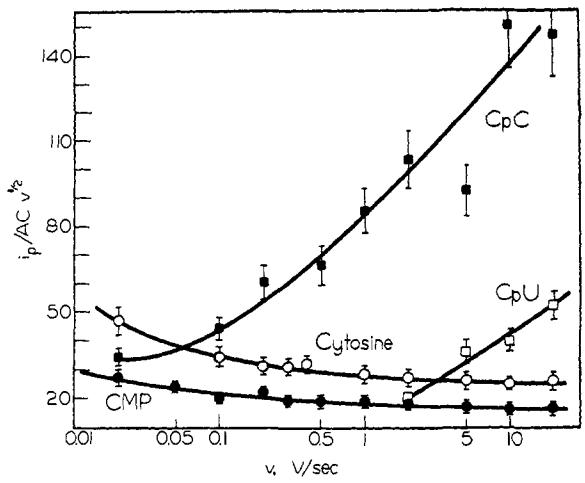

Figure 3. Variation of current function with polarization rate at hmde for cytosine species at 25°. Points are plotted with an indicated error limit of ±10%. Conditions (pH, buffer, ionic strength, and compound concentration): pH 4.1 acetate (0.5 *M*) for cytosine (0.97 m*M*) and CMP (0.99 m*M*); pH 4.5 McIlvaine (0.1 *M*) for CpC (0.088 m*M*) and CpU (0.094 m*M*).

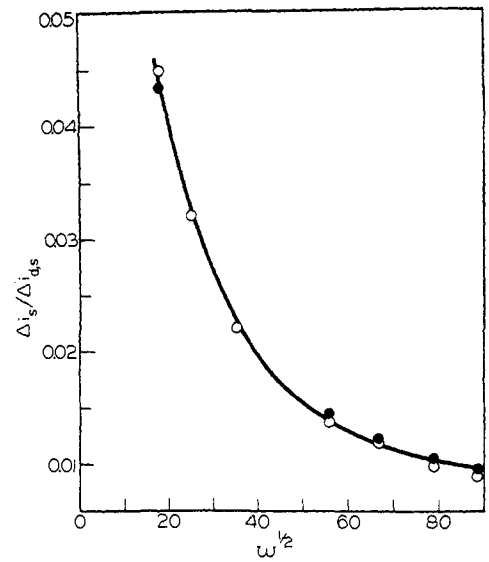

Figure 4. Variation of the ratio of $\Delta i_s/\Delta i_{d,s}$ with frequency for cytosine (open circles) and CMP (solid circles). Conditions are specified in the caption to Figure 3.

chemical reaction assuming that $k_{s,h}$ is large, *i.e.*, the electron-transfer step is faster than the chemical reaction step. When the time scale of the experiment is long enough for the chemical reaction to occur, *i.e.*, when $k_f \gg \omega^{1/2}$, where $\omega = 2\pi f$ and f is the frequency of the applied ac voltage, the ac current will be limited by the value of k_f. However, when $k_f \ll \omega^{1/2}$, the current will be due to the reversible electron-transfer step and becomes dependent only on $\omega^{1/2}$. Thus, a plot of $\Delta i_s/\Delta i_{d,s}$ *vs.* $\omega^{1/2}$, where Δi_s is the observed ac in-phase peak current and $\Delta i_{d,s}$ is the expected current for the diffusion-controlled reversible redox system, reaches a limiting value at high frequencies, from which k_f can be calculated[12] from eq 5 where t is the drop-

$$k_f^{1/2} = \frac{1}{1.387t^{1/2}}\left[\left(\frac{4.78\,\Delta i_{d,s}}{\Delta i_s}\right)^{1/2} - 1\right]^2 \qquad (5)$$

time in seconds. (Although the cytosine reduction involves two chemical reactions intervening between two electron-transfer steps or an ECCE mechanism, an EC mechanism is being effectively observed by ac polarography, since the second chemical reaction is too slow to allow the second electron-transfer step to occur to an appreciable extent; *cf.* subsequent discussion.)

Figure 4 shows such a plot for cytosine ($\Delta i_{d,s}$ was calculated from the equation on page 47 of ref 13 for a 2e process). Since the ratio never reaches a limiting value at high frequencies, it is evident that the rate of the intervening chemical reaction is relatively fast. However, if this limiting value is estimated to be about 0.008, k_f is calculated to be about 5×10^4 sec^{-1} (this k_f is subsequently identified as k_p).

This apparent contradiction between k_f values calculated from cyclic voltammetry and ac polarography can be explained if two chemical reactions occur between the electron-transfer steps with the more rapid reaction occurring first. Since purine and pyrimidine are initially reduced in 2e processes at the equivalent 1,6 and 3,4 N=C bond, respectively, to form carbanions which are then protonated to form the dihydro compounds, and the k_f values for these protonation steps are about 5×10^3 and 5×10^4 sec^{-1}, respectively,[14] this step more than likely also occurs in cytosine and is the faster step based on the identical k_f value obtained for pyrimidine. The second and slower step in the cytosine reduction is, therefore, the deamination reaction to form 2-HP.

To avoid confusion, the rate constant for the carbanion protonation will be designated as k_p and that for deamination as k_d. Thus, k_p for cytosine is 5×10^4 sec^{-1} and k_d is 10 sec^{-1}.

Since ac polarographic studies show that the ratio of $\Delta i_d/\Delta i_{d,s}$ does not reach unity even at frequencies as high as 1250 Hz, the electron transfer reaction must be relatively fast; hence, the irreversibility of the overall electrode process is controlled by the intervening chemical reactions. Thus, both the cyclic voltammetric and the ac polarographic measurements suggest that the initial 2e reduction is close to reversible behavior. For such a situation, the $k_{s,h}$ values for the present situation could be estimated to exceed 0.1 cm sec^{-1}.

Reaction Path. Based on all of the available data, the mechanism outlined in Figure 5 is proposed for the reduction of cytosine. The basis for the essential pattern of the cytosine reduction pathway (2e reduction, rapid deamination, and subsequent 1e reduction) is evident from a consideration of the half-wave potentials and faradaic n values tabulated in Table II. Thus, addition of an amino group at C(4) in pyrimidine (equivalent to C(6) in purine) makes the initial reduction at the 3,4 N=C double bond more difficult, which results in the initial reduction occurring only at sufficiently negative potential to cause the first two reduction steps to merge, *e.g.*, compare pyrimidine and 4-aminopyrimidine, and purine and adenine.

The presence of the 2-hydroxy substituent on py-

(12) G. H. Aylward and J. W. Hayes, *Anal. Chem.*, 37, 195, 197 (1965); T. G. McCord, H. L. Hung, and D. E. Smith, *J. Electroanal. Chem.*, 21, 5 (1969).

(13) B. Breyer and H. H. Bauer, "Alternating Current Polarography and Tensammetry," Interscience, New York, N. Y., 1964, p 47.

(14) P. J. Elving, S. J. Pace, and J. E. O'Reilly, *J. Amer. Chem. Soc.*, 95, 647 (1973).

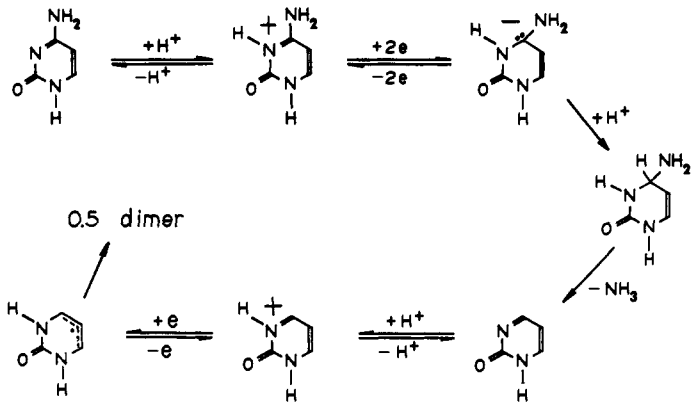

Figure 5. Proposed reaction pathway for the reduction of cytosine in aqueous media.

Table II. Comparative Reduction Patterns for Cytosine and Pertinent Compounds[a]

Compound		pH dependence of $E_{1/2}$,[b] V	n	$E_{1/2}$ at pH 4.2, V
Pyrimidine	I	−0.576–0.105 pH	1	−1.017
	II	−1.142–0.011 pH	1	−1.188
2-Hydroxypyrimidine		−0.530–0.078 pH	1	−0.858
4-Aminopyrimidine	I	−1.13 at pH 1.2	2?	
	II	−1.23 at pH 1.2	1?	
Cytosine (4-amino-2-hydroxypyrimidine)		−1.125–0.075 pH	3	−1.440
Purine	I	−0.697–0.083 pH	2	−1.046
	II	−0.902–0.080 pH	2	−1.238
Adenine (6-aminopurine)		−0.975–0.090 pH	4	−1.353

[a] Data taken from tables in ref 1. [b] Roman numbers refer to the wave sequence.

rimidine removes the 1,2 N=C bond from the ring due to ketonization and causes the initial reduction of the 3,4 N=C bond to occur energetically more easily than in pyrimidine, accompanied apparently by very rapid dimerization of the resulting free radical. The presence of the 2-hydroxy substituent also seems to accelerate the deamination process in the pyrimidine; *e.g.*, the initial reduction wave for 4-aminopyrimidine is apparently only a 2e process. Unfortunately, the behavior of 2-hydroxy-6-aminopurine (isoguanine), whose pyrimidine ring configuration is similar to that of cytosine, cannot be used for comparison due to the fundamental differences in the isoguanine reduction mechanism.[15] However, the deamination process in adenine is quite slow; *e.g.*, a 4e wave appears under the brief time conditions of dme polarography but prolonged large-scale electrolysis yields a faradaic n of 6.[16]

The trend in the behavior of cytidine indicates that its reduction mechanism does not differ significantly from that of cytosine.

CMP more than likely is also reduced by a mechanism similar to that of cytosine except that the rate of deamination reaction, k_d, is slower as evidenced by the lower degree of curvature of the $i_p/ACv^{1/2}$ *vs.* v curve (Figure 3) and the slow electrolysis reaction; k_d is estimated to be 3 sec^{-1}. However, the rate of the protonation reaction for CMP is estimated from Figure 4 to be about the same as that for cytosine.

(15) B. Janik and P. J. Elving, *J. Electrochem. Soc.*, **116**, 1087 (1969).
(16) D. L. Smith and P. J. Elving, *J. Amer. Chem. Soc.*, **84**, 1412 (1962).

Based on an electrolytic n value of four, it appears reasonable that each ring of CpC is reduced by the initial 2e mechanism and that the deamination step occurs very slowly or not at all. Proof of the lack of deamination and product analysis are extremely difficult and the viewpoint just enunciated must be regarded as tentative. However, its reasonableness is supported by the following considerations. (Unfortunately, it is difficult to use cyclic voltammetry and ac polarography to further elucidate the reduction mechanism of CpC due to interference by adsorption as indicated by the rapid increase in the $i_p/ACv^{1/2}$ value with scan rate[17] (Figure 3).) At 25° in neutral or slightly alkaline pH, CpC exists in an equilibrium between stacked single stranded and disordered conformations.[18] Charge-transfer complexes stabilized by van der Waals–London forces were observed[19] in frozen solutions (77°K) of cytidine, CpC, and poly C at pH around pK_a. It is possible that similar complexes, although in much lesser extent and more loosely bound, exist in solutions above 0°C. Due to stacking and possible charge-transfer interaction, the reduction sites in CpC may be partially shielded and, consequently, deamination proceeds very slowly. Higher pH would simulate the deamination. The final decrease and disappearance in the reduction current around pH 10 (Figure 2) can be explained similarly to cytosine, *i.e.*, recombination with H^+ and complete loss of protonated form at high pH.[3] It is also possible that deamination of CpC may be inhibited by strong adsorption of the dihydrocytosine product.

Association in Solution

Pyrimidine nucleosides and nucleotides have been found to associate in aqueous solution by osmotic pressure lowering.[20] However, the concentration shifts of the proton resonances are negligible,[21] indicating that the pyrimidine nucleosides do not support ring

(17) R. H. Wopschall and I. Shain, *Anal. Chem.*, **39**, 1514 (1967).
(18) J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, **25**, 465 (1967).
(19) T. Montenay-Garestier and C. Hélène, *Biochemistry*, **9**, 2865 (1970).
(20) (a) P. O. P. Ts'o, J. S. Melvin, and A. C. Olson, *J. Amer. Chem. Soc.*, **85**, 1289 (1963); (b) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *ibid.*, **89**, 3612 (1967); T. N. Solie and J. A. Schellman, *J. Mol. Biol.*, **33**, 61 (1968); M. E. Magar and R. F. Steiner, *Biochem. Biophys. Acta*, **224**, 80 (1970); M. E. Magar, R. F. Steiner, and R. Kolinski, *Biochem. J.*, **11**, 387 (1971).
(21) M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **87**, 5241 (1965); M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *ibid.*, **90**, 1042 (1968).

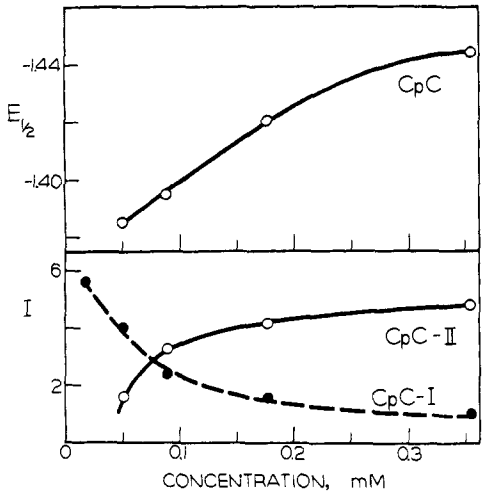

Figure 6. Variation of $E_{1/2}$ and I with concentration of CpC in pH 5.0 McIlvaine buffer (ionic strength: 0.13 M) at 0.5°. Roman numerals refer to the observed waves.

currents as do the aromatic purine bases. Likewise, CpC and CpU show little concentration dependence of chemical shift.[22] In general, purine nucleosides and nucleotides associate to a greater extent than pyrimidine nucleosides and nucleotides.

In purine derivatives, the extent of association increases with concentration and the predominant mode is vertical stacking due to hydrophobic interaction of bases.[23] Since the standard free energy for this association is of the order of the thermal energy, the stacks must break and re-form rapidly, and higher stability can be expected at lower temperatures; *e.g.*, the decrease of the diffusion current constant (I) for the adenine nucleoside–nucleotide series with increasing concentration is about two to three times greater at 1.5° than at 25°, indicating the considerable greater association at the lower temperature.[24] The decreasing reversibility of the electrode process (increasingly negative value of $E_{1/2}$) with increasing concentration is in agreement with additional energy being involved; *e.g.*, association increases the activation energy for charge-transfer kinetics, making the reduction more difficult.

Similar studies on di- and oligonucleotides[22,25] indicate strong intramolecular interactions as well as intermolecular association. The mode of association of dinucleotides is similar to that of the nucleotides.

It is difficult to interpret the I data for the cytosine series because of the concomitant occurrence of adsorption. However, even after maximum coverage of the electrode area with the reduced adsorbed layer has been reached (*ca.* 0.3 mM), $E_{1/2}$ for the CpC reduction continues to become more negative with increasing concentration (Figure 6). Thus, these changes may be due to the presence of intermolecular interactions (*cf.* next paragraph).

Extrapolation of the $E_{1/2}$ *vs.* concentration curves for CpC (Figure 6), and ApC and CpA[25] to zero concentration (and, thus to zero degree of association), results in a more or less common $E_{1/2}$ at about −1.37 V. This value corresponds to $E_{1/2}$ values for adenosine and cytidine of −1.38 V at the pH involved. The increasing differences in $E_{1/2}$ among the three dimers with increasing concentration can be explained in terms of increasing differences in the degree of interaction, *e.g.*, association, with the latter being greatest for CpA, at least at concentrations below 0.2 mM.

Adsorption on Mercury Electrodes

Although the limiting current is essentially diffusion-controlled for all of the cytosine derivatives examined, all of the electrochemical techniques employed strongly support involvement of adsorption phenomena in the electrode process for each cytosine species. The extent of adsorption and nature of the adsorbing molecule depend upon the species involved and its concentration, pH, and the potential of the electrode.

The broad depression in the quadrature base current below background at the potential of the ecm[26] (−0.5 V) for all of the compounds suggests adsorption of an uncharged portion of the molecule.[27] In pH 4 acetate buffer at 25°, the depth of this depression increases in the order: cytosine < cytidine, CMP (Figure 1); based on measurements of double-layer capacity,[30a] the surface activities of these compounds increase in the same order. This indicates that the ribose and ribosophosphate groups strongly influence the adsorption of the molecule through production of additional adsorption sites in the molecule as a result of the added ribose moiety and/or through the effect of the ribose group on adsorption sites in the cytosine ring. The latter possibility has some validity since the above order for the depression depth is the same as that for the increasing ease of reducibility under the same experimental conditions (Table I; ref 3).

As potential becomes more negative than the ecm, gradual reorientation of the molecule occurs as indicated by the broad quadrature peak around −1.0 V. Again, the influence of the ribose and ribosophosphate groups is seen because cytosine does not display this peak (Figure 1). Because of the negatively charged phosphate group, the depression around the ecm and the following peak for CMP are shifted to more positive potentials from those of cytidine.

Depression of the quadrature current at a potential just prior to reduction indicates that the molecule is probably oriented so that the protonated ring nitrogen is closest to the electrode surface. It occurs for all of the compounds studied in the pH 4 acetate buffer at 25° and is compatible with the argument that the protonated form of the molecule is the reducible species.

The dinucleoside phosphates (CpC, CpU, and CpG) adsorb much more strongly than cytosine, cytidine, and CMP (Figures 1, 7, and 8). The depression near the ecm is much deeper for the dinucleoside phosphates and the following reorientation peak is hardly visible for cytosine and its nucleoside and nucleotide at comparable conditions. This increased adsorption is probably due to the presence of more adsorption sites in a dinucleoside phosphate and possible ring interactions when it is in the stacked configuration.[22]

(22) P. O. P. Ts'o, N. S. Kondo, and M. P. Schweizer, *Biochemistry*, **8**, 997 (1969).
(23) P. O. P. Ts'o, G. K. Helmkamp, and C. Sander, *Proc. Nat. Acad. Sci. U. S.*, **48**, 686 (1962); P. O. P. Ts'o and P. Lu, *ibid.*, **51**, 17 (1964); V. Vetterl, *Experientia*, **21**, 9 (1965).
(24) B. Janik and P. J. Elving, *J. Amer. Chem. Soc.*, **92**, 235 (1970).
(25) P. J. Elving, B. Janik, and J. W. Webb, private communication.
(26) The ecm or electrocapillary maximum occurs at the potential of net zero charge on the electrode.
(27) Reference 13, p 271.

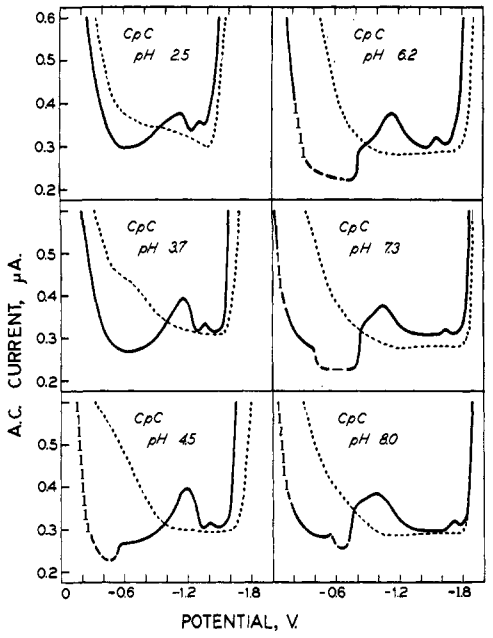

Figure 7. Total ac polarograms for *ca.* 0.05 m*M* CpC in McIlvaine buffer (pH indicated) at 25°. Dashed lines denote distorted current oscillations, dotted line denotes background electrolyte base current.

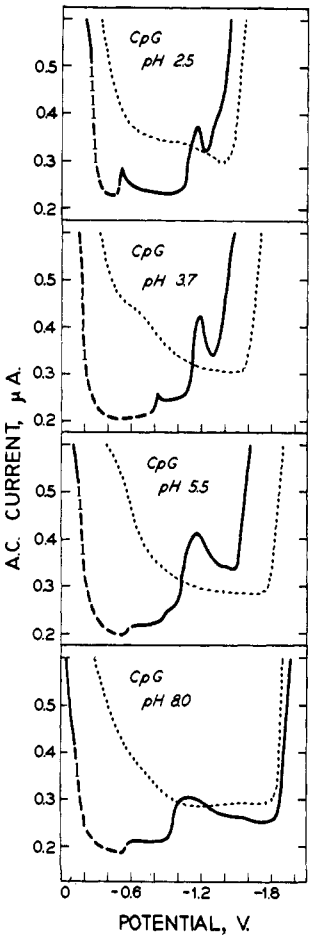

Figure 8. Total ac polarograms for *ca.* 0.05 m*M* CpG in McIlvaine buffer (pH indicated) at 25°. Dashed lines denote distorted current oscillations, dotted line denotes background electrolyte base current.

The greater changes in the double-layer capacity as indicated by the larger quadrature peaks for the dinucleoside phosphates probably indicate more complex changes and orientations at the solution–electrode interface than for cytosine, cytidine, or CMP. The dinucleoside phosphates also exhibit smaller peaks inside the first depression, especially at higher pH (Figures 7 and 8); this suggests that, over certain pH ranges, two different forms of the depolarizer are adsorbed, which may require its orientation in different ways on the electrode. Since equilibria between species with zero, one, and two charges are possible (the species may be zwitterionic or charged positively or negatively),[28] simultaneous adsorption of more than one form is likely with one form, however, being probably predominant, *e.g.*, positively charged and uncharged forms below pH 4 to 5 and zwitterionic and negatively charged forms above pH 5 to 6.

While cytosine, cytidine, and CMP produce only a single polarographic reduction wave, CpC and possibly CpU and CpG produce two waves in a pattern (Figure 1) in which only wave I may appear at very low concentration, *e.g.*, below 0.02 m*M*. With increasing concentration, wave I grows in height and then levels off at still a low concentration, *e.g.*, 0.2 or 0.3 m*M*, whereas wave II at more negative potential continues to grow (Figure 6). This pattern is the consequence of the adsorption of the dinucleotide and its reduction product at the interface.

Adsorption of Reducing Product. Assignment of the prewave (wave I) to reduction to an adsorbed product is supported by its proportionality to drop-time, extremely small temperature coefficient, and distorted current oscillations and characteristic *i–t* curve shapes on its limiting portion, as well as by disappearance of the normal wave at low concentrations. $E_{1/2}$ for this process is constant with increasing concentration and more positive than that of wave II, since it is an energetically easier process.[29] The decrease of the apparent *I* for wave I with increasing concentration indicates blockage of the effective reduction sites by increasing formation of adsorbed product until maximum coverage of the effective electrode area is reached, *e.g.*, at about 0.3 m*M* for CpC (Figure 6).

Consequently, further reduction necessitates diffusion of the reducible species through this adsorbed layer and subsequent desorption of the reduction product and/or reduction *via* electron transmittal through the adsorbed layer; in either event, the process is energetically more difficult, accounting for the occurrence of wave II at a more negative potential than wave I. As the bulk nucleotide concentration increases, the fraction involved in producing the adsorbed reduced layer decreases even though more of the product is adsorbed in covering a larger fraction of the available interface; as a result, an increasing fraction of the nucleotide is reduced at more negative potential. Thus, *I* for wave II increases with increasing concentration and $E_{1/2}$ becomes more negative until attainment of maximum coverage of the electrode by the adsorbed layer and then is constant (Figure 6). The sum of *I*

(28) H. Simpkins and E. G. Richards, *Biochemistry*, **6**, 2513 (1967); Y. Inoue and K. Satoh, *Biochem. J.*, **113**, 843 (1969).

(29) L. Meites, "Polarographic Techniques," Wiley, New York, N. Y., 1965, p 187.

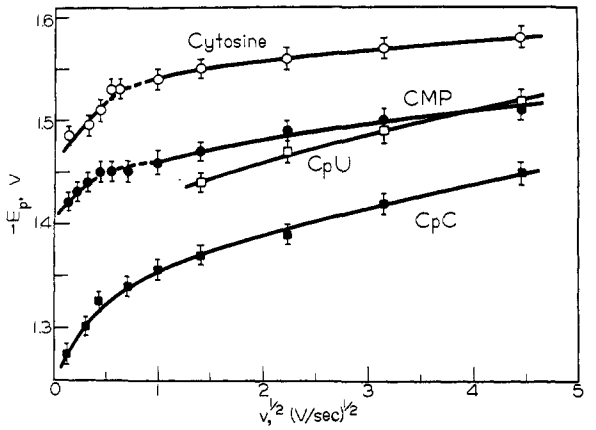

Figure 9. Variation of peak potential with polarization rate at hmde for cytosine species at 25°. Points are plotted with an indicated error limit of ±10 mV. Conditions are specified in the caption to Figure 3.

for waves I and II, however, remains constant with change in concentration.

The presence of a small depression for CpC in the quadrature current component just after the potential where the depolarizer is reduced to an adsorbed product suggests that the adsorption process is slow. A slow process would also explain the presence of wave II at concentrations before the height of wave I becomes constant, as the result of competition between the processes producing adsorbed and unadsorbed product.

The reduction product of CpC is adsorbed over the whole pH range where reduction occurs (Table I). Strong adsorption of this product is supported by the fact that the voltammetric peak at the hmde, which appears only on the first scan (on subsequent scans only a more negative shoulder is formed), is not restored after stirring the solution.

Association in Adsorbed State. Concomitant with adsorption at the interface is film formation or association of the adsorbed molecules. Since this phenomenon occurs at the electrode surface (as opposed to association in solution), it is potential dependent; consequently, when the film forms and breaks with changing potential, marked changes occur in the double layer capacity and more or less well-developed pits or wells appear in the ac quadrature current component (Figures 7 and 8). This effect has been observed for the nucleosides which commonly occur in nucleic acids (adenosine, guanosine, cytidine, uridine, and thymidine), at relatively high bulk solution concentration (generally above 1 mM).[30] The purine nucleosides have a greater tendency to associate than the pyrimidine nucleosides; *e.g.*, at pH 7.0, where adsorbed cytidine does not associate even at a bulk concentration of 17 mM (deoxycytidine associates at 0.9 mM), adenosine is associated at 4.4 mM.[30c,d]

The cytosine-containing dinucleoside phosphates associate much more than the cytosine nucleoside and nucleotide; *e.g.*, CpC associates at pH 7.3 even at a solution concentration below 0.05 mM (Figure 7). However, those dinucleoside phosphates containing adenine associate to a greater extent than those containing cytosine; *e.g.*, at low temperature and pH 5, CpC does not associate at bulk concentrations up to 0.9 mM while ApA associates at concentrations as low as 0.05 mM.[25]

The potential range covered by the pit can also be used to evaluate the extent of association at the interface. For the cytosine dimers studied, this range increases in the order CpC < CpU < CpA, ApC, ApG.[25] This order parallels that for the tendency of nucleosides and nucleotides to associate in solution, *i.e.*, pyrimidine–pyrimidine < purine–pyrimidine < purine–purine,[20a] and, consequently, may indicate similar modes of association at the interface and in solution, *i.e.*, vertical overlapping or stacking of the bases.

Since the principal mode of self-association in the solid state is also base stacking,[31] it appears reasonable that the molecules in the adsorbed state undergo similar modes of interaction.

Effect of Structural Organization

In pH 5 McIlvaine buffer at 0°, the difficulty of reducibility follows the order cytidine < CpC < CMP < cytosine. For cytosine and its nucleoside and nucleotide, this order parallels the electron-withdrawing effect of the ribose and phosphate groups due to the close proximity of the substituents to the reduction site.

In pH 4 acetate buffer at 25°, the order for difficulty of reducibility is cytidine, CMP < cytosine. Since the half-wave potentials for cytosine and CMP are not affected by the buffer system or temperature, the major reason for the change in order of relative reducibility is pH. The change of $E_{1/2}$ of cytidine and cytosine with increasing pH is about −70 mV/pH; that for CMP exceeds 100 mV/pH.[3,4] All three cytosine species are protonated at the N(3) position of the cytosine ring (pK_a values are about 4).[32] CMP, however, has two other acidic protons on the phosphate group with pK_a values of about 1 and 6. Thus, at pH 4 or 5, there exist in equilibrium a CMP species with a negative charge on the phosphate, a zwitterion species with a negative charge on the phosphate and a positive charge on the ring, and a species with two negative charges on the phosphate and with or without a positive charge on the ring; *e.g.*, at pH 4, the N(3) position is about 50% protonated and the phosphate is about 99% protonated in the single anionic form. The result is a rather complex equilibrium system with a change in pH affecting the concentrations of all species and, thus, the nature of the predominant species which is reduced at the electrode. Consequently, the structural effect on the relative ease of reducibility of cytosine, cytidine, and CMP is dependent upon pH.

The position of CpC in the series indicates the involvement of other factors which make the dinucleoside phosphates more easily reducible than the nucleotide or base. One factor may be interaction between the rings due to the stacked configuration of the dinucleoside.[20] Although CpU reduction at the dme is obscured by background discharge, its E_p at high cyclic scan rates (Figure 9) is between those of CpC and cytosine, possibly indicating different degrees of interaction between the cytosine and uridine rings.

(30) (a) V. Vetterl, *Collect. Czech. Chem. Commun.*, 31, 2105 (1966); (b) *ibid.*, 34, 673 (1969); (c) *J. Electroanal. Chem.*, 19, 169 (1968); (d) *Biophysik*, 5, 255 (1968).

(31) C. E. Bugg and J. M. Thomas, *Biopolymers*, 10, 175 (1971).

(32) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963, pp 104 and 14.

Orientation near the Interface. Another reflection of structural organization is the experimental diffusion coefficient, D, which is related to the orientation of the molecule as it diffuses toward the electrode surface, *i.e.*, its effective cross-sectional area. D can be calculated from the Ilkovic equation, using dme polarographic I values (Table I) and $n = 3$ for cytosine, cytidine, and CMP, and 4 for CpC. D can also be calculated from cyclic voltammetry (c.v.), using the Randles–Sevcik equation (p 122 of ref 33) and the limiting $i_p/ACv^{1/2}$ value at high scan rates since the current is then due only to the initial 2e reduction for cytosine and CMP. D values from c.v. are not as reliable as those from polarography because of the difficulty in estimating the limiting $i_p/ACv^{1/2}$ value, but there is general agreement between the two methods (Table III).

Table III. Diffusion Coefficients for Cytosine and Its Nucleosides

Compd	Temp, °C	$D \times 10^5$, cm²/sec dc[a]	$D \times 10^5$, cm²/sec c.v.[a]	Limiting current Temp coeff,[b] %/°C	Limiting current Activation energy, kcal/mol
Cytosine	25	1.18	1.1	1.5	5.0
	0	0.58			
Cytidine	25	1.20		1.7	5.6
	0	0.51			
CMP	25	0.64	0.4–0.6	1.7	5.6
	0	0.31			
CpC	25	1.69		2.3	7.6
	0	0.53			

[a] Experimental basis: dc = dme polarography; c.v. = cyclic voltammetry. [b] Calculated by compound interest formula.

The dc limiting currents themselves are clearly diffusion controlled for cytosine, cytidine, and CMP; *e.g.*, the activation energies are within the expected 4 to 6 kcal/mol range.[34] The slightly greater temperature coefficient for CpC probably reflects the expected change in intramolecular association with temperature.

The diffusion coefficient data indicate that the ribose group has little effect on the diffusing species while the bulky phosphate group slows down the diffusion by about a factor of 2. Thus, it appears reasonable that the cytosine species diffuse to the electrode with the plane of the ring perpendicular to the plane of the electrode surface. In this model, the ribose group is behind the cytosine ring and adds little to the effective cross-sectional area of the molecule. The phosphate group, however, sticks out to one side of the molecule, increasing the diffusional cross-sectional area.[24]

The diffusion coefficient of CpC indicates that its effective cross-sectional area may be similar to that of cytosine or cytidine, suggesting that CpC is oriented somewhat differently than cytosine as it diffuses to the interface since the effective cross-sectional area of CpC in the stacked configuration has to be greater than CMP if the molecule diffuses with its rings perpendicular to the electrode surface. Unfortunately, there are not sufficient data to be more specific concerning the orientation of CpC.

(33) R. N. Adams, "Electrochemistry at Solid Electrodes," Marcel Dekker, New York, N. Y., 1969, p 135.

(34) G. H. Aylward and J. W. Hayes, *Bull. Chem. Soc. Jap.*, **38**, 1794 (1965); A. A. Vlček, *Collect. Czech. Chem. Commun.*, **24**, 3538 (1959).

Experimental Section

Chemicals. The reported analytical data and spectrophotometric assay for the cytosine compounds (Nutritional Biochemicals Corp. and Calbiochem) indicated sufficient purity for polarographic study.

Buffer solutions were prepared from analytical reagent grade chemicals (J. T. Baker and Mallinckrodt); a small amount of chloroform, which was added to prevent microorganism growth, was completely removed on the preliminary nitrogen purging. 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. Current–voltage and current–time curves were automatically recorded with a three-electrode potentiostat and a Moseley Model 7001A(S) X–Y recorder.

The potentiostat was a multipurpose instrument,[35] based on solid-state operational amplifiers, with only certain functions being used in any given mode; details of its construction, operation, and performance are given elsewhere.[36] A conventional arrangement was employed for dc polarography; for phase-sensitive ac polarography, a Princeton Applied Research Model 122 lock-in amplifier was incorporated. Automatic compensation for series resistance was achieved by positive feedback.[37] 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,[38] kept at 25.0 ± 0.1° or about 0.5°; the latter temperature is that of a circulating ice–water bath. Agar salt bridges were inserted on the counter and reference sides of the medium porosity glass frits separating the compartments. The reference compartment contained a saturated calomel reference electrode (sce); the counter compartment contained a platinum mesh electrode immersed in saturated KCl solution. DME capillaries (marine barometer tubing) had drop-times, measured at potentials of interest, between 6 and 7 sec and an m value of 1.0 mg/sec. A Metrohm E-140 microburet assembly was used to generate the hanging mercury drop electrode (hmde) employed in cyclic voltammetry.

Coulometry used a mercury pool electrode (3 cm²) at the temperature of interest in a water-jacketed cell (25-ml capacity), fitted with a Teflon cap, which allowed introduction of sample, salt bridges, and deaeration tubing. Reference and counter electrodes were thermostatted and identical with those used for voltammetry except that they 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 glass rod (Corning Code 7930 porous glass). The number of coulombs passed during electrolysis was found by measuring the area of the current–time curve with a Gelman Model 39231 compensating polar planimeter.

Ultraviolet spectra were obtained with a Beckman Model DB spectrophotometer and a Photovolt Model 43 recorder. All pH measurements were made with a Beckman Model G pH meter.

All potentials reported are *vs.* the sce at the experimental temperature.

Polarographic and Voltammetric Procedures. Test solutions were prepared by diluting known volumes of stock solutions in volumetric flasks with the desired buffer. About 10 ml of test solution was transferred to the cell, purged with N_2 for 15 min and then examined electrochemically with N_2 passing over the solution. Except where indicated, the background current, obtained on an identically treated buffer solution, was subtracted algebraically from the total current.

A natural drop-time was employed for dc polarography; $E_{1/2}$ and i_d were determined graphically, utilizing the average recorder

(35) D. D. DeFord, Division of Analytical Chemistry, 133rd National Meeting of the American Chemical Society, San Francisco, Calif., April 1958; W. M. Schwarz and I. Shain, *Anal. Chem.*, **35**, 1770 (1963); D. E. Smith, "Electroanalytical Chemistry," A. J. Bard, Ed., Vol. I, Marcel Dekker, New York, N. Y., 1966, pp 1–155.

(36) C. O. Schmakel, Ph.D. Thesis, University of Michigan, 1971.

(37) P. Delahay and I. Trachtenberg, *J. Amer. Chem. Soc.*, **80**, 2094 (1958); R. De Levie and A. A. Husovský, *J. Electroanal. Chem.*, **20**, 181 (1969).

(38) J. E. Hickey, M. S. Spritzer, and P. J. Elving, *Anal. Chim. Acta*, **35**, 277 (1966)

Table IV. Cyclic Voltammetry of Cytosine and Its Nucleosides[a]

Compd	Concn, mM	A, mm^2	pH	v, V/sec	$-E_p$, V	i_p, μA	$i_p/ACv^{1/2}$
Cytosine	0.106–1.06	5.3[b]	4.6	0.062		3.3–24.2	34.1
				0.26		8.8–58	33.2[c]
	0.53		3.7–7	0.026	1.14 + 0.084 pH		
				0.26	1.17 + 0.082		
	0.97	2.2	4.1	0.02–20	(Figure 9)		(Figure 3)
CMP	0.99	2.2	4.2	0.02–20	(Figure 9)		(Figure 3)
CpC	0.088	2.2	4.5	0.02–20	(Figure 9)		(Figure 3)
	0.05	3	2.0–5.3	0.3	1.14 + 0.042 pH		
			5.3–6.0		0.23 + 0.210 pH		
			6.0–8.0		0.09 + 0.101 pH		
			9.0–10.0		1.08 + 0.076 pH		
CpU	0.094	2.2	4.5	2–20	(Figure 9)		(Figure 3)

[a] All data taken at 25°. [b] From ref 39. [c] The ratio, $i_p/ACv^{1/2}$, decreases with increasing concentration.

Table V. Ac Polarography of Cytosine and Its Nucleosides (Total Current)[a]

			Depression				Peak I			Peak II	
			Δi_{min},[f]		ΔE,[g]						
Compd	Concn, mM	pH	pH	μA	pH	V	pH	$-E_s$,[h] V	Δi_s, μA	pH	$-E_s$, V
Cytosine[b]	2	1.05				0.27					
	2	4.6				0.91					
	2	5.6				0.65					
	0.5–2.5	1–6						1.27 + 0.054 pH[c]	0.1–0.14[d]		
CpC[e]	0.05		4.5	0.21	3.7	0.80	2.0–4.5	1.08 + 0.019 pH		2.0–4.2	1.21 + 0.041 pH
					5.5	0.73	4.5–5.7	1.18		4.2–8.0	1.02 + 0.108 pH
							5.7–8.0	1.72 + 0.096 pH			
							9.0–10.0[i]	1.11 + 0.019 pH			
CpG[e]			5.5	0.31	3.5	0.80	2.0–3.7	1.11 + 0.015 pH			
					7.7	0.82	3.7–9.6[j]	1.24 + 0.018 pH			
			4.5	0.25	4.5	0.60	2.5–5.5	1.13 + 0.007 pH			
							5.5–9.2[j]	1.30 + 0.038 pH			

[a] Cytosine data at 4 mV rms amplitude; all others at 3.5 mV. [b] Cytosine data taken from ref 39; chloride and acetate buffers (μ = 0.5) at 25°. [c] At 0.99 mM concentration. [d] At pH 4.7. [e] Data for *ca.* 0.05 mM solutions in chloride and McIlvaine buffers (μ = 0.5) at 25°. [f] Data are the maximum values of Δi_{min} taken from Δi_{min} *vs.* pH plots, where the difference in current from the background electrolyte base current was measured at the potential of the lowest part of the depression. Blank spaces indicate that the maximum Δi_{min} value could not be estimated from the plot. A second value in the case of CpC is for the second maximum observed on the Δi_{min}–pH plot. The maximum errors in estimating Δi_{min} are ±0.3 pH and ±0.1 μA. [g] The width of the depression, ΔE, is the difference between potentials at the 0.05-μA level above the minimum of the depression or above that of a pit or step if the latter occur. Data are the maximum widths obtained from ΔE–pH plots; blank spaces indicate that ΔE could not be estimated from such plots. The maximum errors in estimating ΔE are ±0.3 pH and ±50 mV. [h] Maximum deviations from the equations are 25 mV for peak I and 15 mV for peak II. [i] Ammonia and carbonate buffers. [j] McIlvaine and carbonate buffers.

trace. For ac polarography, a controlled 3-sec drop-time was used and the instantaneous ac current amplitude at the end of drop-life recorded; an applied ac frequency of 5 mV peak amplitude (3.54 mV rms) was employed.

Cyclic voltammetric potential and current data are reported for the first scan unless otherwise indicated. Single scan voltammograms could generally be obtained up to a scan rate of 20 V/sec; at higher scan rates, only steady-state voltammograms were usually obtained. The hmde had a surface area of 0.022 cm^2.

Coulometric Procedures. An aliquot of buffer solution was added to the cell and deoxygenated for 20 min; Hg was then introduced and the solution electrolyzed at a potential identical with that to be employed for electrolysis, until the current fell to a minimum value (0.01 to 0.03 mA). The potential was then shifted to −0.6 V and an aliquot of stock solution containing only the electroactive species added; N_2 was again vigorously bubbled through the solution until the current level dropped to zero (5 min); electrolysis was then begun at the desired potential and was stopped when a constant current level was reached. Nitrogen was continuously passed through the cell during electrolysis. Current was automatically recorded as a function of time.

Immediately after each electrolysis, an aliquot of the electrolyzed solution was examined spectrophotometrically. Dilution was accomplished with distilled water; an identical concentration of buffer was used in the reference cell.

Cytosine. (1) Dc Polarography. Cytosine gives a fairly well-defined reduction wave at 25° in pH 3.7 to 5.7 acetate buffer[4] (Figure 1). $E_{1/2}$ becomes more negative and I remains approximately constant with increasing pH (Table I). There is no appreciable shift in $E_{1/2}$ between 0 and 25°, when the sce is at the experimental temperature. In a pH 5 McIlvaine buffer, no wave is observed and only a slight inflection occurs on the background discharge at 25°.

(2) Cyclic Voltammetry. Cytosine shows one cathodic peak of pH-independent i_p; E_p varies linearly with pH[39] (Table IV). The ratio of $i_p/ACv^{1/2}$ decreases with increasing scan rate (v) up to about 1 V/sec, above which the ratio becomes relatively constant (Figure 3). E_p becomes more negative with v; a plot of E_p *vs.* $v^{1/2}$, which seems to consist of two linear segments with the greater slope between 0 and 1 V/sec, may be exponential in shape (Figure 9). On steady-state scanning, there is no evidence for electroactive unstable intermediates or other electroactive transitory species at v up to 200 V/sec.

(3) Ac Polarography. At 25° in pH 4.2 acetate buffer, the total ac polarogram shows a weak depression from background current centered around −0.4 V and a peak at about −1.5 V which becomes more negative with increasing pH and whose height is proportional to concentration (Table V). Phase-selective ac polarography, however, shows that this peak consists of a small depression followed by a peak in the quadrature component and a single peak in the in-phase component (Tables VI and VII; Figure 1). The summit potential, E_s, of the in-phase peak becomes more negative with increasing frequency, but its height remains constant up to 1250 Hz. The quadrature peak also becomes more negative in potential with increasing frequency, but its height decreases up to 250 Hz above which it is no longer visible.

At 0° in pH 5 McIlvaine buffer, no in-phase peak is visible but the quadrature current component exhibits the depression around −0.5 V, a weak rounded elevation around −0.7 V (peak I), a second depression at −1.1 V, and peak II at −1.4 V. In pH 3.5 McIlvaine

(39) G. Dryhurst and P. J. Elving, *Talanta*, **16**, 855 (1969).

Table VI. Ac Polarography of Cytosine and Its Nucleosides (Quadrature Component)

Compd	Concn, m*M*	pH	Temp, °C	Freq, Hz	Depression I: $-E_{min}$, V	Depression I: Δi_s, μA	Peak I: $-E_s$, V	Peak I: Δi_s, μA	Depression II: $-E_{min}$, V	Depression II: Δi_s, μA	Peak II: $-E_s$, V	Peak II: Δi_s, μA
Cytosine	0.1	3.5[c]	0	50	0.54	0.13	0.95	0.04				
	0.1	5.0[c]	0	50	0.49	0.27	0.7	0.01	1.05	0.02	1.35	0.04
	0.97	4.2[d]	25	20	0.4	0.04			1.43	0.01	1.45	0.13
				50	0.4	0.1			1.46	0.03	1.47	0.1
				100	0.4	0.2			1.47	0.1	1.48	0.08
				250	0.4	0.4			1.48	0.1	1.49	0.2
				500	0.4	1.0			1.49	0.2		
				714	0.4	1.4			1.48	0.2		
				1000	0.4	2.0			1.48	0.4		
Cytidine	0.1	5.0[c]	0	50	0.4	0.27	1.1	0.4	1.38	0.04		
	1.00	4.1[d]	25	50	0.77	0.03	1.06	0.08	1.39	0.09	1.48	0.06
CMP	0.1	5.0[c]	0	50	0.4	0.34	0.9	0.02				
	0.99	4.1[d]	25	50	0.55	0.18	0.93	0.11	1.39	0.03	1.50	0.06
CpC[e]	0.018–0.89	5.0[c]	0	50	0.4–0.6	0.12–0.2	1.25–1.27	0.03–0.16	1.39–1.45[a]	0.01–0.02	1.54–1.59[b]	0.04–0.07

[a] Observed above 0.018 m*M* concentration. [b] Observed above 0.18 m*M* concentration. [c] McIlvaine buffer of $\mu = 0.13$ *M*. [d] Acetate buffer of $\mu = 0.5$ *M*. [e] Because of the relatively small variation with concentration, only the ranges are shown.

Table VII. Ac Polarography of Cytosine and Its Nucleosides (In-Phase Component)

Compd	Concn, m*M*	pH	Temp, °C	Freq, Hz	Peak I: $-E_s$, V	Peak I: Δi_s, μA	Peak III: $-E_s$, V	Peak III: Δi_s, μA	Peak III: $-E_s$, V	Peak III: Δi_s, μA
Cytosine	0.1	5.0[a]	0	50			no peaks observed			
	0.97	4.2[b]	25	20	1.45	0.64				
				50	1.50	0.62				
				100	1.51	0.63				
				250	1.52	0.61				
				500	1.53	0.60				
				714	1.53	0.62				
				1000	1.54	0.61				
				1250	1.54	0.62				
Cytidine	0.1	5.0[a]	0	50	1.40	0.05				
	1.00	4.1[b]	25	50	1.43	0.52				
CMP	0.1	5.0[a]	0	50	1.50	0.02				
	0.99	4.1[b]	25	50	1.43	0.37				
CpC	0.018	5.0[a]	0	50	1.32[c]	0.013	1.43[d]	0.037	1.62[e]	0.13[e]
	0.89					0.060	1.54	0.5		
	0.096	4.5[a]	25	50	1.28	*f*	1.4	*f*		

[a] McIlvaine buffer of $\mu = 0.13$ *M*. [b] Acetate buffer of $\mu = 0.5$ *M*. [c] Average value throughout concentration range. [d] Observed above 0.018 m*M*. [e] Observed above 0.36 m*M*. [f] Peaks occur as shoulders on background discharge and are difficult to measure.

buffer, peak I is larger and at more negative potential (Table VI); the second depression and peak are not seen.

At 25° in pH 4.2 acetate buffer, the second harmonic at 50 Hz has a positive peak at −1.5 V and a negative peak at −1.6 V with E_0 at −1.57 V; the positive to negative peak height ratio is about 13:1. At 500 Hz and above, the negative peak disappears and the positive peak broadens. The second harmonic ac data thus provide additional evidence that the overall process is irreversible.

An additional argument for the presence of an irreversible follow-up chemical reaction as the cause of the overall irreversibility is the dependence of the faradaic phase angle, ϕ, on the dc potential. Cot ϕ increases at potentials more positive than $E_{1/2}$, indicating a chemical reaction involving the reduced species.[40]

(4) Controlled Potential Electrolysis. Electrolyses in pH 4.2 and 4.7 acetate buffer at 25° show a three-electron reduction; *n* values are between 2.5 and 3.3, depending on whether the current flows are corrected for the current blank in the presence of the final concentration of the product or for the average background current in the absence of product. It has been suggested[4] that *n* is actually between the two corrected values due to the accumulation of product during electrolysis. Electrolyses at 0°, where the pre- and post-electrolysis backgrounds are at about the same level, also yield an *n* value of 3 (2.6 and 3.2, depending on concentration).

Examination of the electrolyzed solutions by ultraviolet spectrophotometry shows that the cytosine peak at 275 nm has been replaced by one at 249 nm. The latter peak also appears after electrolysis of 2-hydroxypyrimidine.[4]

(40) D. E. Smith, *Anal. Chem.*, **35**, 602 (1963).

Cytidine. (1) Dc Polarography. In pH 4.1 acetate buffer at 25°, cytidine gives a well-defined wave whose $E_{1/2}$ is more positive and *I* value is identical with those of cytosine (Table I; Figure 1). At 0° in pH 5.0 McIlvaine buffer, $E_{1/2}$ is similar to that in pH 4.1 acetate buffer at 25°.

(2) Ac Polarography. The in-phase component exhibits one peak more negative than the corresponding $E_{1/2}$ (Table VII; Figure 1) in both pH 4.1 acetate buffer at 25° and pH 5.0 McIlvaine buffer at 0°.

At 0° in pH 5.0 McIlvaine buffer, the quadrature current component exhibits depressions I and II and peak I, similar to those shown by cytosine. Depression I covers a larger potential region than that for cytosine, but, instead of being rounded as with cytosine, the current decreases linearly with more negative potential with a somewhat rounded portion at about −0.8 V. Peak I and depression II occur at more negative potential and are higher and deeper, respectively, relative to background than those of cytosine (Table VI).

In pH 4.1 acetate buffer at 25°, the quadrature current component shows the same series of peaks and depressions as in pH 5.0 McIlvaine buffer at 0°, except that depression I is more rounded and peak II is visible (Figure 1).

CMP. (1) Dc Polarography. In pH 5.0 McIlvaine buffer at 0°, $E_{1/2}$ is more negative and *I* is lower than those of cytidine. However, at 25° in pH 4.1 acetate buffer, the half-wave potentials for both compounds are similar (Table I; Figure 1). There is no appreciable shift in $E_{1/2}$ for CMP between 0 and 25°.

(2) Cyclic Voltammetry. In pH 4.1 acetate buffer at 25°, CMP shows one reduction peak whose $i_p/ACv^{1/2}$ ratio decreases to a limiting value above 0.1 V/sec (Figure 1), which is about 30% less

than that of cytosine. E_p becomes more negative with $v^{1/2}$ similar to cytosine except that the curve is shifted about 80 mV more positive (Figure 9). No steady-state anodic peaks are observed at scan rates up to 200 V/sec.

(3) Ac Polarography. The in-phase component shows one peak of intensity less than that of cytidine in pH 4.1 acetate buffer at 25° and pH 5.0 McIlvaine buffer at 0° (Table VII; Figure 1).

In McIlvaine buffer at 0°, the quadrature current component shows depression I and a weak peak I similar to those of cytosine under the same conditions; peak II and depression II are not observed (Table VI).

In the acetate buffer at 25°, CMP exhibits a quadrature curve similar to that of cytidine, except that depression I and peak I occur at more positive potential (Figure 1).

(4) Controlled Potential Electrolysis. Electrolysis of CMP in pH 3 acetate buffer at 0 and 25° yields n values between 2 and 4, depending on whether the currents are corrected for the post- or preelectrolysis background. In all but one experiment, examination of the electrolyzed solution by dc polarography and ultraviolet spectrophotometry indicated the presence of some unreduced CMP although the final electrolysis current appeared to be constant. In addition to the CMP peak at 275 nm, the electrolyzed solution showed a peak at 242 nm. In one experiment, no CMP was left as indicated by the absence of a dc polarographic wave and the 275-nm peak; the 242-nm peak was present. In this case, the n values were 2.6 and 4.2 on correction for post- and pre-electrolysis current backgrounds, respectively.

These results indicate the occurrence of a slow reaction during the CMP reduction; *i.e.*, at low concentrations of unreduced CMP, the current due to its reduction cannot be distinguished from that of background. On this basis, the electrolysis n value of CMP is about 3.

CpC. (1) Dc Polarography. CpC shows one generally moderately well defined cathodic wave, which in certain pH and concentration ranges may be distorted by being split into two waves (Table I; Figure 2).

At 25°, the rising parts of the normal wave of a *ca.* 0.05 m*M* CpC solution may be distorted in the sense that the initial portion appears to constitute a more positive prewave. The limiting current portion of the latter (wave I) is characterized by abnormal current oscillations and is usually hard to define; its $E_{1/2}$ becomes more negative with increasing pH similar to the normal wave. $(E_{1/4} - E_{3/4})$ is *ca.* 33 mV compared to *ca.* 50 mV for the normal wave slope.[41]

$E_{1/2}$ of the normal reduction wave (wave II) becomes more negative with increasing pH (Table I); a plot of $E_{1/2}$ *vs.* pH at 25° consists of three linear segments with inflection points at *ca.* pH 5.9 and 8.0, whose slopes, $d(E_{1/2})/d(pH)$, are 0.06, 0.10, and 0.05 V/pH.[42] $E_{1/2}$ values in acetate buffer usually fall on the $E_{1/2}$–pH plot for McIlvaine buffer; however, the wave shape shows a small rounded maximum on the limiting portion.

The wave height, or total height if the prewave appears, gradually decreases up to pH 7, followed by an increase to a maximum at pH 9 and disappearance at pH 11 (Figure 2).

$E_{1/2}$ and height of both waves do not change significantly with ionic strength between 0.25 and 0.75 *M*; at low ionic strength, *e.g.*, below 0.2, the limiting portion of the prewave is usually better defined but the current oscillations are more distorted.

The normal wave height (total height if prewave appears) is (within experimental error) directly proportional to $h^{1/2}$ but the i_l–$h^{1/2}$ plots do not pass through the origin. $E_{1/2}$ usually becomes 0 to 15 mV more negative over a 40-cm increment in h. The height of the prewave is directly proportional to h.

The i–t curves recorded at various potentials along the rising part of the normal wave have a smooth parabolic shape and give more or less linear log i–log t plots, but i–t curves recorded at the limiting portion of the prewave, when the original wave still exists, exhibit a small decrease in current at the end of the drop-life.

In pH 5.0 McIlvaine buffer at 0°, the prewave appears throughout the concentration range studied (0.018 to 0.89 m*M*); its height increases up to about 0.4 m*M*, above which it remains constant; $E_{1/2}$ is independent of concentration (Figure 6). $E_{1/2}$ of wave II becomes more negative with increasing concentration with an inflection point on the $E_{1/2}$–concentration plot (Figure 6). Above 0.18 m*M*, ill-formed wave III appears at very negative potential and blends with the background above 0.7 m*M*; I and $E_{1/2}$ are difficult to measure but remain approximately constant in the concentration range over which the wave is seen.

(2) Cyclic Voltammetry. At 25° and scan rates between 0.02 and 20 V/sec, CpC exhibits a single cathodic peak at the foot of background discharge on the first scan from zero to negative potential; below pH 6, a shoulder or inflection appears on subsequent scans at about 30 mV more negative than the first peak.

The main peak is well defined in acidic solution, is ill defined above pH 4.0 to 5.0, where it begins to disappear, and may only appear as an inflection or shoulder on background discharge in pH 9.0 to 10.5 carbonate buffer (it gives no such indication in pH 9.1 ammonia buffer). It is generally symmetrical in the pH range before it begins to disappear or to merge with background discharge; above pH *ca.* 6, it becomes asymmetric, particularly at lower scan rates, with the current increase being steeper than the current decrease.

With increasing v, $i_p/ACv^{1/2}$ increases sharply (Figure 3) and E_p becomes more negative; the E_p–$v^{1/2}$ plot is similar to that of cytosine except that it is shifted about 170 mV more positive (Figure 9).

(3) Ac Polarography. At 25° below pH 5 (McIlvaine buffer) the general pattern of a total ac polarogram for 0.05 m*M* CpC consists of a depression below the base current, centered at −0.6 to −0.8 V, a broad peak (I) between −1.0 and −1.3 V, and a narrow peak (II) between −1.3 and −1.4 V (Figure 7; Table V). Peak II, which is essentially constant in height up to pH 4.0 to 4.5, decreases sharply at higher pH and disappears at pH 8.

Below pH 4, the depression shows no current or other irregularities (Figure 7); above pH 4, current oscillations are distorted at potentials more positive than −0.5 V and generally at potentials where wells or pits with steep walls or other minima appear (Figure 7). Depth in current below the background curve of the depression and its width and location in potential cannot be estimated precisely due to the variability in its shape (for the sake of comparison, arbitrary points must be often chosen as in Table V). Plots of the depth *vs.* pH pass through a maximum at pH 4.5, followed by a lag above pH 5.5 and a sharp decrease above pH 7.5.

E_s of peak I becomes more negative with increasing pH between pH 2 and 4.5, is constant between pH 4.5 and 5.7, then becomes more positive (Table V). The height of peak I above background current increases with increasing pH and, above pH 5, levels off. E_s of peak II shifts negatively with increasing pH (Table V).

The pattern of ac polarograms recorded in pH 2.1 chloride buffer is essentially the same as in McIlvaine buffer; however, magnitudes of individual parameters usually differ.

CpC shifts the in-phase background discharge about 100 mV more positive at pH 2.0. The magnitude of the shift is *ca.* 30 to 60 mV up to pH 5 and then decreases to 0 at pH 8.

At 0° in pH 5 McIlvaine buffer, the in-phase component exhibits the same peak pattern as the dc waves (Table VII; Figure 1); *i.e.*, E_s for the main peak (peak II) becomes more negative with increasing concentration and is 30 to 45 mV more negative than $E_{1/2}$ of wave II. Peaks I and III occur as shoulders on peak II and, hence, are very difficult to measure. However, peak I is more negative than $E_{1/2}$ of wave I, whereas peak III occurs at about the same potential as $E_{1/2}$ of wave III.

The quadrature component shows a broad depression centered around −0.5 to −0.6 V, and a broad peak between −1.23 and −1.27 V (Figure 1). E_s of the latter becomes more negative and the intensity (Δi_s) increases with increasing concentration up to about 0.35 m*M*, above which both potential and current remain approximately constant. A weak depression, seen between −1.39 and −1.45 V, becomes more negative with increasing concentration. A second peak at about −1.54 V, corresponding to in-phase peak III, whose height is essentially independent of concentration, appears above 0.18 m*M*.

(4) Controlled Potential Electrolysis. Electrolysis in pH 4.5 McIlvaine buffer at 0° yields an n of 3.8. As with cytosine at 25°, the current blank in the presence of the final concentration of the product is higher than the average background current in the absence of product; thus, n is probably somewhat higher, *i.e.*, about 4.

CpU and CpG. (1) Dc Polarography. CpU and CpG produce one to three very ill-defined waves at 25°; the most negative CpG wave, which is up to 30 and 10 times higher than its most positive wave and the sum of the two more positive waves, respectively, is very probably a catalytic hydrogen wave. A relatively well-defined, single wave appears for both compounds at pH 6 to 9; $E_{1/2}$ is 70 to

(41) The theoretical value for a reversible 1e controlling process at 25° is 56 mV; it is 28 mV for a 2e process. The corresponding values at 0° are 54 and 27 mV.

(42) The theoretical value for a reduction involving the same number of electrons and protons in the potential-determining step of the electrode process is 0.06 V/pH.

80 mV more negative and the wave height is about one-half of that of the CpC wave.

At 0° in pH 4.5 McIlvaine buffer, CpU shows one ill-defined wave close to background discharge; I and $E_{1/2}$ are similar to those of CpC wave I (Table I).

(2) Cyclic Voltammetry. At scan rates below 1 V/sec, CpG and CpU produce a faint inflection near background discharge. At higher scan rates, the peak for CpU becomes visible; its E_p becomes more negative and the $i_p/ACv^{1/2}$ ratio increases rapidly with increasing v (Figures 3 and 9; Table IV).

(3) Ac Polarography. At 25°, CpU and CpG have the same general total ac pattern as CpC, showing the depression from background centered around −0.6 V but only rounded peak I at about −1.2 V (Table V; Figure 8).

CpU does not show distorted current oscillations below pH 8; CpG exhibits such oscillations at all pH's in addition to the pitlike formations.

The height of peak I above background for both compounds increases with increasing pH to pH 5 and then decreases. For CpG, the base current is depressed below that of the background at potentials more negative than peak I.

CpG shifts the background discharge to more positive potential. The magnitude of the shift is about 150 mV at pH 2 and, as with CpC, is proportional to pH up to pH 5 and then decreases to vanish at pH 8. CpU shifts background discharge by a relatively small but variable magnitude of 10 to 40 mV, which is apparently pH independent. At pH 4.0, the magnitude of the positive background shift for the dinucleoside phosphates increases in the order CpU < CpC < CpG.

Acknowledgment. The authors thank the National Science Foundation, which helped support the work described. One author (J. W. W.) is grateful to the Electrochemical Society for a Richards Summer Fellowship.

Conformational Analysis. I. Molecular Structure, Composition, Trans–Gauche Energy and Entropy Differences, and Potential Hindering Internal Rotation of Gaseous Oxalyl Chloride as Determined by Electron Diffraction

Kolbjørn Hagen and Kenneth Hedberg*

Contribution from the Department of Chemistry, Oregon State University, Corvallis, Oregon 97331. *Received August 17, 1972*

Abstract: Electron-diffraction patterns obtained from gaseous oxalyl chloride at 0, 80, and 190° reveal it to consist of a mixture of trans and gauche conformers instead of the generally supposed trans and cis. The greater stability of the trans and gauche forms relative to the cis may be understood in terms of the "bent" single-bond pair concept of the carbon–oxygen double bond. Assuming that the geometries of the two conformers differ only in the torsion angle about the C–C bond, some of the more important parameter values at 0° with estimated errors of 2σ are $r_{C=O}$ = 1.182 Å (0.002), r_{C-C} = 1.534 Å (0.005), r_{C-Cl} = 1.744 Å (0.002), ∠CCO = 124.2° (0.3), ∠CCCl = 111.7° (0.2), ϕ (the gauche torsion angle relative to 0° for the trans form) = 125.0° (5.8), $l_{C=O}$ = 0.0380 Å (0.0025), l_{C-C} = 0.0482 Å (0.0026), l_{C-Cl} = 0.0532 Å (0.0026), and δ (the rms torsion amplitude for the trans conformer) = 22.1° (3.3). The mole fractions of the trans conformer at 0, 80, and 190° were found to be 0.676 (0.084), 0.513 (0.100), and 0.424 (0.102), respectively, from which the energy difference ($\Delta E° = E°_g - E°_t$) was calculated to be 1.38 (σ = 0.35) kcal/mol and the entropy difference ($\Delta S° = S°_g - S°_t$) to be 2.3 (σ = 1.0) cal mol^{-1} deg^{-1}. For an assumed hindering potential of the form $2V = V_1(1 - \cos\phi) + V_2(1 - \cos 2\phi) + V_3(1 - \cos 3\phi)$, a value with estimated standard deviation of 13.2 ± 4.2 kcal/mol was obtained for $V^* = V_1 + 4V_2 + 9V_3$. This together with two other conditions on the V_i's imposed by the experimental results gave V_1 = 1.01 ± 0.22, V_2 = 0.85 ± 0.19, and V_3 = 0.98 ± 0.43, all in kcal/mol. The barrier separating the trans from the gauche conformer is 2.00 ± 0.42 and that separating the two gauche forms is 0.61 ± 0.44 kcal/mol.

During the past 2 decades oxalyl chloride has been the subject of a number of experimental and interpretive spectroscopic studies involving infrared work on the gas,[1-5] liquid or solution,[1-3,5] and solid;[5,6] Raman on the liquid[1-5] and solid;[5] and ultraviolet on the gas,[7-10] liquid,[7] and solid.[11] An important concern in most of this work was the composition of the material, *i.e.*, the possible existence of more than one rotational conformer. The solid phase appears to consist only of the expected coplanar s-trans form (symmetry D_{2h}), and although some of the results for the gas and liquid phases have been interpreted[2,4] as consistent with the presence of just this form, the weight of the evidence seems to favor as well the presence in these phases of a second conformer generally agreed to be the coplanar s-cis rotamer (symmetry C_{2v}). The composition of liquid oxalyl chloride at room temperature was estimated[5] to be 15–20% cis with the trans form the more stable by 2.2–2.8 kcal/mol.[1,5] No evidence for a cis form was found in an X-ray diffraction investigation of the crystal[12] nor in

(1) B. D. Saksena and R. E. Kagarise, *J. Chem. Phys.*, **19**, 987 (1951).
(2) J. S. Ziomek, A. G. Meister, F. F. Cleveland, and C. E. Decker, *ibid.*, **21**, 90 (1953).
(3) B. D. Saksena, R. E. Kagarise, and D. H. Rank, *ibid.*, **21**, 1613 (1953).
(4) J. L. Hencher and G. W. King, *J. Mol. Spectrosc.*, **16**, 158 (1965).
(5) J. R. Durig and S. E. Hannum, *J. Chem. Phys.*, **52**, 6089 (1970).
(6) R. E. Kagarise, *ibid.*, **21**, 1615 (1953).
(7) B. D. Saksena and R. E. Kagarise, *ibid.*, **19**, 999 (1951).
(8) J. W. Sidman, *J. Amer. Chem. Soc.*, **78**, 1527 (1956).
(9) B. D. Saksena and G. S. Jauhri, *J. Chem. Phys.*, **36**, 2233 (1962).
(10) W. J. Balfour and G. W. King, *J. Mol. Spectrosc.*, **27**, 432 (1968).
(11) H. Shimada, R. Shimada, and Y. Kanda, *Spectrochim. Acta, Part A*, **23**, 2821 (1967).
(12) P. Groth and O. Hassel, *Acta Chem. Scand.*, **16**, 2311 (1962).