Comparison of Electrochemical and Enzymic Oxidation of 3-Methyluric Acid

Rajendra N. Goyal and (Miss) Madhu Shri Verma
Department of Chemistry, University of Roorkee, Roorkee-247 667, India

The electrochemical oxidation of 3-methyluric acid has been studied in the pH range 3.2–11.3 at pyrolytic graphite and glassy carbon electrodes. The conjugate base is the species oxidized over the whole pH range studied. Intermediates generated have been characterized in terms of their UV spectra and kinetics of decay, and products have been separated and characterized. The intermediates, formed and spectral and kinetics studies, during the peroxidase-catalysed oxidation of 3-methyluric acid indicated identical behaviour to that observed in electrochemical oxidation. It has thus been concluded that the electrochemical and enzymic oxidation of 3-methyluric acid proceed by an identical EC mechanism.

Electrochemical behaviour of biologically important compounds provides useful insights into the generally complex mechanisms of enzymic and perhaps *in vivo* redox reactions. The ongoing studies of McCreery and co-workers [1–3] on the electrochemical oxidation of phenothiazine tranquilizer drugs and Dryhurst [4,5] and others [6,7] on the electrochemical oxidation of indolic neurotransmitters represent examples of biologically relevant information which can be deduced from electrochemical studies. In recent years the electrochemical oxidation of naturally occurring purines has attracted considerable attention, and the pathway and the products for peroxidase, xanthine oxidase and cytochrome P-450 oxidation of purines was found to be similar to the electrochemical oxidation.[8–10] As determination of purines in body fluids has been considered as an indicator of ATP (adenosine triphosphate) depletion,[11] it was considered of interest to study the electroxidation of 3-methyluric acid at solid electrodes.

This paper presents our results on the electrochemical oxidation of 3-methyluric acid at glassy carbon electrode (GCE) and pyrolytic graphite electrode (PGE) using a variety of electroanalytical, spectroscopic and analytical techniques. A comparison of electrochemical and enzymic oxidation of 3-methyluric acid is also presented.

Experimental

3-Methyluric acid was obtained from Adams Chemical Co. (Round Lake, IL) and was used without further purification. *N,N*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and silylation grade acetonitrile were obtained from Supelco, USA. Type VIII peroxidase (R_z *ca.* 3.4) isolated from horseradish peroxidase was obtained from Sigma Chemical Co. and catalase was a product of CSIR Centre for biochemicals, New Delhi.

The instrumentation used for electrochemical studies and method for the fabrication of electrodes were essentially the same as have been described earler.[12,13] The area of electrodes was measured using a potentiostatic method with 4.0 mmol dm^{-3} potassium ferricyanide in 0.5 mol dm^{-3} KCl as reported by Adams.[14] The electrodes were polished on a 600 grit metallographic polishing disc after each voltammogram. This polishing procedure resulted in new mirror-like surface each time. For determining peak current values an average of at least three runs was taken.

UV and IR spectra were recorded using a Beckmann DU-6 spectrophotometer and a Perkin-Elmer 1600 FT-IR spectrometer respectively. Gas chromatography–mass spectrometry was carried out on a Hewlett Packard 5985 B instrument equipped with 3% SE-30 columns. Electron impact (EI) mass spectra were recorded at an electron beam voltage of 70 eV and chemical ionization (CI) mass spectra utilized methane as the reagent gas at a pressure of 2×10^{-4} torr in the source chamber at an electron beam voltage of 150 V. TLC was carried out using Silica Gel-G plates with methanol–benzene (3:1) as irrigant.

Procedure.—The stock solution (1 mmol dm^{-3}) of 3-methyluric acid was prepared in doubly distilled water. Experiments were carried out in phosphate buffers[15] of ionic strength 0.2 mol dm^{-3}. The solutions for recording voltammograms were prepared by mixing 2 cm^3 of the stock solution with 2 cm^3 of the buffer of appropriate pH. Nitrogen gas was bubbled for 5–8 min before recording the curves. All potentials are referred to the SCE at an ambient temperature of 20 ± 2 °C.

Controlled potential electrolysis of 3-methyluric acid at potentials 50 mV more positive than the peak potentials were carried out in a three-compartment cell. Pyrolytic graphite plate (6×1 cm^2) was used as a working electrode, cylindrical platinum gauze as auxilliary and SCE as reference electrode. The value of n, the number of electrons involved in oxidation, was determined by graphical integration of the current–time curve as reported by Lingane.[16]

The enzymic oxidation of 3-methyluric acid was followed in 1.0 cm quartz cells. 1.0 cm^3 of 3-methyluric acid (0.2 mmol dm^{-3}) was mixed with 0.25 cm^3 of horseradish peroxidase (1 μmol dm^{-3}, molecular wt. 20 000) prepared in phosphate buffer having an ionic strength of 0.2 mol dm^{-3}. The enzymic reaction was initiated by addition of 0.5 cm^3 of H_2O_2 (6 mmol dm^{-3}) prepared in the buffer of same pH. When the absorbance at λ_{max} reached 50%, the enzymic oxidation was terminated by addition of 0.1 cm^3 of catalase solution (1 mg, cm^{-3}). All species involved in the enzymic reaction, except 3-methyluric acid, were present in the reference cell.

Product Characterization.—Products of electrooxidation of 3-methyluric acid were characterized at pH 3.0 and 7.0. For this purpose 8–10 mg of the material was oxidised at PGE. The progress of electrolysis was monitored by recording cyclic voltammograms at different time intervals. When the peak current decreases to about 5–10%, electrolysis was discontinued and the electrolysed solution was removed from the cell and lyophilized. The freeze dried material obtained was dissolved in 1–2 cm^3 of water and passed through a glass column (75×15 cm^2) packed with sephadex G-10 (Sigma, bead size 40–120 μm). Distilled water was used as the eluent and fractions of 5 cm^3 each were collected using a SICO fraction collector model FRAC 711. The absorbance of each fraction was monitored at 210 nm and the absorbance *versus* volume plot exhibited three

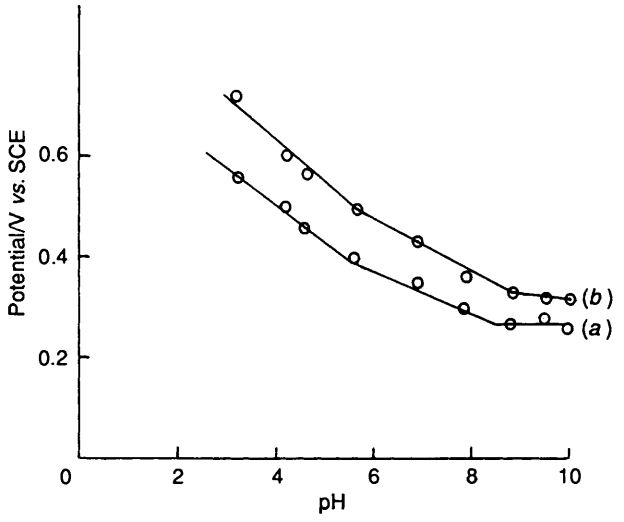


Fig. 1 Variation of the peak potential (E_p) with pH for the voltammetric oxidation of 0.5 mmol dm^{-3} 3-methyluric acid

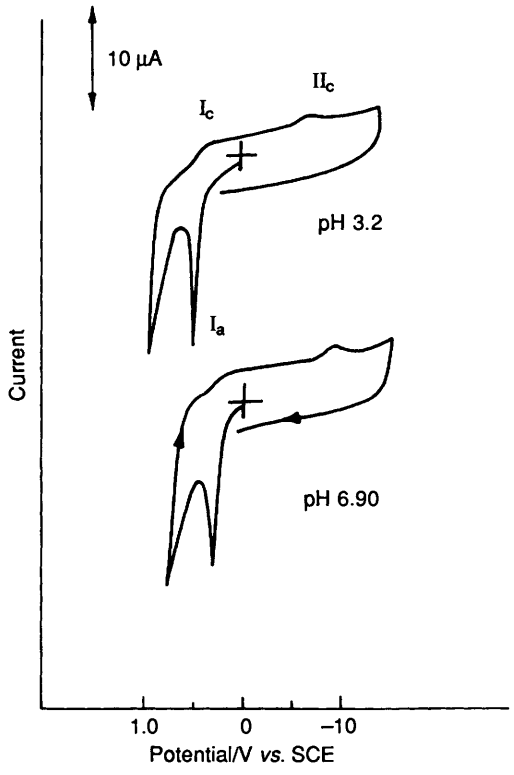


Fig. 2 Cyclic voltammograms of 0.2 mmol cm^{-3} 3-methyluric acid in phosphate buffers of different pH; μ = 0.1 mol dm^{-3}, sweep rate = 150 mV s^{-1}

peaks at pH 3.0. Peaks 1 and 2 (P1, P2) were obtained between 180 and 205 cm^3 and were closely overlapping. These peaks were found to be due to phosphate and hence discarded. The volume collected between 230 and 265 cm^3 (peak 3) was freeze dried and the colourless material obtained was analysed by m.p., IR and mass spectra. In contrast to pH 3.0, only one broad peak was observed, apart from the phosphate peak, in the liquid chromatography at pH 7.0. The first peak between 185 and 205 cm^3 was due to phosphate and hence rejected. The volume collected under peak P4 (220–275 cm^3) was lyophilized and analysed.

The partially separated oxidation products were converted to a volatile species by a formation of trimethylsilyl derivatives. For this purpose about 100–200 μg of the product was treated with 50 mm^3 of acetonitrile and 50 mm^3 of silylating reagent, bis(trimethylsilyl)trifluoroacetamide (BSTFA) in a 3.0 cm^3 vial. The vial was tightly closed and heated in an oil bath at 110 °C for 10–15 min. After cooling, GC-mass spectral studies were carried out.

Results and Discussion

Linear and Cyclic Sweep Voltammetry.—Linear sweep voltammetry of 3-methyluric acid in the pH range 3.2–11.3 exhibited one well-defined oxidation peak (Ia) at PGE and glassy carbon electrode at a sweep rate of 20 mV s^{-1}. The peak at glassy carbon electrode was found to be broad in comparison to PGE. The peak potential of the oxidation peak Ia was dependent on pH and shifted towards less positive potential with increase in pH. Nevertheless, the peak potential of the oxidation peak Ia at GCE was always found to be about 100–125 mV more positive than at PGE. The E_p *versus* pH plots at both electrodes exhibited two breaks at around 5.6 and 8.6 (Fig. 1), which correspond to the pK_a of 3-methyluric acid as reported in the literature.[17] The dependence of E_p on pH can be represented by the following equations:

$$E_p \text{ (pH 3.2–5.6)} = [0.95–0.08 \text{ pH}] \text{ V at GCE}$$
$$E_p \text{ (pH 5.6–8.6)} = [0.78–0.053 \text{ pH}] \text{ V}$$
$$E_p \text{ (pH 3.2–5.6)} = [0.79–0.071 \text{ pH}] \text{ V at PGE}$$
$$E_p \text{ (pH 5.6–8.6)} = [0.62–0.039 \text{ pH}] \text{ V}$$

At pH > 8.6, the peak potential became practically independent of pH. The dependence of E_p on pH indicate that the conjugate base is the species oxidized over the whole pH range studied. This is the species predominating at pH > 8.6, which is rapidly formed from conjugate acid at pH 5.6–8.6 and from species bearing two protons more than the conjugate base at pH < 5.6. The peak current of the oxidation peak Ia was independent of pH in the range 3.2–11.3, indicating that even at pH 3.2 the formation of conjugate base is fast enough.

In cyclic sweep voltammetry at a sweep rate of 150 mV s^{-1} 3-methyluric acid exhibits a well-defined anodic peak Ia. During the reverse sweep two cathodic peaks (Ic and IIc) were observed. Some typical cyclic voltammograms are presented in Fig. 2. Peak Ic formed a quasi-reversible couple with peak Ia and the peak current of peak Ic increased with increase in sweep rate. The ratio of peaks Ic/Ia increased with increase in sweep rate and reached to 0.30 at a sweep af 1.0 V s^{-1} (Table 1). The ratio of peak currents for the peaks Ic/IIc was found to be 0.5 and did not change with increase in concentration of 3-methyluric acid in the concentration range 0.01–0.5 mmol dm^{-3}. Peak current for peak IIc was dependent on the sweep rate. The plot of $i_p = f(V)$ was found to be linear in the sweep range 10–500 mV s^{-1}. However, the ratio Ic/IIc increased from 0.5 to 1.0 when the sweep rate was changed from 10 mV s^{-1} to 500 mV s^{-1}. This behaviour indicated that the product of peak Ic reaction is unstable and competitive chemical reactions play a significant role.

Though the peak current of peak Ia was practically independent of pH, the (i_p) Ic was strongly dependent on pH. The ratio of Ia/Ic was also found to be dependent on pH, and the value decreased with increase in pH up to 7.0 and then increased again (Table 2). The dependence of peak Ic on pH clearly indicated that the reaction of the species involved in peak Ic reduction is acid–base-catalysed. The effect of concentration of 3-methyluric acid on peak Ia was studied in the concentration range 10–700 μmol dm^{-3} at both the electrodes. It was observed that peak current increased with increasing concentration of 3-methyluric acid. The plot of i_p *vs.* concentration was linear up to 0.4 mmol dm^{-3} (Fig. 3) and (i_p) Ic remained constant at higher concentrations. This behaviour indicated the involvement of

Table 1 Peak current values observed at different sweep rates for the redox couple Ic/Ia at pH 6.95

Sweep rate/mV s^{-1}	Ia/μA	Ic/μA	Ic/Ia[a]
5	2	—	—
10	6	0.5	0.07
20	8	0.7	0.08
50	10	1.0	0.10
100	12	1.6	0.14
200	23	4.0	0.17
500	56	20.0	0.28
1000	138	41.0	0.33

[a] Average of at least three replicate determinations.

Table 2 Peak current values observed for peaks Ia and Ic for 0.2 mmol dm^{-3} 3-methyluric acid at different pH values

pH	Ia/μA	Ic/μA	Ia/Ic[a]
3.20	45	6	7.5
4.17	40	6	6.6
4.62	40	6	6.6
5.56	45	7	6.4
6.95	45	6	7.3
7.86	40	4	10.0
8.80	35	3	11.6
9.52	45	4	11.2

[a] Average of at least three replicate determinations.

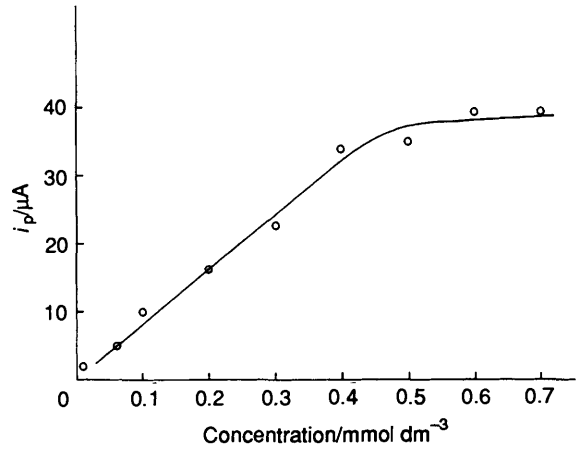


Fig. 3 Peak current *versus* concentration behaviour for the peak Ia oxidation of 3-methyluric acid at PGE; pH = 7.0

adsorption [18,19] in the electrode reaction. The role of adsorption was further confirmed by the linear dependence of peak current (i_p) on the sweep rate (Fig. 4).

The peak potential of peak Ia was also found to shift to more positive potential with increase in sweep rate in the range 5–1.0 V s^{-1} at both the electrodes. The E_p was found to shift 15 mV per ten-fold increase in sweep rate at low sweep values (5–200 mV s^{-1}) whereas the shift decreased to 10 mV at higher sweep rates. The plots of the ratio of Ia/Ic *versus* log V and $\Delta E_{p/2}/\Delta \log V$ *versus* log V were S-shaped at both the electrodes, and hence the nature of the electrode reaction was established as EC, in which charge transfer is followed by an irreversible chemical reaction.[20,21]

Coulometric Studies.—Controlled potential electrolysis of 3-methyluric acid was carried out in phosphate buffer of different pH at PGE as well as at GCE. The nature of the plots of i_p *versus* time was exponential. The plot of log $i_p = f(t)$ for the peak Ia oxidation was a straight line for the first 15 min of oxidation and thereafter a large deviation was observed. This behaviour

Table 3 Coulometric n-values observed for the electroxidation of 3-methyluric acid at different pH

pH	Electrode	Conc./ mmol dm^{-3}	Potential/V *vs.* SCE	n[a]
3.2	PGE	0.2	0.70	1.74
	PGE	0.5	0.70	1.86
	PGE	1.0	0.70	1.92
4.6	PGE	0.2	0.65	2.14
	GCE	0.2	0.70	2.02
5.6	GCE	0.2	0.60	1.82
	GCE	0.5	0.60	1.92
7.0	PGE	0.2	0.55	1.78
	PGE	0.5	0.55	1.86
	GCE	0.5	0.55	1.97
7.8	PGE	0.2	0.45	1.81
	PGE	0.5	0.45	2.01
	GCE	0.5	0.45	1.84
8.8	PGE	0.2	0.40	1.82
	PGE	0.5	0.40	1.94
	GCE	0.5	0.40	1.88

[a] Average of at least two replicate determinations.

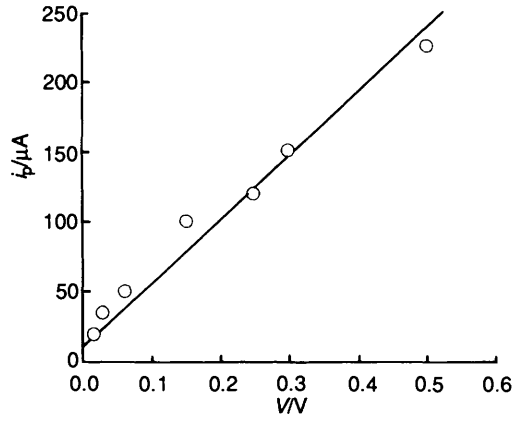


Fig. 4 Variation of the peak current (i_p) for the oxidation peak Ia with the sweep rate (V) for 0.5 mmol dm^{-3} 3-methyluric acid at pH 7.0

indicated that oxidation of 3-methyluric acid follows a simple path only up to 15 min, and thereafter follow-up chemical reactions play an important role as suggested by Meites [22] and Cauquis *et al.* [23] The values of n determined at $t < 15$ min at different pH are presented in Table 3 and clearly indicate that oxidation of 3-methyluric acid involves close to 2 electrons over the entire pH range.

The progress of electrolysis was monitored by recording cyclic voltammograms at different time intervals. The oxidation product formed on the CPE timescale is not electroactive. On the other hand, cyclic voltammetry indicates that on the CV-timescale oxidation products are electroactive.

The progress of electrolysis was also monitored by recording UV spectra at different time intervals. At pH 3.2, a 0.5 mmol dm^{-3} solution of 3-methyluric acid exhibited two broad overlapping maxima at about 220 and 245 nm, and a third maximum at 285–295 nm (curve 1, Fig. 5). The absorbance at 295 nm was about 0.02 AU higher than that at 285 nm. Upon application of potential (0.8 V) more positive to peak Ia, the absorbance at the longer wavelength maximum systematically decreased, whereas at short wavelengths the first band (220 mm) either remained unchanged or was replaced by a band of slightly different λ_{max} and slightly smaller value of ε; this change in spectra results mainly from the decrease in the band at about 245 nm with possible formation of another band at about 230 nm with small ε. For this we observed a small change of absorbance at 220 and 230 nm, and a large change at about 240

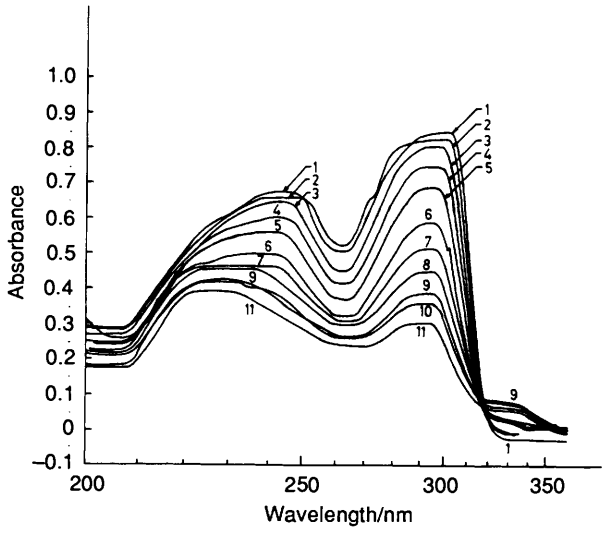


Fig. 5 Observed spectral changes during oxidation of 0.5 mmol dm^{-3} 3-methyluric acid at PGE, pH 3.2, potential 0.8 V. Curves were recorded at intervals of 5 min.

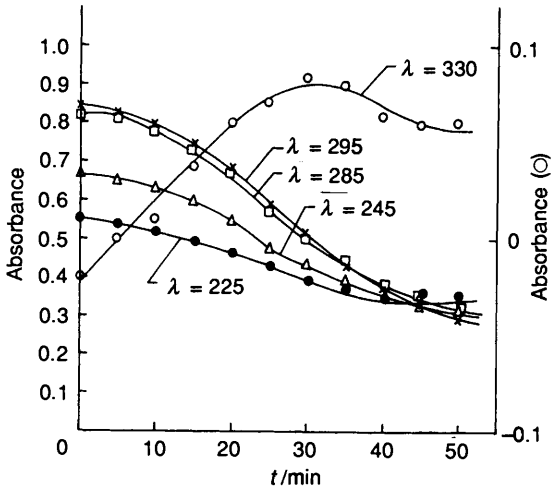


Fig. 6 Observed change in absorbance with time at different wavelengths during electrooxidation of 3-methyluric acid at pH 3.2

nm. An increase in absorbance at longer wavelength (315–350 nm) was also observed for 40 min of electrolysis (curve 7), after which the absorbance systematically decreased. Curve 11 was recorded after 90 min of electrolysis and exhibited broad bands at 225 nm and 285 nm. The changes in absorbance as a function of time are presented in Fig. 6. This behaviour indicated that a UV-absorbing intermediate at longer wavelength (330 nm) was generated during electroxidation of 3-methyluric acid, which then decomposed by follow-up chemical reactions to give products. The most characteristic change, the increase of the absorption band of an intermediate with λ_{max} about 330 nm is difficult to correlate to changes at 220–230 nm, as there is no evidence that the decrease of absorbance at 330 nm is accompanied by an increase of absorbance at any wavelength. The small absorption of products can also be overlapped anywhere between 220–230 nm. Identical UV spectral changes were also observed at pH 7.0 and 10.0.

The kinetics of the decay of the UV-absorbing intermediate were studied at different pH. The procedure employed was to oxidize 3-methyluric acid and monitor the absorbance at selected wavelengths (Table 4) during the course of oxidation. When the absorbance at 295 nm reached about 50% of its initial value (normally after 25–30 min of electrolysis) the applied potential was changed to zero and the decay of the intermediate was monitored as a function of time. The value of A_∞ was established when the values of absorbance became constant for a sufficient period of time (10–15 min). The value of $A_\infty \neq 0$ in the entire range (200–350 nm) due to absorption of products. At all pH values studied the plots of absorbance *versus* time indicated an exponential decay (Fig. 7). The plots of log $(A - A_\infty)$ *versus* time were linear indicating that decay of the UV-absorbing intermediate follows first-order kinetics. The values of rate constants calculated at individual pH values are presented in Table 4. Absorbance measured at different wavelengths at different pH values (Table 4) probably correspond to different reactions. These rate-constants have no simple meaning and most probably are not comparable.

Table 4 Observed rate constants for the decay of the UV-absorbing intermediate of 3-methyluric acid

		$k/10^{-3}$ s^{-1}	
pH	λ/nm	Electrochemical	Enzymatic
3.2	245	2.792	—
	335	6.907	—
5.2	287	2.086	2.636
6.9	260	3.184	3.350
8.8	310	3.688	3.810

Characterization of Products.—The products formed on electrochemical oxidation of 3-methyluric acid at various pH values were freeze dried. Products obtained at pH 3.2 exhibited two spots (R_f = *ca.* 0.41 and 0.63) in TLC. Gel permeation chromatography of the oxidation products yielded three peaks, two of them, P1 and P2, were found to be due to phosphate. The volume collected under peak P3 on lyophilization gave a colourless material with m.p. 151 °C. The mass spectrum of this material gave a clear molecular ion peak at m/z = 174, attributed to 1-methylalloxan hydrate. The other high mass peaks observed in the fragmentation were at 137 (5.4%), 136 (2.9%), 129 (5.8%), 128 (94.7%), 109 (19%) and 100 (52.7%). The IR spectrum of the product exhibited major bands at 3615, 3512, 2827, 2732, 2489, 2386, 2310, 1735, 1453, 1121 and 865 cm^{-1} and was superimposable with the IR spectrum obtained for the authentic 1-methylalloxan.

The formation of 1-methylalloxan at pH 3.2 suggested that the other product of electroxidation of 3-methyluric acid should be urea. However, urea could not be separated from the phosphate owing to its low molecular weight. An authentic sample of urea using the same column and mobile phase eluted at around 190–200 cm^3 and hence it was concluded that peak of urea will be overlapped by peak P1 and P2 of phosphate in gel permeation chromatography. A similar observation has also been reported[24,25] in the case of other purine derivatives. Nevertheless the R_f *ca.* 0.63 obtained in the freeze dried material was similar to urea and hence it was concluded that products of oxidation of 3-methyluric acid at pH 3.2 were 1-methylalloxan and urea.

At pH 7.0, the peak P1 again corresponds to phosphate. The freeze dried material obtained under chromatographic peak P4 was converted to its trimethylsilyl derivative. The silylated derivatives were separated by gas chromatography and analysed by mass spectrometry. Gas chromatography of the derivatized product gave two intense peaks with R_t *ca.* 28.8 and 30.2 min, together with several small peaks due to decomposition of silylating reagents. The EI mass spectrum of the peak at 28.8 min exhibited a small amount of the ion at m/z = 460 (9.0%) and a larger amount of the ion at m/z = 445 (29.0%) indicating the loss of a CH_3 group. Thus, the molar mass of the silylated species is 460, which was further confirmed by the CI mass

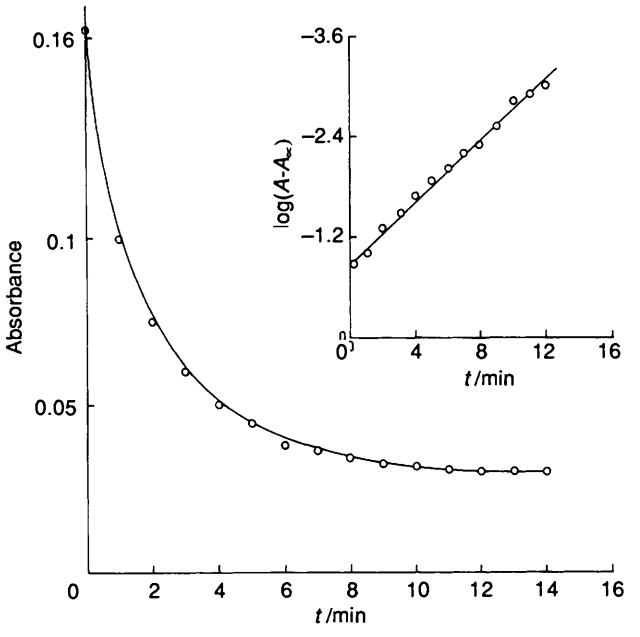
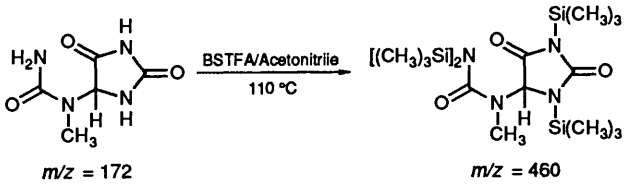
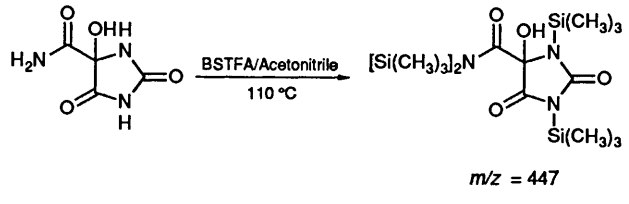


Fig. 7 Change in absorbance at 335 nm with time and log $(A - A_\infty)$ with time observed for the decay of UV-absorbing intermediate generated during oxidation of 3-methyluric acid at pH 3.2; potential applied: 0.8 V for 15 min



spectrum. In the CI spectrum a large amount of the ion at m/z =461 $[(M + H)^+]$ was observed. Characteristic peaks at 489 (10.9%) corresponding to $(M^+C_2H_5)^+$ and 501 (2.8%) corresponding to $(M + C_3H_7)^+$ were also observed. The base peak was found at 445 (100%) and corresponds to a $(M - CH_3)^+$ species. Thus, it was concluded that the species with m/z = 460 is the molecular ion, and corresponds to methyl allantoin.



The second peak in gas chromatography at R_t 30.2 min exhibited a clear molecular ion peak at m/z = 447. The other high mass peaks observed in the spectra were at 433 (8.1%), 432 $(M - CH_3$; 15.1%), 333 (28.1%), 332 (100%), 331 (79.1%) and 316 (12.1%). The molar mass of 447 was further confirmed by the chemical ionization mass spectrometry. The CI spectrum exhibited a $(M + H)^+$ peak at 448 (10%), $(M - CH_3)^+$ at 432, $(M + C_2H_5)^+$ at 476 and $(M + C_3H_7)^+$ at 488. Thus the molar mass of 447 indicated that the molecule possessed four sites which can undergo silylation and hence the compound was identified as 5-hydroxyhydantoin-5-carboxamide.

Enzymic Oxidation of 3-Methyluric Acid.—The peroxidase-catalysed oxidation of 3-methyluric acid by hydrogen peroxide was studied in phosphate buffers (μ = 0.1 mol dm^{-3}) at pH 5.0 and 7.0. Below pH 5.0, the enzymic oxidation was very slow and it required several hours before any significant oxidation occurred. The spectral behaviour observed upon initiation of enzymic oxidation of 3-methyluric acid with hydrogen peroxide indicated a systematic decrease in absorbance at λ_{max} and an increase in absorbance in the region 310–350 nm. Absorption spectra after quenching the enzymic reaction with catalase showed a decrease of the absorption bands at 295 nm and 310–350 nm. The absorbance change at 240 nm could not be monitored due to catalase absorption in this region.

The decrease of the absorbance at λ_{max} corresponding to the decay of the species formed by enzymic oxidation of 3-methyluric acid, followed first-order kinetics. The rate constants of the reaction depended on pH (Table 4). Both the magnitude of these rate constants and their dependence on pH were similar to those observed for cleavage of the product of electrooxidation.

To detect the formation of species responsible for peak IIc during electrochemical oxidation, cyclic voltammograms were also monitored during enzymatic oxidation of 3-methyluric acid and provided further evidence that the enzymatic reaction parallels the electrochemical oxidation. A cyclic voltammogram of a 0.2 mmol dm^{-3} solution of 3-methyluric acid at pH 7.0 is presented by curve (*a*) in Fig. 8. Fig. 8(*b*) represents a voltammogram recorded after adding peroxidase (0.002 mmol dm^{-3}) and shows little difference from curve (*a*). It may be noted that in both the curves (*a*) and (*b*), the initial sweep was towards negative potentials and no peak IIc was observed in the first negative sweep. When H_2O_2 (1.7 mmol dm^{-3}) was added to initiate oxidation and cyclic voltammograms were recorded, it was interesting to observe that peak IIc started appearing. Fig. 8(*c*) represents a cyclic voltammogram recorded after 5 min of adding H_2O_2 and a well defined peak IIc was observed. It was interesting to observe that peaks of the chemically formed product [peak IIc, curve (*c*)] and the electrochemically formed one are practically additive. Thus, it was inferred that enzymatic oxidation of 3-methyluric acid produced a species similar to electrochemical oxidation, which was reduced in the peak IIc reaction.

The similarity in cleavage of the primary product of electroxidation at around 0.6 V at pH 5.2–8.8 to the decay of the primary product of oxidation of 3-methyluric acid by horseradish peroxidase in the same pH range indicates a similarlity of the primary product formed. This indicates a possibility of similar mechanism for the oxidation by enzymes and at an electrode.

Reaction Scheme.—The experimental results indicate that 3-methyluric acid is electrochemically oxidized in a reaction involving close to 2e per molecule to give a UV-absorbing intermediate, which decomposes in a series of reactions to give 1-methylalloxan and urea at pH 3.0 and methylated allantoin and 5-hydroxyhydantoin-5-carboxamide at pH 7.0. It was expected that the value of dE_p/dpH for peak Ia at both the electrodes should be the same. However, in our case the values of dE_p/dpH at GCE were found to be 10–15 mV higher than at PGE. Also peaks Ic and IIc were never observed clearly at GCE. This difference in behaviour is probably not significant, and may be due to the difference in adsorption on two different carbon surfaces.[26] The main argument for 2e, 2H$^+$ oxidation of 3-methyluric acid is the value of n and absence of two separate 1e peaks even at 0.1 mmol dm^{-3} concentration. Also, as no radical species, has ever been detected during chemical, electrochemical or autooxidation of uric acid, it seems reasonable to conclude that oxidation of 3-methyluric acid in a single 2e, 2H$^+$ step gives the diimine species **2**. The dependence of E_p on pH indicated that the conjugate base is the species oxidized over the entire pH range studied.

The diimine species **2** is expected to be unstable as has been

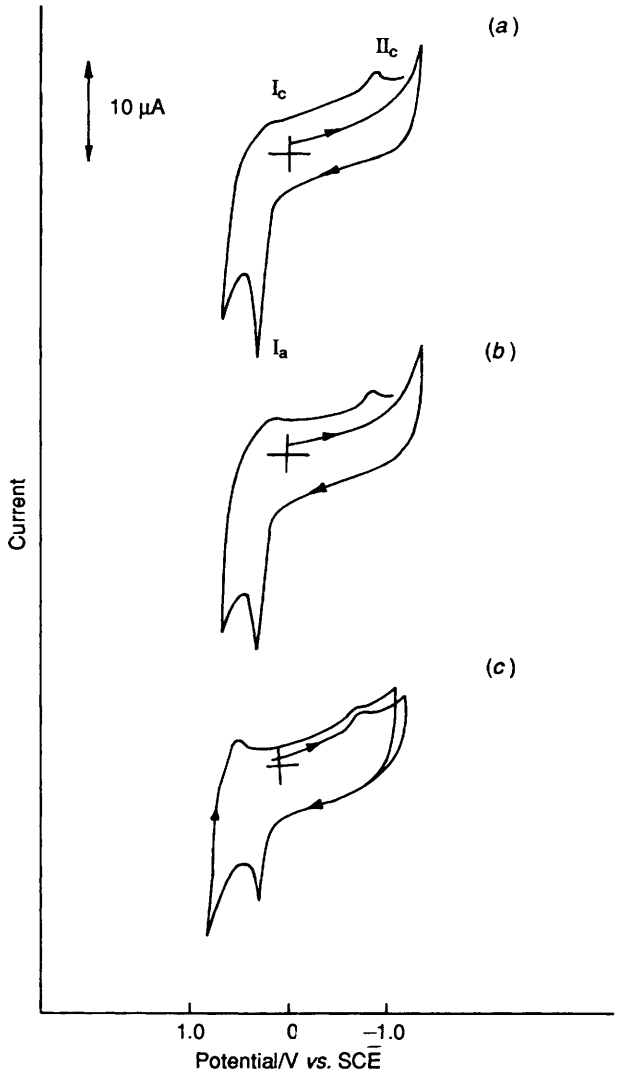
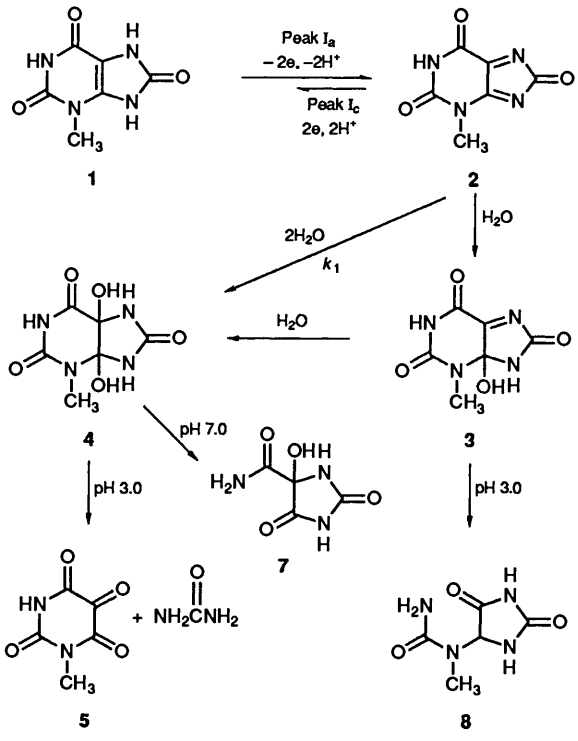


Fig. 8 Cyclic voltammograms observed during enzymic oxidation of 3-methyluric acid at pH 7.0 (*a*) 0.2 mmol dm^{-3} 3-methyluric acid; (*b*) 3-methyluric acid and peroxidase (0.002 mmol dm^{-3}); (*c*) after 5 min of adding H_2O_2 (1.7 mmol dm^{-3})

reported in the case of several purines,[24,25,27] and by analogy with earlier investigations is believed to be the quinonoid diimine (**2**). Peak Ic therefore represents the reduction of species **2** back to 3-methyluric acid. The increase in absorbance observed at longer wavelength during spectral studies seems to be due to the formation of diimine, which possesses an extensive π-conjugation. As peak Ic was clearly observed only at relatively fast sweep rates, and the peak current of peak Ic increased with increase in sweep rate, the quinonoid diimine (**2**) must undergo fast chemical follow-up reactions. Hence, species **2** is readily attacked by water to give imine alcohol **3** or diol **4**. As hydration of species **2** limits the height of reverse peak Ic, the ratio of Ia/Ic was strongly pH dependent. Hence, it was concluded that hydration of **2** is acid–base-catalysed. Reduction peak IIc observed in the reverse sweep of 3-methyluric acid (Fig. 2) is believed to be due to the reduction of species **3**. At pH 3.0, the diol **4** readily decomposes to give 1-methylalloxan and urea which were identified as the major products.

At pH 7.0, methylated allantoin and 5-hydroxyhydantoin-5-carboxamide were obtained as the products. Both putative intermediates **3** and **4** undergo a series of reactions to give the ultimate products. The decompositions of **3** and **4** occur in the pyrimidine ring (Scheme 1) to give methylated allantoin (**8**) and 5-hydroxyhydantoin-5-carboxamide (**7**). The rupture of the



Scheme 1 Tentative mechanism proposed for the electrooxidation of 3-methyluric acid

pyrimidine ring in neutral and alkaline media, and of the imidazole ring in acidic media, are well reported for the chemical oxidation of purines.[28–30] The proposed sequence of reactions are in agreement with known processes, but may correspond to only one of several reaction paths.

Cyclic voltammetry of 3-methyluric acid undergoing enzymic oxidation revealed that reduction peak IIc in the electrochemical oxidation was assigned to the reduction of imine alcohol **3**. It seems reasonable to conclude that the same intermediate is generated during the peroxidase-catalysed reaction. A comparison of the UV spectral changes observed during electrochemical and enzymatic oxidation of 3-methyluric acid further indicates that in both cases the changes are identical and hence the same intermediate and products should be formed in both the oxidations. The identical values of the first-order decay constant also supported the view that the same intermediate is formed in electrochemical as well as enzymatic oxidation.

In conclusion, it is believed that the peroxidase-catalysed oxidation of 3-methyluric acid follows a chemical pathway quite similar to electrochemical oxidation. It is difficult to decide at this stage whether the formation of diimine in the enzymic oxidation takes place by two rapid 1e, $1H^+$ oxidations or a single 2e step. However, it is reported that uricase and peroxidase oxidize uric acid, and such oxidations are characterized by two one-electron oxidations of the substrate.[31,32] It is believed that it would be very difficult to elucidate such detailed mechanisms based solely on the studies of enzymic reactions, and hence it has been concluded that electrochemical investigations can provide uniquely valuable insights into the chemical aspects of the redox reactions involved in enzymic processes.

Acknowledgements

One of the authors (M. S. V.) is thankful to the Council of Scientific and Industrial Research, New Delhi for the award of a Senior Research Fellowship. Financial assistance for this work was provided by the Department of Science and Technology, New Delhi through grant No. SP/S1/G21/91.

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Paper 2/05117F
Received 24*th September* 1992
Accepted 17*th February* 1993