Electroreduction and Related Studies on 2-Aminoacrylic Acid Derivatives. Part I. Electroreactivity of 2-Acetamidocinnamic Acid

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Polarographic, cyclic voltammetric, controlled potential electrolysis, and potentiometric evidence indicates that at low pH values (<4) the mechanism of electroreduction of ethyl α-acetamidocinnamate and α-acetamidocinnamic acid (pK_a −1·03 and −1·13, respectively) involves the protonation of a weak base in a thin layer near the electrode surface or at the surface. As the protonated form of the substrate is removed by electrolysis, the electroactive substrate is replenished by a chemical reaction between the non-protonated substrate and a hydrogen ion. As the pH is increased, the rate of this chemical reaction decreases and two polarographic waves appear. In addition, α-acetamidocinnamic acid exhibited an additional electrochemical acid–base equilibrium, believed to result from the dissociation of the carboxylic proton.

It is well documented that dehydroalanine derivatives are involved in metabolic pathways,[1-4] that they are the products of alkali-catalysed β-elimination reactions of *O*-glycosyl peptides,[5-7] and that a number of microbial peptides contain dehydroalanyl residues.[8-10]

Interest in these important biochemical entities has led us to investigate the electrochemical reduction of two model dehydroalanyl derivatives, ethyl α-acetamidocinnamate (3) and α-acetamidocinnamic acid (4) which are hydrolytically stable phenyl derivatives of the parent 2-aminoacrylate. Several questions about these derivatives of 2-aminocinnamic acid (3) and (4) involved the nature of their electroreactivity. In elucidation of the electrochemical behaviour of (3) and (4), the study of such parameters as dependence of the reaction on pH,

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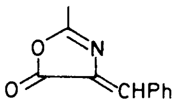
product identification, pK_a values of substrates, and reduction mechanism was essential. This paper deals with the more hydrolytically stable *N*-acetylenamine, α-acetamidocinnamic acid. Part II[11] considers the electroreduction of the more hydrolytically labile α-acetamidoacrylic acid, which is hydrolysable to acetamide and pyruvic acid at acid pH values. Utilization of the analytical techniques described herein should contribute to elucidation of biochemical mechanisms of the above mentioned α-aminoacrylate derivatives.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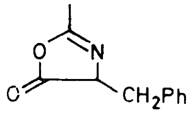
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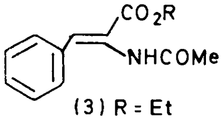
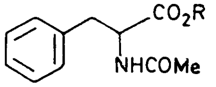

(3) R = Et

(4) R = H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EXPERIMENTAL

T.l.c. was carried out using the ascending technique on Eastman Chromagram sheets (type 6060, silica gel, and type 6065, cellulose, both with fluorescent indicator). Solvent systems were: chloroform–ethyl acetate (1 : 1) (solvent A), benzene (solvent B), light petroleum–ethyl acetate (1 : 1) (solvent C), and ethanol (solvent D). Zones were located by u.v. light. M.p.s were obtained using Thomas-Hoover and Mel-Temp apparata and are uncorrected. N.m.r. spectra were recorded on a Varian A60 60 MHz spectrometer for deuteriochloroform and deuterium oxide solutions using tetramethylsilane (TMS) and sodium 4,4-dimethyl-4-silapentanesulphonate (DSS), respectively, as internal standards. U.v. spectra were recorded with a Bausch and Lomb model 505 spectrometer.

Determination of pK_a *Values.*—The pK_a values for (3) and (4) were determined by potentiometric titration in glacial acetic acid using 1M-perchloric acid as the titrant and the chloranil electrode of Hall and Werner.[12] In this work, 0·05M solutions of the substrate were used so that pK_a values in water would be consistent with the results of Hall.[13]

Chloranil was obtained from Eastman Kodak, m.p. 284° (lit.,[14] 284°), R_F 0·81 (silica gel; solvent C; u.v. light).

Hydrochloranil was prepared by reduction of chloranil with $SnCl_2$,[15] m.p. 241° (lit.,[14] 236—237°), R_F 0·52 (silica gel; solvent C; u.v. light). Perchloric acid (1M in glacial acetic acid) was prepared according to ref. 12 and standardized with potassium acid phthalate using Crystal Violet as an indicator.[16]

Polarographic Measurements in Aqueous Buffers.—Polarographic measurements were made in Britton–Robinson buffers[17] of ionic strength 0·5 except where noted. All solutions contained 0·005% gelatin (Difco). Polarograms were recorded at the Oak Ridge National Laboratory using an ORNL D.C. polarograph Q 2792[18] coupled with an ORNL drop time controller Q 2942.[19] A 15 ml cell, thermostatted at 25·0 ± 0·2° and sealed against the atmosphere, was used.[19] A Smoler 90° dropping mercury electrode (DME) with a flow rate of 1 mg s^{-1} and a controlled drop time of 0·50 s was used in all measurements. Derivative polarograms and average current polarograms were run at scan rates of 1 V min^{-1} and 200 mV min^{-1}, respectively, using as a reference electrode the SCE described by Adams.[20] All solutions were sparged with argon.

Polarographic Measurements in Sulphuric Acid.—The H_0 values of sulphuric acid solutions were in accordance with the data of Paul and Long.[21] A saturated mercurous sulphate electrode (SME) in 17M-H_2SO_4 was used as the reference electrode.[22] Polarographic curves were obtained with a Heathkit polarography system model EUW 401[23] (Heath Co.) using an N.L.S. model X-3 digital voltmeter to monitor the initial potential of the working electrode. Capillary characteristics were: 45° Smoler DME, flow rate 1·50 mg s^{-1}, drop time 1·20 s at −1·50 V *vs.* SCE in 2·5M-H_2SO_4 at 25·0 ± 0·2°. The polarographic cell (10 ml) was similar in design to the cell used at the Oak Ridge National Laboratory[19] except that a 4 mm diameter carbon rod was used for the counter electrode. The solutions were sparged with nitrogen which was scrubbed by a train of vanadous chloride solutions.[24] The temperature was maintained at 25·0 ± 0·2°.

Cyclic Voltammetric Measurements.—Linear sweep and cyclic voltammograms were obtained with a controlled potential and controlled current cyclic voltammeter.[25] The cell (30 ml) was similar in design to that used for polarographic measurements in sulphuric acid. The sparging tubes, DME, HMDE assembly, and counter electrode were sealed into the cap with Borden's Flexite tub caulk. The SCE and spoon (used for collection of mercury drops from the DME and dislodging drops from the HMDE assembly) were fitted with rubber O rings which were seated into depressions machined into the cap. All measurements were made at ambient temperature.

Controlled Potential Electrolysis (*CPE*).—The cell (100 ml wide mouth specimen jar) was fitted with a Plexiglass cap with provisions for a DME, sparging tubes, SCE, Hg contact tube, and a separate anode compartment. The anode compartment was a filter tube with a 10 mm fine porosity

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frit covered with a layer of agar (1 cm) containing KCl. The anolyte was a saturated solution of KCl. A carbon rod (6 mm diameter, 10 cm long) served as the anode. The cathode was a mercury pool, *ca.* 28 cm^2 in area. Stirring was with a magnetic stirrer. The potentiostat employed was a Fisher controlled potential electroanalyser, model 40. Potentials were monitored with a N.L.S. model X-3 digital voltmeter. A nitrogen–hydrogen gas coulometer [26,27] was employed in all CPE investigations. Typical concentrations utilized in the electrolysis cell were 1 mmole of substrate dissolved in 25—50 ml of buffer. At the end of the electrolysis, signified either by DME measurements or by an observation of the cessation of gas evolution in the hydrogen–nitrogen coulometer, the solution was extracted with chloroform or ether and the products were identified by n.m.r. spectroscopy.

Instantaneous Current–Time Curves.—All measurements were made with the ORNL cyclic voltammeter [25] equipped with an oscilloscope and Polaroid camera. Preliminary polarograms were made at the slowest scan rate possible with this instrument (5 mV s^{-1}) so that appropriate potentials could be selected at which to investigate the current–time behaviour.

4-*Benzylidene*-2-*methyl*-2-*oxazolin*-5-*one* (1).—Compound (1) was prepared by the method of Dakin.[28] Purification was effected by recrystallization from benzene, m.p. 150° (lit.,[28,29] 154—155, 148—150°); R_F 0·83 (silica gel; solvent B; u.v. light).

4-*Benzyl*-2-*methyl*-2-*oxazolin*-5-*one* (2).—Compound (2) was prepared by the procedure of Bergmann *et al.*,[30] b.p. 125° at 1 mmHg (lit.,[30] 118° at 0·8 mmHg), R_F 0·67 (silica gel; solvent A; u.v. light).

Ethyl α-*Acetamidocinnamate* (3).—Compound (3) was prepared by a modification of Nicolet's method.[31] Compound (1) (5 g) was suspended in absolute ethanol (100 ml) and a catalytic amount of sodium was added. The resulting dark brown, homogeneous solution was allowed to react for 4 h. Ethanol (75 ml) was removed under vacuum, water (100 ml) was added, and the solution was extracted with ethyl ether. The extract was dried ($MgSO_4$), and compound (3) was precipitated by the addition of light petroleum (b.p. 30—60°). Repeated crystallizations (CCl_4) were required before a compound of sufficient purity was obtained, m.p. 102—103°; R_F 0·85 (silica gel; solvent A; u.v. light); $\lambda_{max.}$ [Britton–Robinson buffer (μ 0·5; pH 7)] 208 (ε ~ 11,000) and 274 nm (17,000), $\lambda_{max.}$ [Britton–Robinson buffer (μ 0·5; pH 0·5)] 207 (ε 10,600) and 274 nm (16,800); δ ($CDCl_3$) 1·3 (3H, t, *J* 7 Hz, OCH_2CH_3), 2·0 (3H, s, $NCOCH_3$), 4·3 (2H, q, OCH_2), 7·2—7·5 (6H, m, PhCH), and 7·7—7·9 (1H, m, NH, disappeared upon shaking with D_2O).

α-*Acetamidocinnamic Acid* (4).—Compound (4) was obtained from D. G. Doherty of the Biology Division, Oak Ridge National Laboratory, Oak Ridge, m.p. 188° (lit.,[28] 190—192°), δ (sodium salt in D_2O) 2·2 (3H, s, $NCOCH_3$), 7·3 (1H, s, CH), and 7·4—7·6 (5H, m, Ph), R_F 0·58 (silica gel; solvent D; u.v. light). Compound (4) was converted to (1) by the action of hot acetic anhydride.[32]

±-*Ethyl* N-*Acetylphenylalanate* (5).—Compound (5) was prepared similarly to (1). Crystallization was effected from chloroform–light petroleum (b.p. 30—60°), m.p. 66° (lit.,[33] 68°), R_F 0·62 (silica gel; solvent A; u.v. light), δ ($CDCl_3$) 1·26 (3H, t, *J* 7 Hz, OCH_2CH_3), 1·93 (3H, s, $NOCH_3$), 3·09 (2H, d, *J* 7·5 Hz, CH_2CH), 4·11 (2H, q, OCH_2CH_3), 4·80 (1H, q, CH_2CHNH, collapsed to a t upon shaking with D_2O), 6·25 (1H, d, NH, disappeared upon shaking with D_2O), and 7·15 (5H, s, Ph).

RESULTS

Polarographic Studies of Ethyl α-*Acetamidocinnamate* (3).—Compound (3) serves as a hydrolytically stable model to investigate the electrochemical reduction of the dehydroalanine derivatives. The reduction waves at all pH values were well defined and electrochemical parameters could be obtained over the entire range of the Britton–Robinson buffer system [17] (pH 2—12).

Two reduction waves are exhibited for (3). The limiting current of the first wave decreases in the form of a dissociation curve as the pH is increased, and is replaced by a second more cathodic wave. The sum of the currents of the two waves remains essentially constant except at pH values >10, where there is a slight decrease. Wave I, the more anodic wave, exhibited a maximum on polarograms recorded at pH values near 2. This maximum was reduced by the use of gelatin (0·005% by weight), although it was not completely eliminated. No maximum was observed at pH $\leqslant 0{\cdot}5$ or $>3{\cdot}0$. The limiting currents for wave II, the more cathodic wave, were extrapolated to zero time to account for saponification of the ester. At pH <11, the rate of saponification of (3) was insignificant. However, at pH 12, the half-life for saponification was *ca.* 15 min.

Both waves exhibit irreversibility as evidenced by the change in the value of the slope of the log instantaneous current–log time plots along the rising portion of the polarographic waves. For example, for the wave obtained in 0·25M-H_2SO_4, the slope of the log i–log t plot changed from 0·60 near the foot of the wave to 0·21 at the plateau; for the wave obtained at pH 10·5, the slope changed from 0·44 to 0·21. For an irreversible wave the slope of the log i–log t plot varies from 0·66 at the foot to 0·19 at the plateau, while that of a reversible process is 0·19 at all points.[34]

In addition to the change in slope of the log i–log t plots for individual drops, the log $i_d/(i_d - i)$ *vs.* E plots were linear for wave I with αn values of 1·18 and 1·19 at pH 0·5 and 3·0, respectively. The values of αn were 0·98 and 1·03 at pH 7 and 9, respectively, for wave II. As shown below, n is 2. Also cyclic voltammograms, which will be discussed later, did not exhibit anodic waves for either wave I or II.

A plot of i_k/i_d, where i_k is the current at any pH and i_d is the maximum current recorded at pH 0·85, and of the half-wave potentials as a function of pH, is shown in Figure 1. In this plot, the pH range has been extended to a H_0 value of 0·2 using 0·5M-HCl and to a pH of 0·85 using sulphuric acid (*ca.* 0·1M). Figure 2 is a plot of half-wave potentials from an H_0 value of $-3{\cdot}3$ to a pH of 6. The observed

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variation of i_k/i_d and $-E_{\frac{1}{2}}$ with pH points to acid-base kinetic behaviour.[35-41]

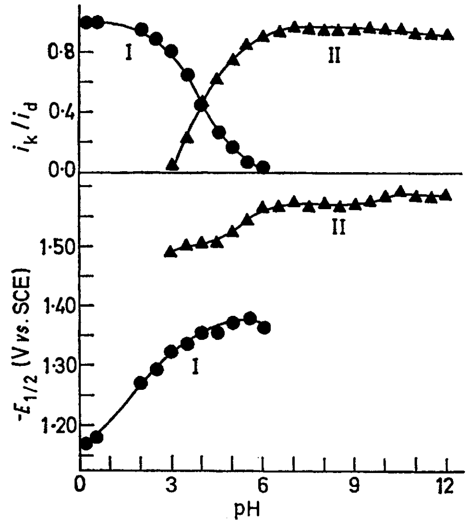


FIGURE 1 Dependence of limiting currents and half-wave potentials on pH for (3)

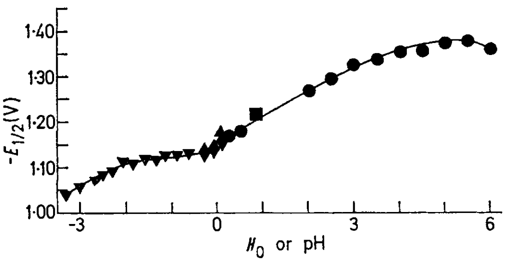
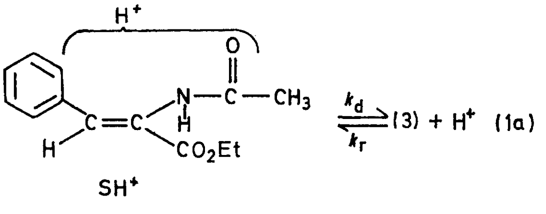


FIGURE 2 Dependence of half-wave potentials on pH or H_0 for (3). The symbols represent data obtained with the following instruments: ●, ORNL D.C. polarograph Q 2792; ▲, ORNL cyclic voltammeter; ■, ORNL cyclic voltammeter and Heathkit polarography system EWU 401; ♦, Heathkit polarography system using a SCE reference electrode; ▼, Heathkit polarography system using a saturated mercurous sulphate reference electrode



$$SH^+ + H^+ + 2e \xrightarrow{E_1} (5) \qquad (1b)$$

$$(3) + 2H^+ + 2e \xrightarrow{E_2} (5) \qquad (1c)$$

SCHEME

It is helpful to view three pH regions separately: $H_0 < \phi$, pH ϕ—6, and pH > 6. It is believed that the electrochemical behaviour exhibited in these regions can be described by the Scheme where k_d/s^{-1} = dissociation constant and $k_r/l\ mol^{-1}\ s^{-1}$ = recombination constant (potential $E_1 >$ potential E_2; $K_a = k_d/k_r$).

In the region $H_0 < \phi$, the electrochemical reduction can be described by reactions (1a) and (1b). This represents a system where the rate of protonation is fast compared to the electrode process. The current in this range is described by the Ilkovič equation and the half-wave potentials are independent of pH. This range was not experimentally accessible with the Britton–Robinson buffer system, as seen in Figure 1.

It was for this reason that the investigations were extended to sulphuric acid solutions as shown in Figure 2. Current–time curves were recorded for a 2mM solution of (3) in 1·0M-H_2SO_4. The slope of the log i–log t plot at the plateau of the polarographic wave was 0·19 after subtraction of background, which is in agreement with theoretical predictions for a diffusion controlled wave.[34]

The half-wave potentials for this region can be described[36] by equation (2) where c is a constant. When $[H^+] \gg K_a$,

$$E_{\frac{1}{2}} = c + \frac{RT}{\alpha nF} \ln \frac{[H^+]}{K_a + [H^+]} \qquad (2)$$

$E_{\frac{1}{2}}$ is independent of pH. This behaviour is approximated, but not completely realized, as shown in Figure 2. The $E_{\frac{1}{2}}$ values are essentially constant from a H_0 −2 to 0.

When the two linear portions in Figure 2, *i.e.* the range where $E_{\frac{1}{2}}$ is independent of H_0 (−2 to 0) and where $E_{\frac{1}{2}}$ is dependent on pH (0·5—2·5), are extended, they intersect at an H_0 value of *ca.* −0·5. Using Zuman's criteria,[36] this value of −0·5 should correspond to the potentiometric pK_a. The pK_a value determined by a potentiometric titration using the chloranil–hydrochloranil couple in glacial acetic acid for (3) was −1·03; thus the approximate polarographic pK_a value is in reasonable agreement with the potentiometric pK_a value.

CPE of (3) performed in 2·0M-H_2SO_4 gave as the product ±-ethyl *N*-acetylphenylalanate (5), which was isolated by extracting the reaction solution with ethyl ether. Thin layer chromatograms of the ether extract indicated that the only product was (5), which was then identified by comparing the n.m.r. spectrum of the electrolysis product with that of an authentic sample prepared by an independent method. The potential was held at −1·20 V *vs.* SCE; and since the background current was >25% of the total current, no valid *n* value could be obtained. In view of the fact that the only product isolated and detected was (5), the *n* value was assumed to be 2.

In the pH range 1—6, the first polarographic wave is of a quasi-diffusion nature,[41] *i.e.* $E_{\frac{1}{2}}$ is a function of pH and another wave appears at pH 3. According to Mairanovskii,[41] with a sufficient increase in pH of the solution, the limiting current should decrease and assume a kinetic nature when i_k/i_d is $\leqslant 0·20$. This behaviour was exhibited by compound (3). At pH 5·5, where i_k/i_d was 0·06 (Figure 1), the slope of the log i–log t plot taken at the plateau of the wave was 0·58, indicating control by the rate of the chemical reaction.[36] Linear $E_{\frac{1}{2}}$ plots for regions where pH >

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(pK_a' + 1) and pH > (pK_a + 1) should intersect at the polarographic pK_a' value.[36] (The polarographic pK_a' is the pH where $i_k/i_d = 0{\cdot}5$.) The point of intersection for (3) is near pH 4, which also corresponds to the pH where $i_k/i_d = 0{\cdot}5$.

Since kinetic behaviour was also observed in the cyclic voltammetry studies (see below), it is believed that the mechanism of reduction at pH 1—6 can be described by reactions (1a—c). The Scheme represents a system in which the rate of protonation of the substrate is comparable to the rate of the electrode reaction, *i.e.* two waves appear. The more anodic wave corresponds to the reduction of the protonated form and the more cathodic wave corresponds to the reduction of the conjugate base.

The height of wave I in the pH range 1—6 does not correspond to the analytical concentration of (3) because it is augmented by the recombination of the conjugate base with hydrogen ions in the reaction layer in the case of a ' volume '[37,38] reaction or at the surface of the electrode in ' surface ' reactions.[41]

When the concentration of (3) was increased in the pH region where the two waves were observed, the ratio of the height of the two waves and the $E_{\frac{1}{2}}$ values remained constant. This is an indication that no other chemical reaction was occurring other than the acid-base equilibrium; for example, if dimerization occurs, $E_{\frac{1}{2}}$ is proportional to the logarithm of the concentration.[42]

CPE performed using (3) in a 1M-acetate buffer at pH 4·7 with the potential held on the plateau of wave I (—1·50 V *vs.* SCE) gave an *n* value of 2. The only product isolated and detected by t.l.c. was (5).

In the third region (pH >6) a single wave was observed (Figure 1). This wave is believed to be due to the reduction of the unprotonated form of (3). The half-wave potentials and limiting currents remain essentially independent of pH except at pH >10. CPE performed on (3) in a 1M-NH_3-NH_4 buffer at pH 9·2 gave an *n* value of 2. The only product isolated was (5).

Cyclic Voltammetric Studies of (3).—Cyclic voltammograms were recorded at pH 2·0, 5·5, and 9·0. The absence of a reverse wave at all scan rates indicates that the process is irreversible. At pH 2·0 cyclic voltammograms recorded at scan rates < 100 mV s^{-1} exhibited sharp maxima which precluded the tabulation of the current function. This anomalous behaviour was also encountered in the recording of polarograms at the same pH.

An examination of the plot of $i_p/v^{\frac{1}{2}}$ as a function of scan rate as shown in Figure 3A reveals that the function $i_p/v^{\frac{1}{2}}$ is independent of the scan rate. Such behaviour may be interpreted as involving an irreversible charge transfer without coupled chemical reactions.[42] This behaviour is consistent with the polarographic and CPE results as kinetic character is observable only at the pH where polarographic i_k/i_d is $\leq 0{\cdot}2$.

At pH 9·0 cyclic voltammograms of (3) exhibited an adsorption prewave which was detected at scan rates $\geq 0{\cdot}50$ V s^{-1} as the principal diffusion wave. The prewave was not observed at slow scan rates possibly since a moderately high concentration of (3) was used (2mM). A combination of slow scan rates and high concentration of (3) reduced the prewave to negligible proportions compared to the total current. Only at faster scan rates could the effect be seen. The following experimental evidence indicates that the prewave is caused by strong adsorption of the product.[43-45] The function $i_p/v^{\frac{1}{2}}$ as a function of scan rate (Figure 3C) and a plot of the ratio of the prewave peak to diffusion peak both increase.

The presence of an adsorption prewave [46] was not detected in the polarographic investigations. This was probably due to the combination of the use of a 0·005% (by weight) gelatin and the high concentration of (3) which was used in order to obtain a profile over the entire pH range of the Britton–Robinson buffer system. As stated previously, gelatin was not used in the cyclic votammetry experiments.

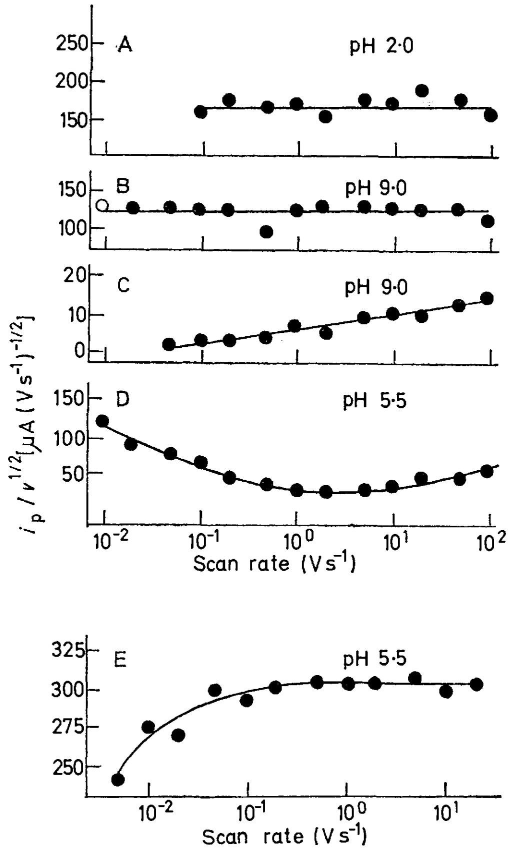

FIGURE 3 Variation of $i_p/v^{\frac{1}{2}}$ with scan rate for (3) in Britton–Robinson buffers: A, 2mM solution of (3) at pH 2·0 (corresponding polarographic wave I); B, 2mM solution of (3) at pH 9·0 (corresponding polarographic wave II); C, adsorption prewave for B; D, 6mM solution of (3) at pH 5·5 (corresponding polarographic wave I and the prewave for the corresponding polarographic wave II); E, 6mM solution of (3) at pH 5·5 (corresponding polarographic wave II)

The $i_p/v^{\frac{1}{2}}$ *vs.* *v* plot for the diffusion peak has a slope of zero indicating an irreversible charge transfer [47] (Figure 3B) with no chemical complications.

Additional evidence for kinetic behaviour in the pH range 1—6 was collected from cyclic votammetry experiments performed at pH 5·5 at a concentration of 6mM. Results of the polarography experiments (Figure 1) show that the current of the first wave is <20% of the total

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current at this pH. Two peaks were observed in the cyclic voltammograms. At scan rates >1 V s^{-1}, the more anodic peak which corresponds to the more anodic polarographic wave appears to be replaced by the prewave peak observed for (3) at pH 9·0, as evidenced by the behaviour of the $i_{\mathrm{p}}/v^{\frac{1}{2}}$ *vs.* v plot (Figure 3D). As can be seen, the function $i_{\mathrm{p}}/v^{\frac{1}{2}}$ goes through a minimum.

The first portion of the function as shown in Figure 3D corresponds to the reduction of the protonated form of (3). As the scan rate increases, the chemical reaction, *i.e.* protonation of the substrate, cannot supply enough of the protonated form because its rate is too slow; thus, the function $i_{\mathrm{p}}/v^{\frac{1}{2}}$ decreases in the manner predicted.[47] At fast scan rates, *i.e.* >1 V s^{-1}, wave I no longer contributes appreciably to the current and the cyclic voltammograms contain a peak due to the reduction of the nonprotonated species. However, as shown above, at fast scan rates the nonprotonated species exhibits an adsorption prewave with a peak potential nearly identical to that of the protonated form. For the adsorption prewave, the function $i_{\mathrm{p}}/v^{\frac{1}{2}}$ increases as the scan rate increases (see Figure 3D). It was also found that the ratio of the prewave peak current to the diffusion peak current increases at fast scan rates.

Figure 3E is a plot of the function $i_{\mathrm{p}}/v^{\frac{1}{2}}$ for the second wave *vs.* scan rate. Examination of this plot also reflects the occurrence of protonation. The function $i_{\mathrm{p}}/v^{\frac{1}{2}}$ increases as the scan rate increases to the point where the removal of the nonprotonated form due to protonation is negligible. At fast scan rates, the chemical reaction cannot supply a sufficient amount of the protonated form; thus, the electrochemical reduction observed is that of the nonprotonated form.

Electrochemical Reduction of α-Acetamidocinnamic Acid (4). Three polarographic waves were detected for (4). The total current remained essentially constant with pH except at higher pH values where there was a slight decrease. The first wave decreased with pH and was replaced by a second, more cathodic wave which also decreased and was replaced by a more cathodic third wave. The third wave occurred at very cathodic potentials and could only be analysed by means of derivative polarography. Plots of $i_{\mathrm{k}}/i_{\mathrm{d}}$ and half wave potentials *vs.* pH are shown in Figure 4.

Wave I was found to be irreversible using the criterion of the change in the slope of the log i–log t plots along the rising portion of the wave. In 2·0M-sulphuric acid the slopes of the log i–log t plots were 0·44, 0·54, and 0·20 at potentials corresponding to 0·24, 0·74, and 1·00 of the height of the polarographic wave. Also the cyclic voltammograms which were obtained (see below) did not exhibit anodic peaks. The electrochemical behaviour of wave I for (4) paralleled that of wave I for (3). At low pH values, the current was diffusion controlled,[48] *i.e.* log i–log t plots on the plateau of the polarograms exhibited slopes of *ca.* 0·2 in 2·5M-sulphuric acid and pH 2·0. The half-wave potentials were a function of pH, which is consistent for a quasi-diffusion wave [41] and shown to be the case for wave I of (3). Wave I exhibited kinetic characteristics when the parameter $i_{\mathrm{k}}/i_{\mathrm{d}} < 0{\cdot}2$ as evidenced by the independence of the limiting current on the mercury pressure. The pK_{a} value determined potentiometrically was $-1{\cdot}13$ as compared to $-1{\cdot}03$ for (3).

Wave II was found to be irreversible using the criterion of change in shape of the log i–log t curve along the rising portion of the polarographic wave. At pH 5·0, the slopes of the log i–log t plots were 0·60, 0·54, and 0·20 at potentials corresponding to 0·24, 0·70, and 1·00 of the height of the polarographic wave.

The limiting currents for waves I and II were directly proportional to the concentration and the half-wave potentials were not a function of the concentration.

Cyclic voltammetry investigation was limited to pH 2·0. Compound (4) did not exhibit maxima on the voltammograms at slow scan rates at pH 2·0 as was the case for (3).

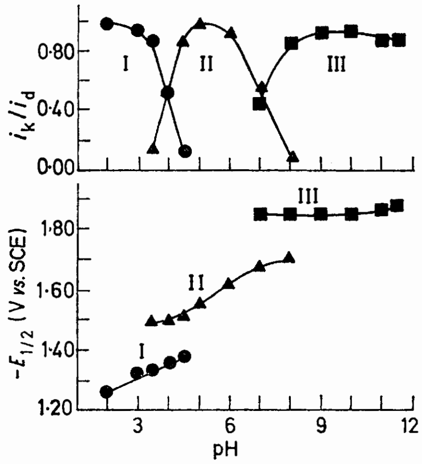

FIGURE 4 Dependence of limiting current and half-wave potentials on pH for (4)

The function $i_{\mathrm{p}}/v^{\frac{1}{2}}$ was independent of scan rate. The absence of a reverse wave at all scan rates indicates that the process is irreversible.

DISCUSSION

As noted above, the experimental results obtained for the reduction of (3) indicates that an acid–base equilibrium described by the Scheme is operative. Evidence for the mechanism of reduction of a protonated weak base has been given by Pospisil and Kuta [49] who have studied fumaric and maleic acids. They have presented evidence for the reduction of the triprotonated species of fumaric and maleic acids, *i.e.* the undissociated acids are further protonated to the species AH_3^+. The pK_{a} values for these triprotonated species as determined by u.v. spectroscopy were found to be $-0{\cdot}88$ and $-1{\cdot}03$ for fumaric and maleic acid, respectively.[50]

Because (5) was the sole reduction product of (3) and because the total limiting current for (3) was essentially constant over the entire pH range (0·5—12), dimer formation does not appear to be a major side reaction as it is in the reduction of alkyl aryl ketones [51] in acid media. Further evidence that dimer formation is not a factor at low pH values is that the log $i/(i_{\mathrm{d}} - i)$ *vs.* E plots were linear at pH 0·5, 2·5, and 3·0. For reduction mechanisms which involve dimerization, either log

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$i^{\frac{2}{3}}/(i_d - i)$ [52,53] or $\log i/(i_d - i)^2$ [43] as a function of E is linear.

Brand and Fleet [54] using microcoulometric experiments with a DME did not isolate a dimer in the reduction of *trans*-cinnamic acid at pH 6.

When surface reactions are operative, the following experimental behaviour should be observed: (1) the slope of the $E_{\frac{1}{2}}$–pH [40,41] plot differs from reciprocal slope of the log analysis plot, *i.e.* $\log i/(i_d - i)$ *vs.* E, and (2) the value of k_r exceeds 10^8 l mol^{-1} s^{-1}.[55]

The parameter, αn, calculated from the slope of the $\Delta E_{\frac{1}{2}}/\Delta$pH plot, as shown in Figure 1 for wave I (linear portion from pH 0·5 to 3·0), is 0·83. The value of αn calculated from the reciprocal slope of the semi log plot of $\log i/(i_d - i)$ *vs.* E was 1·18 at pH 0·5 and 1·19 at pH 3·0. Because the values of these two slopes differ, then the protonation may be partially or completely of a surface nature.[41]

The values of the rate constants [56] calculated from a pK_a of $-1·03$ and pK_a' of 4·0 are $2·8 \times 10^9$ l mol^{-1} s^{-1} for k_r and $2·9 \times 10^{10}$ s^{-1} for k_d. According to Brdicka,[38,39,57] the polarographic method for the determination of rate constants can only be reliable if $k_d \leq 10^8$ s^{-1} and k_r does not exceed 10^{11} l mol^{-1} s^{-1}. The large difference between the polarographic pK_a' (4·0) and the potentiometric pK_a ($-1·03$) also points to ' surface ' protonation.[40,41,55]

In Figure 2, the $E_{\frac{1}{2}}$ values are dependent on acidity at H_0 values < -2. A second chemical equilibrium could be operative at $H_0 < -2$. Goldfarb and his coworkers [58,59] investigated the base strength of acetamide and several derivatives. The values of the pK_a of acetamide and all homologous *N*-butylacetamide derivatives were close to zero, *i.e.* $-0·46$ to $+0·37$. These authors demonstrated [59] further that a second equilibrium was present which was not associated with the binding of a second proton and was explained by a solvent effect through hydrogen bonding.

The decrease of the height of wave II and the appearance of wave III on the polarograms for (4) point to another acid-base equilibrium (Figure 4). The likely process in this equilibrium is the dissociation of the carboxylic proton. This type of behaviour has been well documented,[60] the classical example being the reduction of pyruvic acid.

A rough approximation of the pK_a of this dissociation for (4) can be arrived at by an examination of Figure 4. The intersection of the two linear portions of the $E_{\frac{1}{2}}$–pH plot [36] for wave II, *i.e.* the extension of the lower portion near pH 3·5 and the rising portion from pH 4·5 to 6·0, is approximately at pH 4. The pK_a of *ca.* 4 for the dissociation of the carboxylic proton of (4) is not out of line with the pK_a values for other α,β-unsaturated acids. Some representative pK_a values [61] are: *trans*-cinnamic acid, 4·44; *cis*-cinnamic acid, 3·88; acrylic acid, 4·71; *trans*-crotonic acid, 4·71; *cis*-crotonic acid, 4·41; α-methylacrylic acid, 4·66; β-ethylacrylic acid, 4·69; and fumaric acid, 3·03 and 4·44.

Azlactone formation could possibly be a factor in the electrochemical investigations of (4) at low pH values but was deemed insignificant in the pH range 2—12. One procedure for preparing an azlactone is to heat a solution of an *N*-acyl unsaturated acid in glacial acetic acid.[32]

Compound (1), the azlactone of (4), was prepared by allowing a concentrated sulphuric acid solution of (4) to stand overnight at room temperature.[62] A 1mM solution of (1) was then analysed polarographically at pH 3·0. Three waves were detected at this pH with $E_{\frac{1}{2}}$ values of $-0·910$, $-1·160$, and $-1·320$ V, respectively. The waves at the more anodic potentials were of equal height and decreased rapidly. Within 5 min they had disappeared and were replaced by the wave at $-1·320$ V. The height of the more cathodic wave was 4·1 μA. The $E_{\frac{1}{2}}$ and limiting current at this pH were identical to a 1mM solution of (4). The decomposition of (1) is consistent with the known behaviour of azlactones in dilute acid media.[63]

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