Oxidation Chemistry of Adenine and Hydroxyadenines at Pyrolytic Graphite Electrodes

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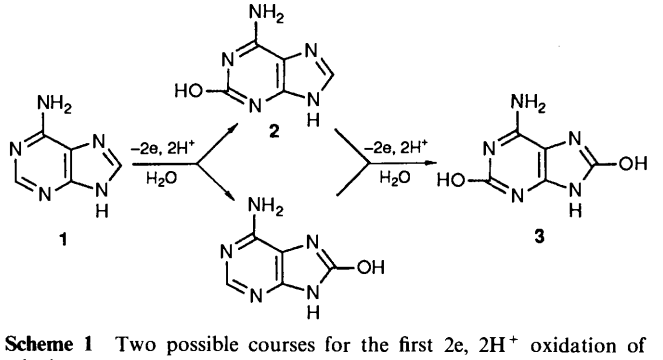
The electrochemical oxidation of adenine and hydroxyadenines has been studied in aqueous solutions in the pH range 3.0–11.2 using a pyrolytic graphite electrode. The initial course of the electrode reaction has been deduced to involve a 2e, $2H^+$ reaction to give 2- and not 8-hydroxyadenine, further oxidation of which gives 2,8-dihydroxyadenine and then diimine species which undergo a series of chemical reactions to give different products. The major products of oxidation at pH 3.0 {urea, alloxan [2,4,5,6(1*H*,3*H*)-pyrimidinetetrone] and parabanic acid (imidazolidinetrione)} and at pH 7.0 [allantoin (5-ureidohydantoin)] have been isolated using HPLC and column chromatography and their structures elucidated by spectroscopic techniques. The probable EC mechanisms for their formation have also been suggested.

Adenine [6-amino-(1*H*)-purine] (**1**) is one of the two important purines most commonly found in nucleic acids and hence intimately involved in protein synthesis and transfer of genetic information. Adenine has also been found as a component of a number of coenzymes; coenzyme I (NAD^+), coenzyme II ($NADP^+$) and coenzyme A (pantothenic acid) contain the adenine moiety in their structure and are often involved in oxidation–reduction processes in biological systems. As electron transfer properties of these compounds *in vitro* provide useful information about *in vivo* behaviour, the electrochemical behaviour of purines has been the subject of investigation for a long time. The first systematic electroreduction of purines was reported by Smith and Elving in 1962[1] and later several papers appeared in the literature on the reduction as well as the determination of purines using electrochemical methods.[2–8] In contrast to electrochemical reduction, the oxidation of purines attracted little attention. Dryhurst and Elving[9] studied the oxidation of adenine at pyrolytic graphite electrodes; however, the studies were carried out in 1 mol dm^{-3} acetic acid, which usually provides information far removed from biological systems and the products were identified on the basis of indirect identification methods such as TLC and colour reactions. Also, the first two-electron oxidation of adenine can yield 2-hydroxyadenine (**2**) or 8-hydroxyadenine (Scheme 1) and no



Scheme 1 Two possible courses for the first 2e, $2H^+$ oxidation of adenine

attempt has so far been made to detect the primary course of the electrode reaction of adenine.

The various studies on the basis of mammalian xanthine oxidase oxidation of adenine and hydroxyadenines simply indicated that neither of the potential intermediates can be excluded on the basis of kinetic studies.[10,11] In view of the limited information available on the oxidation behaviour of adenine, it was considered desirable to study the oxidation chemistry of adenine and to compare it with the oxidation of hydroxyadenines to obtain information on the primary electrode reaction of adenine. The present paper presents the results obtained from the electrochemical oxidation of adenine, 2-hydroxyadenine, 8-hydroxyadenine and 2,8-dihydroxyadenine at a pyrolytic graphite electrode (PGE) using various electrochemical techniques. The products of oxidation have been isolated and characterized using high performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS) and mass spectrometry and mechanisms for their formation have been suggested.

Experimental

Adenine and hydroxyadenines were obtained from Sigma and were used as received. The solutions of desired concentrations of all the compounds were prepared in double-distilled water. The equipment used for linear and cyclic sweep voltammetry, controlled potential electrolysis and spectral studies were essentially the same as reported earlier.[12,13] The stationary PGE was prepared by the reported method[14] and had area 2 mm^2. All measurements were made in phosphate buffers[15] of ionic strength 0.5 mol dm^{-3} at 22 ± 1 °C and all potentials are referred to SCE. IR spectra of the products were recorded as KBr pellets using a Beckmann IR-20 spectrometer and ^1H NMR spectra were recorded on a Varian XL 300 spectrometer in [$^2H_6$]DMSO.

The separation of products was achieved by using LC in which a glass column (80 × 1.5 cm i.d.) packed with Sephadex G-10 (Sigma, bead size 40–120 μm) was used. The exhaustively electrolysed solution was lyopholized and the dried material was dissolved in 1–2 cm^3 of water and passed through the column using water as eluent. This arrangement allowed separation of phosphate from the products as reported by Toth *et al.*[16] Fractions of 5 cm^3 each were collected using a SICO fraction collector (model FARC 711). The absorbance of different fractions was measured at 210 nm using a Hitachi video display spectrophotometer and absorbance *vs.* volume was plotted.

HPLC was used to identify the products at pH 3.0, using a Bio-Rad gradient instrument having a Rheodyne Model 7125

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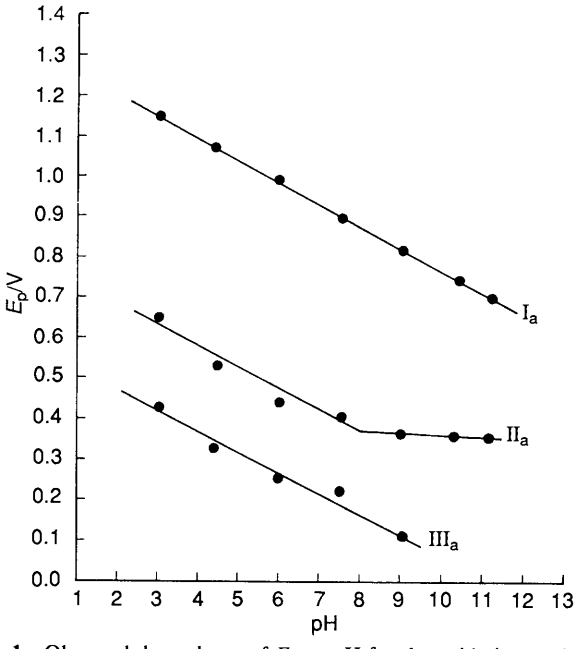


Fig. 1 Observed dependence of E_p *vs.* pH for the oxidation peaks of adenine

loop injector and reversed-phase Brownlee column (RP, 18.5 μm). The mobile phase solvents were prepared as follows: solvent 1 was 1% acetonitrile adjusted to pH 2.1 by addition of concentrated HCl and solvent 2 was 100% acetonitrile. The gradient employed was: 0–10 min, linear decrease of solvent 1 from 100% to 95% at the flow rate of 2.0 cm^3 min^{-1}; 10–20 min, linear increase of solvent 2 to 25% with increase in flow rate to 2.5 cm^3 min^{-1}; 20–30 min, linear increase of solvent 2 to 40% and 30–40 min, increase of solvent 2 to 60%. The final composition was maintained for 7 min and then returned to 100% solvent 1 in 3 min. Solvent 1 was passed for another 10 min to equilibrate the column before the next injection was made.

LC-MS was carried out using a Kratos MS 25/RFA instrument equipped with a thermospray source. The mobile phase was 0.1 mol dm^{-3} ammonium acetate with pH adjusted to 4.1 and the jet temperature was 250 °C. The mass spectra of the samples were recorded on a Hewlett Packard 5985B instrument at an electron beam voltage of 70 eV.

Results

Voltammetric Studies.—Adenine. Linear sweep voltammetry of a 0.25 mmol dm^{-3} solution of adenine at a sweep rate of 5 mV s^{-1} exhibited a single well defined peak when the sweep was initiated in a positive direction. The peak potential of this peak shifted to a less positive potential with increase in pH. In cyclic sweep voltammetry at a sweep rate of 100 mV s^{-1}, adenine exhibited a well defined pH dependent oxidation peak I_a. In the reverse sweep three cathodic peaks II_c to IV_c were observed, which formed quasi-reversible couples with peaks II_a, III_a and IV_a observed in the subsequent sweep towards positive potentials. Cyclic voltammograms of adenine were also recorded by initiating the sweep in a negative direction, and it was found that peaks IV_c and IV_a are not related to the fate of oxidation products, due to a reduction of adenine and subsequent oxidation of the product formed, and hence will not be discussed here. Peaks II_c and III_c are well separated at pH 3.0 but overlap at higher pH values and form a round peak. Peaks II_a and III_a were clearly observed in the pH range 3.0–9.2. At pH > 9.2, peaks II_c, II_a, III_c and III_a were not observed clearly. Some of the typical cyclic voltammograms of adenine are presented in Fig. 2. The potentials of the oxidation peaks I_a to III_a are shifted to more negative values (Fig. 1) and their shifts can be represented by the equations presented in Table 1. The dependence of E_p on pH for peak II_a shows two linear segments with slopes given by the equations in Table 1. The intersection of the two linear segments at pH *ca.* 8.0 probably corresponds to the acid dissociation constant of the reduced form.

The peak current of the oxidation peak I_a was found to increase with increase in concentration of adenine. The plot of peak current *vs.* concentration at pH 3.0 and 7.0 was practically linear below 0.5 mmol dm^{-3} and had a tendency to limit above 0.5 mmol dm^{-3} [Fig. 3(*a*)]. This behaviour suggested the involvement of adsorption in the electrode reaction,[17] which was further confirmed by the increase in peak current function $(i_p/V)^{\frac{1}{2}}$ with increase in sweep rate[18] as shown in Fig. 3(*b*). The peak potential of peak I_a was also found to shift to a more positive potential with increase in sweep rate. Fig. 3(*c*) presents the plot of E_p *vs.* log V and the shift in E_p is consistent with an EC behaviour. The linear regression analysis has a slope of 0.030 and intercept 0.867 [Fig. 3(*c*)].

Hydroxyadenines. The solubility of hydroxyadenines in aqueous buffers was much less than for adenine, and hence studies on 2-hydroxyadenine were carried out at 0.25 mmol dm^{-3}. For 8-hydroxyadenine and 2,8-dihydroxyadenine, saturated solutions of less than 0.10 mmol dm^{-3} (at room temperature) were used. In cyclic voltammetry the first sweep initiated in a positive direction yielded an anodic peak II_a and on the reverse cathodic sweep two cathodic peaks II_c (at pH < 4.0) and III_c (in the entire pH range studied) were observed. In the subsequent sweep towards positive potential a new anodic peak (III_a) was observed which formed a quasi-reversible couple with peak III_c. Potentials of peak II_a have shown the pH dependence to be practically identical with that found for adenine except that a break at pH 4.3 is observed. The plots of E_p *vs.* pH for peak III_a differed and can be described by the equations given in Table 1. The anodic peak II_a was complicated by adsorption phenomena,[17] as with the corresponding peak for adenine. The shift of E_p to more positive potentials with increase in sweep rate was somewhat smaller with $dE_p/d\log V = 0.014$ V s^{-1}.

The linear sweep voltammetry of 8-hydroxyadenine at 5 mV s^{-1} gave one well-defined, pH-dependent oxidation peak (V_a) in the entire pH range studied. In cyclic voltammetry at a sweep rate of 100 mV s^{-1} this peak V_a was accompanied on the reverse sweep by three cathodic peaks III_c, IV_c and V_c. Peak III_c formed a quasi-reversible couple with peak III_a observed in the subsequent sweep towards positive potentials at pH below 7.0, whereas at higher pH peak III_c was not observed, even at a higher sweep rate (20 V s^{-1}). The shift of the potential of peak V_a to more negative values with increasing pH showed two linear segments, following the equations presented in Table 1. The intersection of these two segments at pH 5.8 corresponds to a pK_a value of the reduced (*i.e.*, initial) form of 8-hydroxyadenine.

The peak height of peak V_c also increased with increase in sweep rate and the ratio of V_c/V_a increased from 0.02 to 0.20 with increase in sweep rate from 100 mV s^{-1} to 20 V s^{-1}. The species formed in the oxidation process corresponds to peak V_a and is reduced in peak V_c, hence this undergoes a fast chemical reaction. This competing process prevented occurrence of this peak at sweep rates below 100 mV s^{-1}. The increase in the value of peak current function with increasing sweep rate at pH 3.0 and 7.0 indicated that the process is complicated by adsorption. 2,8-Dihydroxyadenine is oxidized in a single anodic peak III_a, the potential of which (pH 3.0–11.2) follows the equation presented in Table 1. In a reverse sweep in cyclic voltammetry a

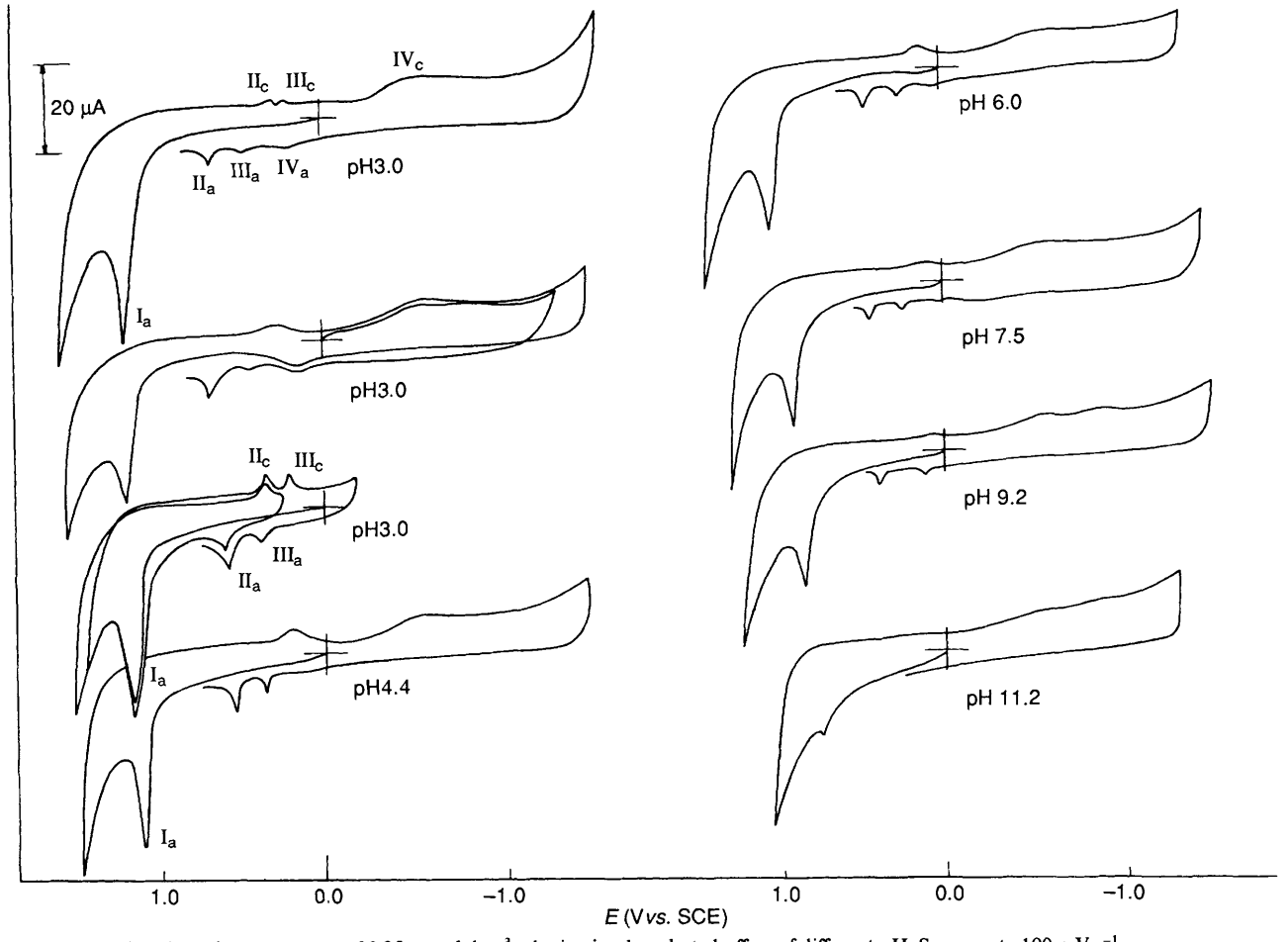


Fig. 2 Typical cyclic voltammograms of 0.25 mmol dm^{-3} adenine in phosphate buffers of different pH. Sweep rate 100 mV s^{-1}.

Table 1 Observed E_p *vs.* pH relations for the oxidation peaks of adenine and hydroxyadenines

Compound	Oxidation peak	E_p, pH relation, V *vs.* SCE
Adenine	I_a	E_p (pH 3.0–11.2) = (1.31 − 0.055 pH)
	II_a	E_p (pH 3.0–8.0) = (0.78 − 0.052 pH)
		E_p (pH 8.0–11.2) = (0.42 − 0.005 pH)
	III_a	E_p (pH 3.0–9.2) = (0.58 − 0.051 pH)
2-Hydroxyadenine	II_a	E_p (pH 3.0–4.4) = (1.08 − 0.025 pH)
		E_p (pH 4.4–9.0) = (0.80 − 0.055 pH)
	III_a	E_p (pH 3.0–11.2) = (0.60 − 0.036 pH)
8-Hydroxyadenine	V_a	E_p (pH 3.0–5.8) = (0.78 − 0.095 pH)
		E_p (pH 5.8–11.2) = (0.64 − 0.043 pH)
2,8-Dihydroxyadenine	III_a	E_p (pH 3.0–11.2) = (0.058 − 0.033 pH)

well-defined quasi-reversible reduction peak (III_c) is observed sometimes accompanied by a round peak at a more negative potential. Deviations from a linear $i_p V^{-1} = f(\log V)$ plot at pH 3.0 and 7.0 indicated strong adsorption of 2,8-dihydroxyadenine at the surface of the PGE. The limited solubility of this compound prevented studies involving variations in concentration of the 2,8-dihydroxyadenine.

Coulometric Studies.—Controlled potential electrolysis of adenine and hydroxyadenines in phosphate buffers of different pH were carried out at potentials more positive than the oxidation peak. The nature of the plot of decrease in current with time for all the compounds was essentially similar. The plot of $\log i_p = f(t)$ for the oxidation peak of adenine was a straight line for the first 20 min of electrolysis ($\Delta \log i_p/\Delta t = 0.020$) and thereafter a large deviation was observed. This behaviour clearly indicated that oxidation followed a single path up to *ca.* 20 min and is an indication of follow-up chemical reactions as suggested by Cauquis *et al.*[19] Table 2 presents the coulometric *n* value observed for various adenines at different concentration and pH. The oxidation of all these compounds was very slow and usually required more than 6 h for complete oxidation. Even a 0.25 mmol dm^{-3} solution of adenine required electrode cleaning several times due to strong adsorption. The coulometric *n* value observed clearly indicates that oxidation of adenine involves close to six electrons in the entire pH range, whereas 2- and 8-hydroxyadenines were found to involve 4.0 ± 0.20 (average ± standard deviation). 2,8-Dihydroxy-

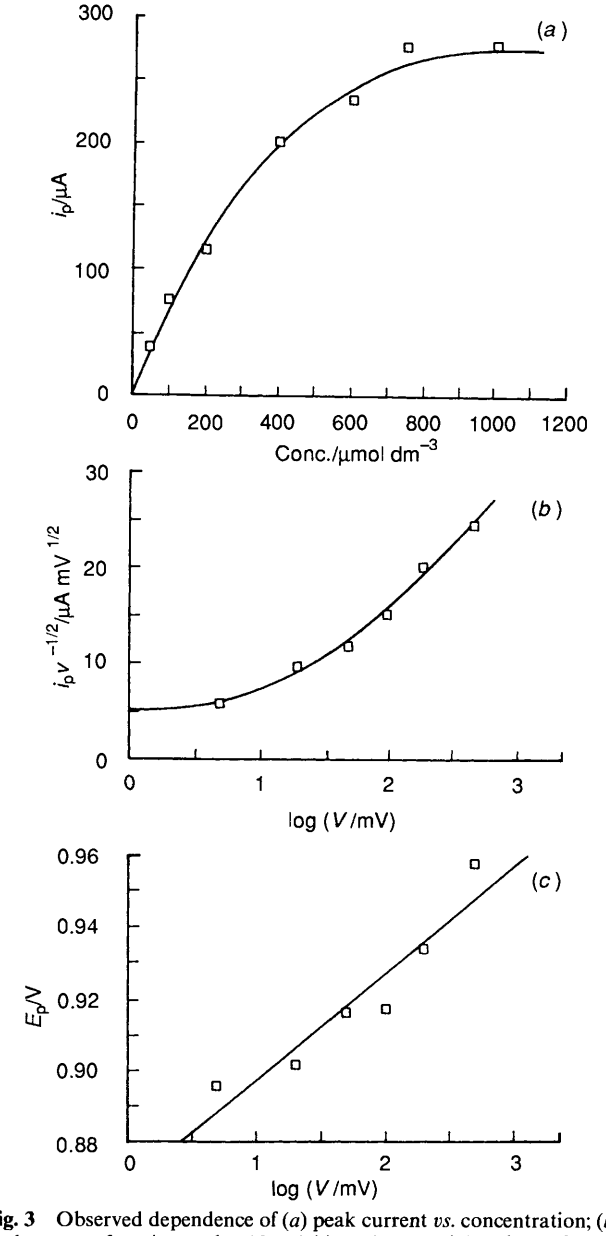


Fig. 3 Observed dependence of (*a*) peak current *vs.* concentration; (*b*) peak current function *vs.* log *V* and (*c*) peak potential *vs.* log *V* for the oxidation peak I_a of adenine

adenine was found to involve only 2.0 ± 0.30 electrons to give the products. The large deviation in the case of 2,8-dihydroxyadenine is probably due to the small concentration of the species in the solution and hence required a large background correction. The progress of electrolysis of adenine and hydroxyadenines was monitored by recording cyclic volammograms at different time intervals at pH 3.0, 7.0 and 10.2. It was observed that all the four compounds exhibited an almost identical behaviour in which the main oxidation peak systematically decreased and no new peak appeared in the cyclic voltammograms. In exhaustively electrolysed solutions there was neither an electroactive species present, nor was one formed on standing for several hours at 25 °C.

Spectral Studies.—The UV spectra of all four adenines were recorded at different pH values to get an idea of the pK_a of these molecules. From the dependence of λ_{max} on pH, the pK_a values for adenine (4.2 and 9.6), 2-hydroxyadenine (4.4 and 9.1), and 8-hydroxyadenine (5.8) were determined. Similar studies on 2,8-dihydroxyadenine could not be made because of poor solubility. The progress of electrolysis was monitored by recording UV

Table 2 Observed coulometric *n* values for the oxidation of adenine and hydroxyadenines

Compound	pH	Potential V *vs.* SCE	Experimental *n* value[a]
Adenine	3.0	1.2	6.10
(0.5 mmol dm^{-3})	4.4	1.2	6.16
	6.0	1.0	5.90
	7.5	1.0	6.20
	9.0	0.9	6.14
2-Hydroxyadenine	3.0	1.2	4.10
(0.1 mmol dm^{-3})	4.4	1.2	3.86
	6.0	1.0	3.92
	7.0	1.0	4.14
	7.5	1.0	3.92
8-Hydroxyadenine	3.0	1.2	4.16
(0.01 mmol dm^{-3})	4.4	1.2	4.20
	6.0	1.0	3.86
	7.0	1.0	4.28
	7.5	1.0	4.12
2,8-Dihydroxyadenine	3.0	1.2	2.22
(0.01 mmol dm^{-3})	4.4	0.8	1.96
	6.0	0.8	2.28
	7.0	0.8	2.24

[a] Average of at least two replicate determinations.

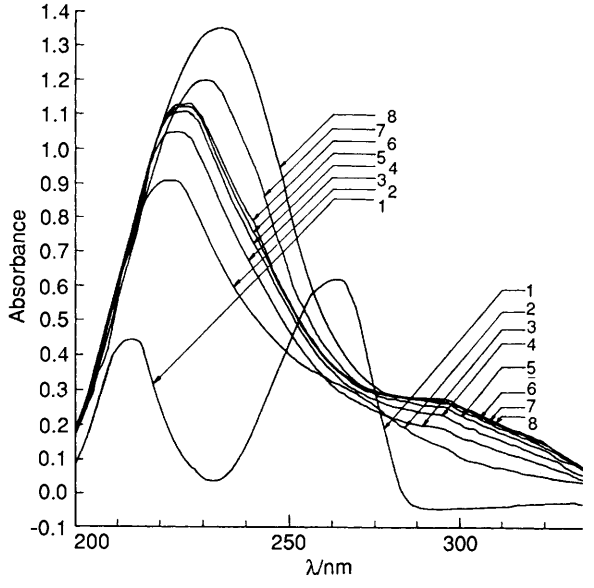


Fig. 4 Spectral changes observed during oxidation of 0.05 mmol dm^{-3} solution of adenine at pH 3.0. Curves 1–5 were recorded at an interval of 10 min. Curve 6 (1 h); 7 (2 h) and 8 (6 h) of electrolysis. Potential 1.2 V *vs.* SCE.

spectra at different time intervals. For example, the UV spectrum of 0.05 mmol dm^{-3} adenine at pH 3.0 is presented by curve 1 in Fig. 4 and exhibited two λ_{max} values at 215 and 263 nm. Upon application of a potential more positive to peak I_a, the broad bands of individual species—both in the region of 210–240 and 270–320 nm regions—overlap and deconvolution yielding the concentration of individual species would be difficult. Overlap of several bands results in the apparent shift of λ_{max} in the 220–240 nm range as electrolysis proceeds. Thus, Fig. 4 indicates two interesting facts: (*a*) very little of the original adenine is present after 10 min of electrolysis (curves 1 and 2); and (*b*) the absence of an isosbestic point at *ca.* 270 nm indicates the presence of several species formed in parallel or competing reactions. In the case of 2-hydroxyadenine two UV bands at 212 and 287 nm were observed before electrolysis. As electrolysis

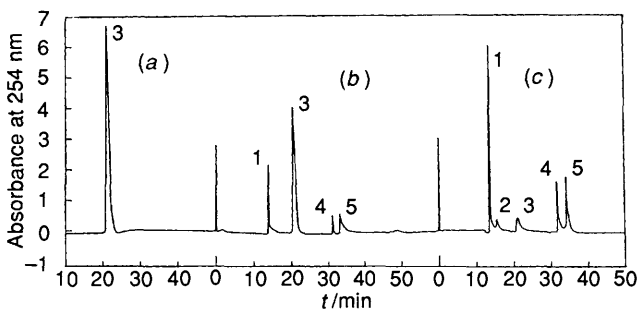


Fig. 5 HPLC chromatogram observed during oxidation of adenine at different times of electrolysis at pH 3.0: (*a*) 0 min; (*b*) 1 h; and (*c*) after 6 h of electrolysis

proceeded, the absorbance at 287 nm first decreased (for 20 min) and then increased systematically. After 2 h electrolysis a band at *ca.* 230–234 nm and a shoulder at 285–290 nm was observed. Similar studies on 8-hydroxyadenine gave a UV spectrum with a band at *ca.* 220–224 and a shoulder in the region 285–290 nm in the exhaustively electrolysed solution. 2,8-Dihydroxyadenine (λ_{max} 222, 238 and 308 nm) on electrolysis indicated a continuous decrease in absorbance at λ_{max}, whereas the absorbance at 215–260 nm decreased for *ca.* 30 min and then increased. The absorbance in the longer wavelength region (330–350 nm) showed a continuous decrease. An exhaustively electrolysed solution of 2,8-dihydroxyadenine showed a band at 228–232 nm and a shoulder at *ca.* 290–296 nm. Thus, it was observed that the exhaustively electrolysed solutions of all the four adenines gave almost identical spectra at pH 3.0.

Spectral changes during the oxidation of adenine and hydroxyadenines were also recorded at pH 7.5. The observed changes for all the four compounds indicated an almost identical trend as that found at pH 3.0. Thus, for example, adenine first indicated a decrease in absorbance at λ_{max}(263 nm) for *ca.* 20 min followed by a systematic increase to give a broad band at *ca.* 224 nm after 2 h electrolysis. Hydroxyadenines also exhibited identical spectra in the exhaustively electrolysed solutions, indicating thereby the formation of the same products at pH 7.5.

Product Identification.—The products of electrooxidation of adenine and hydroxyadenines were identified at pH 3.0 and 7.5. It was interesting to observe that all four compounds gave essentially the same products. Some typical results observed during separation and identification using gravity flow sephadex G-10 column and HPLC are summarized.

The freeze-dried material obtained after lyopholization of an exhaustively electrolysed solution of adenine at pH 7.5 on passing through a Sephadex G-10 column (see experimental) gave 3 peaks. Peaks 1 and 2 were due to phosphate and hence were discarded. The volume collected under peak 3 exhibited a broad UV band at 200–220 nm. The lyopholized white dried material obtained had m.p. 239 °C. The mass spectrum of the product exhibited a clear molecular ion peak at *m/z* 158 and indicated the compound to be allantoin. The high mass peaks observed in the spectrum were at 156 (5.1%), 141 (7.3%), 130 (34.1%), 115 (16.2%), 114 (8.2%), 113 (4.8%), 100 (4.4%), 99 (4.1%), 98 (14.8%) and 87 (55.2%). The ^{1}H NMR spectrum of the material indicated signals at δ = 5.28 (d, 1 H); 5.8 (s, 3 H); 6.89 (d, 1 H); 8.04 (s, 1 H) and 10.52 (s, 1 H) and hence suggested the structure as allantoin. A comparison of the IR spectra of the material obtained and an authentic sample of allantoin indicated them to be superimposable and hence further supported the identification.[20] Hydroxyadenines also indicated the formation of the same product at pH 7.5.

In contrast to pH 7.5, where only one oxidation product was identified, the LC of adenine oxidation products at pH 3.0 exhibited two peaks in addition to phosphate peaks. The freeze-dried material obtained under these peaks exhibited R_f = 0.40 and 0.62 by TLC. The material with R_f = 0.40 was a white solid with m.p. 237 °C and was characterized as alloxan by comparing the IR spectra and m.p. with an authentic sample. The second product had m.p. 242 °C and was identified as parabanic acid by comparing the UV and IR spectra and m.p. with an authentic sample.

The formation of alloxan and parabanic acid suggested the formation of urea as the third product at pH 3.0. However, the peak of urea in LC was not observed in the case of adenine or hydroxyadenines, probably because urea elutes with phosphate due to its low molecular weight. A similar observation has also been reported in the literature for a variety of purines and thiopurines.[21–24] Hence, at this point it was felt necessary to use HPLC to detect the formation of all the products of the oxidation of adenine and hydroxyadenines at pH 3.0. To eliminate the difficulty of buffer constituents, the studies were carried out in a dilute solution of HCl (pH 3.0) in which the cyclic voltammetric behaviour was similar to the pH 3.0 buffer and hence it was believed that the product formation would not be affected by the change from pH 3.0 (HCl) to pH 3.0 (buffer). The HPLC chromatogram of adenine just before oxidation at pH 3.0 is presented in Fig. 5(*a*) and exhibits a peak at 21 min. With the progress of electrolysis three new peaks were observed (peaks 1, 4 and 5), which increased with time [Fig. 5(*b*) and (*c*)]. After *ca.* 2 h of electrolysis an additional small peak at *ca.* 12 min was also noticed. As the eluting solvents contained only acetonitrile, the volume collected under different peaks was directly lyopholized and analysed by analytical techniques. The volume collected under peak 2 was never enough to give reliable mass or ^{1}H NMR spectra and hence it was analysed by LC-MS, whereas all other peaks were analysed by UV, MS and m.p. determination.

The UV spectrum of HPLC peak 1 in eluting solvent did not indicate any band. The white dried material had m.p. 132 °C and exhibited a clear molecular ion peak at *m/z* = 60 (100%) with another high mass peak at 44 (89%). This material was identified as urea by comparing its m.p. with that reported in the literature.[25]

The volume collected under HPLC peak 2 was very small and gave a band at *ca.* 286 nm in the UV spectrum. The LC-MS of this peak exhibited a pseudo-molecular ion peak at *m/z* 152 (100%; MH^+); peaks at 303 (32.4%; $2M + H^+$) and 454 (8.5%; $3M + H^+$) were also noticed. Hence the molar mass of the material was 151, which corresponds to either 2- or 8-hydroxyadenine. To confirm the formation of 2- or 8-hydroxyadenine from the oxidation of adenine, the retention time of both the hydroxyadenines were determined. It was found that 2-hydroxyadenine elutes at *ca.* 12 min, whereas 8-hydroxyadenine elutes at 18 min under similiar HPLC conditions and hence it was concluded that peak 2 is due to the formation of 2-hydroxyadenine.

HPLC peak 4 exhibited a broad band at *ca.* 200–225 nm in the UV spectrum and this UV spectrum was similar to that reported for parabanic acid in the literature. The dried material obtained had a m.p. 244 °C and gave a clear molecular ion peak at *m/z* = 114, indicating the product to be parabanic acid. The other high molecular mass peaks observed in the fragmentation were at 87 (1.8%); 86 (58.8%); 71 (5.2%) and 70 (12.2%). The UV spectrum of HPLC peak 5 also gave a broad band with λ_{max} 210 nm. The white freeze-dried material had m.p. 254 °C and exhibited a molar mass of 141.9 (MS) and was identified as alloxan[26] by comparison with an authentic sample.

2-Hydroxy-, 8-hydroxy- and 2,8-dihydroxy-adenines also gave the same products at pH 3.0 and thus clearly indicated that the ultimate products of the oxidation of adenine and hydroxyadenines are identical. It was interesting to observe

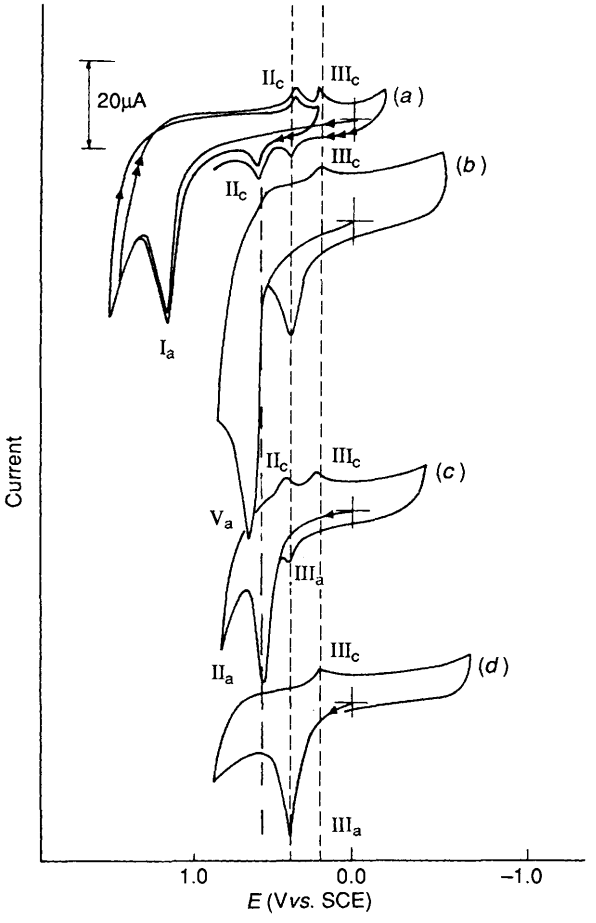
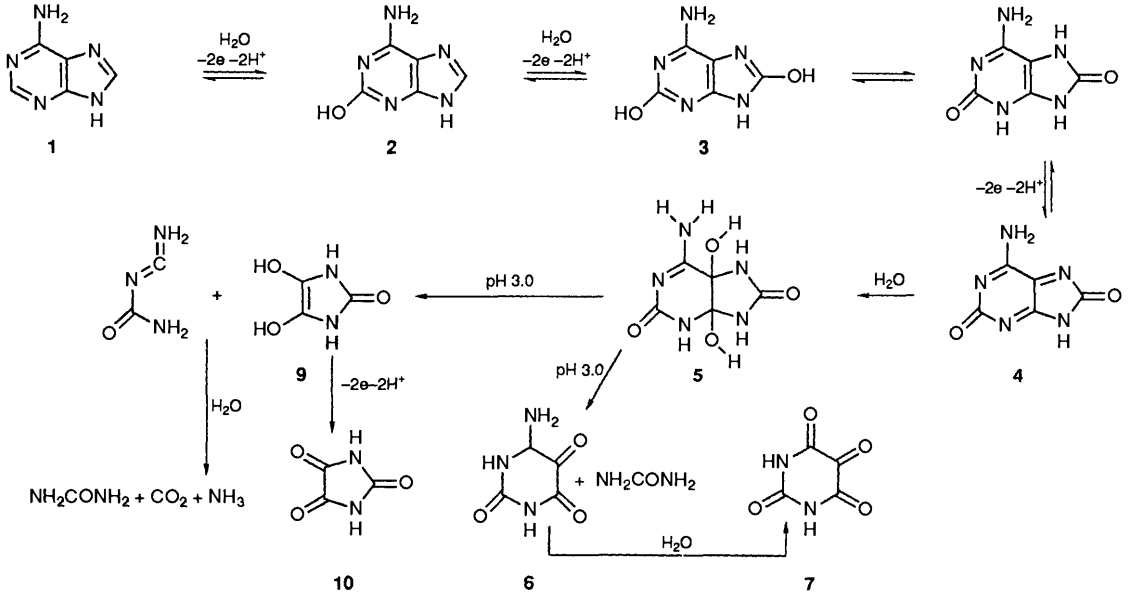


Fig. 6 A comparison of peak potentials of the cyclic voltammograms of (*a*) adenine; (*b*) 8-hydroxyadenine; (*c*) 2-hydroxyadenine; and (*d*) 2,8-dihydroxyadenine at pH 3.0. Sweep rate 100 mV s^{-1}.

that the peak of 2,8-dihydroxyadenine was never observed in HPLC during the oxidation of adenine or 2- or 8-hydroxyadenines. One of the possible reasons for the non-appearance of 2,8-dihydroxyadenine is that it is oxidized at a less positive potential (at least 500 mV less) than adenine and is hence immediately oxidized as soon as it is formed at the surface of the electrode to give the products.

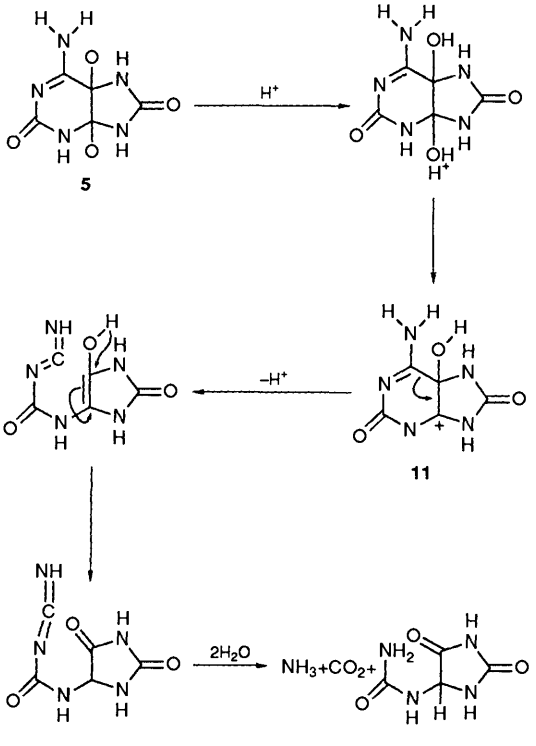
Reaction Scheme.—The above studies clearly indicated that the oxidation of adenine is a 6e, $6H^+$ process *via* the formation of 2-hydroxyadenine to give parabanic acid, alloxan and urea at pH 3.0 and allantoin at pH 7.5. In order to further confirm the formation of 2-hydroxyadenine during the oxidation of adenine, a comparative cyclic voltammetric behaviour of all the four adenines at pH 3.0 is presented in Fig. 6, and it can be deduced that the peak potentials of peaks III_c and III_a of adenine are similar to 2,8-dihydroxyadenine. The oxidation peaks III_a and III_c of 2- and 8-hydroxyadenines also corresponded to the formation of 2,8-dihydroxyadenine. The main oxidation peak of 8-hydroxyadenine (V_a) was always found at a higher positive potential (50–75 mV) than peak II_a of adenine. On the other hand, the peak potential of the oxidation peak of 2-hydroxyadenine was always similar to peak II_a of adenine and hence it further supported the view that the first 2e, $2H^+$ oxidation of adenine gives 2-hydroxyadenine and not 8-hydroxyadenine. The limited solubility of 8-hydroxy- and 2,8-dihydroxyadenines did not permit a comparison of peak currents at the same concentration for all four compounds; however, the ratio of peak currents for adenine and 2-hydroxyadenine was always found to be 3:2.

To propose the reaction scheme it is necessary to rationalize the observed cyclic voltammetric, coulometric and spectral behaviour and the products formed. It seems reasonable to conclude that adenine (**1**) initially undergoes a 2e, $2H^+$ oxidation to give 2-hydroxyadenine (**2**), which has been detected by HPLC. The 2e, $2H^+$ oxidation of 2-hydroxyadenine then gives 2,8-dihydroxyadenine (**3**) which has been confirmed by the anodic peaks in cyclic voltammograms. As 2,8-dihydroxyadenine is more readily oxidized than adenine or 2-hydroxyadenine it rapidly oxidizes in a 2e, $2H^+$ step to give the corresponding diimine **4**. The formation of diimine and its very short half-life (30–40 ms) has been reported in the case of various purines[21–24] at gold, platinum or carbon electrodes. Thus, it is believed that such a diimine will be readily attacked by water to give the 4,5-diol species **5**. The decomposition of diol **5** at pH 3.0 can occur by two different courses to give alloxan or parabanic acid as the major products. In the first course, the cleavage in the five-membered ring will give urea and **6**, which



Scheme 2 Tentative mechanism proposed for the formation of alloxan, parabanic acid and urea from the oxidation of adenine at pH 3.0

on hydrolysis gives alloxan and ammonia, as shown in Scheme 2. In the second course of decomposition, the cleavage in the six-membered ring will lead to the formation of species **8** and **9**. The hydrolysis of **8** will readily give carbon dioxide, ammonia and urea, whereas 4,5-dihydroxy-3*H*-imidazol-2(1*H*)-one (**9**) is easily oxidizable in a 2e, $2H^+$ process to give parabanic acid (**10**). It must be stressed, however, that the formation of parabanic acid from adenine is an overall 8e, $8H^+$ reaction and hence the formation of parabanic acid from adenine or hydroxyadenines is only a side reaction observed under controlled potential electrolysis conditions. The formation of alloxan from adenine has not been reported previously. In contrast to pH 3.0, only one product was formed from the oxidation of adenine or hydroxyadenines at pH 7.0. The diol **5** again serves as the precursor, and is attacked by a proton and loses a water molecule, thereby forming a carbonium ion species **11**. The shift of hydrogen followed by hydrolysis gives allantoin (**12**), ammonia and carbon dioxide as shown in Scheme 3. The formation of the above products indicated that compound **5** at pH 7.0 fragments following protonation to give allantoin, but under acidic conditions (pH 3.0) the fragmentation of protonated **5** occurs in the imidazole ring giving alloxan upon hydrolysis. Such hydrolysis reactions of purine derivatives in acidic and alkaline media to cause the opening of pyrimidine or imidazole rings to give the corresponding products is well documented in the literature.[27–30] Hence, the formation of products observed during the oxidation of adenine or hydroxyadenines is in accordance with the properties of purines. Reaction Schemes 2 and 3 suggested for the formation of products are the most probable routes possible for their formation; however, it must be realized that more than one pathway is always possible for the formation of these products. A comparison of electrochemical oxidation of adenine with radiation[31,32] as well as photochemical oxidation[33,34] clearly indicated that the products of electrochemical oxidations are different. In the case of photochemical oxidation, hypoxanthine has been suggested as the major product whereas in the case of radical oxidation attack at the 4,5-bond is suggested to cause ring opening and ring rearrangement. No information on the formation of 2- or 8-hydroxyadenine as an intermediate has been presented during the oxidation of adenine in the above studies. The present studies, on the other hand, clearly established that the first 2e, $2H^+$ oxidation of adenine gives 2-hydroxyadenine which subsequently oxidized in a 2e, $2H^+$ reaction to 2,8-dihydroxyadenine and then *via* a diimine species to yield the final products.



Scheme 3 Tentative mechanism proposed for the formation of allantoin from the oxidation of adenine at pH 7.5

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