Electroanalytical Chemistry and Interfacial Electrochemistry, 62 (1975) 415–431

ELECTROCHEMICAL OXIDATION OF BARBITURIC ACIDS AT LOW pH IN THE PRESENCE OF CHLORIDE ION

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(Received 22nd November 1974; in revised form 4th March 1975)

ABSTRACT

Barbituric acid, 1-methylbarbituric acid and 1,3-dimethylbarbituric acid are electrochemically oxidized at the pyrolytic graphite electrode by way of a single voltammetric peak at pH 1 in the presence of chloride ion. At least four products are formed as a result of the reaction, the three major products, accounting for more than 80–90% of the oxidized barbituric acid, are the appropriately N-methylated 5,5′-dichlorohydurilic acids, 5,5-dichlorobarbituric acids and alloxans. The mechanism appears to proceed by an initial potential-controlling $1e/1H^+$ oxidation of the barbituric acids to give a barbituric acid radical. This can dimerize to hydurilic acid, which is then further electrochemically oxidized. However, this appears to be a minor route. The barbituric acid radical appears to be mainly further electrooxidized ($1e$) to a carbonium ion which further reacts with nucleophiles such as chloride ion to give 5-chlorobarbituric acid, or with water to give dialuric acid. Further electrochemical oxidation and chemical reactions of the latter species results in formation of the ultimate products.

INTRODUCTION

We have recently initiated a series of investigations into the electrochemical behavior of barbiturates. During the course of these studies it has been found that at low pH in the presence of chloride ion, barbituric acids are electrochemically oxidized at the pyrolytic graphite electrode (PGE) principally to chlorinated products. Such behavior appears to be quite different both in terms of products and mechanism to that observed at higher pH.

Electrooxidative halogenations of N-heterocyclic molecules have not been studied extensively although Meinert and Cech[1] have recently reported that uracil can be electrochemically oxidized to 5-halogenouracils in methanol containing the proper inorganic salts as supporting electrolyte.

In this paper we have described the electrochemical oxidation of barbituric acids at low pH in the presence of chloride ion in terms of the voltammetry, products and electrode and related chemical mechanisms. The work provides the basis for a very simple, one-step electrochemical synthesis of various 5,5′-dichloro-

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hydurilic acids. The behavior of barbiturates at higher pH values is considerably more complex and appears to involve both oligomerization[2] and degradation processes[3], and will be reported in detail at a later time.

EXPERIMENTAL

Chemicals

Chemicals used in this study were synthesized by methods according to authors indicated: barbituric acid (Biltz and Wittek[4]), 1-methyl- and 1,3-dimethylbarbituric acid (Stein *et al.*[5]), 5-chlorobarbituric acid and 5,5′-dichlorobarbituric acid (Bock[6]), 1,3-diethylbarbituric acid (Biltz and Hamburger[7]), 5,5′-dichlorohydurilic acid (from hydurilic acid, Bredereck *et al.*[8]) (Biltz and Hamburger[9]), alloxan monohydrate (Biilmann and Berg[10]), 5,5′-dichloro-1-methylbarbituric acid from monomethylvioluric acid and 5,5-dichloro-1,3-dimethylbarbituric acid from dimethylvioluric acid (Biltz and Hamburger[7,9]), 5,5′-dichloro-1,1′-dimethylhydurilic acid (Biltz and Heyn[11]) from 1,1′-dimethylhydurilic acid (Bredereck *et al.*[8]), 5,5′-dichloro-1,1′,3,3′-tetramethylhydurilic acid (Biltz and Heyn[11]) from 1,1′,3,3′-tetramethylhydurilic acid (Blicke and Godt[12]). Column chromatography utilized Dowex 50W-X8 resin, H^+ form, 20–50 mesh (J. T. Baker Chemical Co.) a 35×2.4 cm column, pre-washed with anhydrous methanol. This column was used primarily to remove inorganic salts from mixtures of reaction products. Product separations were carried out with Sephadex G-10 (Pharmacia Fine Chemicals), 40–120 μm, 37×2.2 cm prewashed with 0.1 *M* acetic acid.

The supporting electrolyte (generally referred to as buffer solution) used in these studies had an ionic strength of 0.5 and a pH of close to 1.0 and was prepared by dissolving 9.5 ml of concentrated HCl and 29.82 g of potassium chloride in 1 l of deionized water.

Apparatus

Polarography, linear sweep voltammetry, and cyclic voltammetry were carried out with an instrument based on conventional operational amplifier design[13]. A Sargent model XVI polarograph was also used on occasion. Voltammetry and polarography were carried out at 25°C. The preparation of pyrolytic graphite electrodes for voltammetry, coulometry and mass electrolyses has been described elsewhere[14]. Coulometry and mass electrolyses utilized a Wenking model 66TAl potentiostat. Current integration during electrolysis was achieved by measuring the voltage drop across a standard resistor by means of a voltage-to-frequency converter and scalar counter. Coulometry and mass electrolyses were carried out in a two compartment cell. The working electrode compartment (175 ml volume) was separated from the counter electrode compartment (100 ml volume) by a fine porosity sintered glass disc and a saturated KCl–agar salt bridge. The counter electrode was a large platinum gauze immersed in the same solvent-supporting electrolyte used in the working electrode compartment. A Beckman fiber tip SCE reference electrode was immersed directly into the solution in the working electrode compartment. All potentials are referred to the SCE at 25°C.

Ultraviolet absorption spectra were obtained on a Perkin-Elmer Hitachi model 124 spectrophotometer using 1.00 cm quartz cells, i.r. spectra were recorded

on a Beckman IR-8 spectrophotometer using KBr pellets. N.m.r. (60 MHz) and mass spectra were obtained on a Varian model T-60 and Hitachi model RMU-6E spectrometers respectively.

Oxidation of barbituric acid in chloride buffer pH 1.0

For mass electrolyses typically about 150 ml of a 10 m*M* solution of the various barbituric acid derivatives were electrolyzed. Yields of products were generally reproducible to better than 10%.

Barbituric acid (182.5 mg, 1.425 mmole) was electrolyzed at pyrolytic graphite electrodes in chloride buffer pH 1.0 (150 ml) at 1.00 V under nitrogen with continuous stirring for 24 h. A white precipitate was formed which was collected by filtration, washed with water to remove the electrolyte, and dissolved in methanol boiling under reflux (50 ml). The resulting solution was filtered to remove carbon particles formed when the electrodes were scraped to remove adhering white solid. The filtrate was evaporated to give a white precipitate (99 mg, 0.276 mmole as dihydrate, yield 38.7%) which was recrystallized from aqueous methanol, m.p. 360°C (turns brown at *ca.* 300°). Analysis calculated for $C_8H_4N_4O_6Cl_2$ (323.07): C 29.42%, H 1.52%, N 17.34%. Found: C 29.74%, H 1.25%, N 17.35%. I.r. spectrum (cm^{-1}, KBr pellet): 3560, 3500 (H_2O), 3225, 3120 (NH), 2830, 1720 (C=O, broad and strong), 1630, 1425, 1385, 1340, 1240, 1010, 945, 820, 755, 670 and 600. Mass spectrum (70 eV, 195°C, *m/e*): 324 (0.2, M^+), 322 (0.5, M^+), 287 (1.5), 251 (2.5), 203 (3.5), 201 (10.5), 160 (3.5), 158 (9), 89 (5), 87 (16), 80 (5), 70 (15), 68 (13), 44 (79), 43 (100), 42 (48), 38 (45), 37 (6), 36 (86), 35 (17), 29 (18.5) and 27 (8). N.m.r. spectrum (60 MHz, δ, d_6-DMSO): 12.2, singlet, NH, exchangeable. Comparison of the above spectral and analytical data of this electrolysis product with those of authentic 5,5′-dichlorohydurilic acid revealed them to be identical.

The electrolyte and washings obtained after filtering 5,5′-dichlorohydurilic acid were combined and freeze-dried. The resultant solid was dissolved in methanol and passed through a column of Dowex 50W-X8 ion exchange resin (H^+ form), previously washed with redistilled methanol, and eluted with 900 ml of methanol. The eluate was evaporated and dried under vacuum to give a pale pink solid (156 mg), which was passed through a Sephadex G-10 column (see earlier discussion of preparation) and eluted with 0.1 *M* acetic acid. After passing 150 ml of eluent the first component was eluted (53 ml) which gave, upon freeze-drying, a pale pink powder (48 mg, 0.30 mmole, 21.1%) which had a molecular ion in the mass spectrum (70 eV, 180°C) of mass 142. Comparison of the mass and i.r. spectrum revealed that this component was alloxan monohydrate. The next 130 ml of eluate on freeze drying gave a white solid (16 mg, unidentified). The last component to appear was, after freeze drying, a white powder (86 mg, 0.437 mmole, 30.6%), which was identified as 5,5′-dichlorobarbituric acid by comparison of its mass spectrum (70 eV, 120°C, *m/e*): 198 (11.5, M^+), 196 (15.5, M^+), 112 (81.5, $Cl_2C{=}C{=}O$), 110 (100, $Cl_2O{=}C{=}O$) with the authentic material. The i.r. and u.v. spectra of this electrolysis product also were identical to authentic 5,5′-dichlorobarbituric acid.

Oxidation of 1-methylbarbituric acid in chloride buffer pH 1.0

1-Methylbarbituric acid (213 mg, 1.500 mmole) was electrolyzed at 1.00 V under nitrogen for 23 h. The resulting white precipitate was filtered, washed with

water and dissolved in methanol boiling under reflux (150 ml), filtered again and evaporated to give a white solid (72 mg, 0.205 mmole, 27.3% yield). Recrystallization from methanol gave white needles (34 mg), m.p. 307–313°C (d). Analysis calculated for $C_{10}H_8N_4O_6Cl_2$ (351.11): C 34.20%, H 2.30%, N 15.96%; found: C 34.27%, H 2.61%, N 15.73%. I.r. spectrum (KBr pellet, cm^{-1}): 3420 (broad, possibly H_2O), 3280 (NH), 1730 (C=O), 1695, 1440, 1380, 1340, 1270, 1175, 1070, 980, 850, 810, 745, 705, 695 and 640. Mass spectrum (70 eV, 175°C, *m/e*): 352 (1, M^+), 350 (2, M^+), 317 (8, M^+–Cl), 315 (22, M^+–Cl), 217 (15), 215 (53.5), 174 (5), 172 (16), 160 (6), 158 (14.5), 89 (13), 87 (30.5), 80 (20), 76 (7), 70 (18.5), 58 (8), 57 (12), 56 (18), 52 (12.5), 44 (50), 43 (12), 38 (36.5), 36 (100). The spectral and other data reported above agreed exactly with those observed for authentic 5,5′-dichloro-1,1′-dimethylhydurilic acid.

The filtrate and washings from the initial separation of the latter compound were collected and freeze-dried, dissolved in a small quantity of methanol and eluted with methanol (800 ml) through a methanol-washed Dowex 50W-X8 column (H^+-form). Evaporation of the methanol eluate gave a pale red solid (233 mg), which when treated with 2 ml of 0.1 *M* acetic acid and allowed to stand at 5°C overnight gave a white precipitate which was filtered and dried (98 mg, 0.464 mmole, 30.9%). This product had the following mass spectrum (70 eV, 115°C, *m/e*): 212 (15, M^+), 210 (24, M^+), 112 (88, $Cl_2C{=}C{=}O$), 110 (100, $Cl_2C{=}C{=}O$). Comparison of this spectrum with that of 5,5-dichloro-1-methylbarbituric acid revealed them to be identical. The melting point (136°C) and i.r. spectrum of the product also agreed with those of the latter compound.

The filtrate was freeze-dried to give an orange-yellow solid (103 mg) which was passed through a Sephadex G-10 column and eluted with 0.1 *M* acetic acid. The first component eluted after 105 ml of eluent has been passed and was collected from the next 32 ml to give, after freeze-drying, an unidentified orange-yellow syrup (13 mg). The second remaining component was then eluted in the next 24 ml and after freeze-drying a pale brown solid was produced (55 mg, 0.316 mmole, 21.1%) which was identified by mass spectrometry (70 eV, 135°C, $M^+ = 156$) and by its u.v. ($\lambda_{max} = 215$ nm in water) and i.r. spectra as 1-methylalloxan monohydrate by comparison with the authentic sample.

Oxidation of 1,3-dimethylbarbituric acid in chloride buffer pH 1.0

1,3-Dimethylbarbituric acid (234,3 mg, 1.500 mmole) was electrolyzed in chloride buffer pH 1.0 at 1.00 V under nitrogen for 44 h. The resulting white precipitate was filtered, washed with water and dried. The white solid was then dissolved in boiling methanol (150 ml), filtered and the filtrate evaporated to give a white solid (156 mg, 0.412 mmole, 54.9%). This was recrystallized from methanol (50 ml) to give white crystals (124 mg), m.p. 272–274°C. Analysis calculated for $C_{12}H_{12}N_4O_6Cl_2$ (379.16): C 38.01%, H 3.20%, N 14.78%; found: C 38.01%, H 3.29%, N 15.14%. I.r. spectrum (KBr pellet, cm^{-1}): 3430 (broad), 2960, 1720, 1680, 1440, 1420, 1365, 1280, 1245, 1110, 1090, 980, 850, 800, 740, 655. Mass spectrum (70 eV, 145°C, *m/e*): 380 (2, M^+), 378 (3, M^+), 345 (12.5), 343 (40), 280 (12.5), 231 (28), 229 (100), 222 (10.5), 172 (24.5), 80 (34), 56 (21). The above spectral and analytical data agreed with those observed for authentic 5,5′-dichloro-1,1′,3,3′-tetramethylhydurilic acid.

The filtrate and washings from the above separation were freeze-dried, dissolved in methanol and passed through a methanol pre-washed column of Dowex 50W-X8 and eluted with methanol (900 ml). Evaporation of the methanol gave a yellow solid (142 mg) to which was added 0.1 *M* acetic acid (2 ml). The resultant solution was stored at 5°C overnight when a white precipitate (79 mg, 0.351 mmole, 23.4%) was formed which was collected by filtration and washed with small amounts of 0.1 *M* acetic acid. This material was identified as 5,5-dichloro-1,3-dimethylbarbituric acid on the basis of its melting point (161–164°C after recrystallization from aqueous methanol), mass spectrum [(70 eV, 130°C, *m/e*): 226 (20, M^+), 224 (26, M^+), 112 (75, $Cl_2C{=}C{=}O$), 110 (100, $Cl_2C{=}C{=}O$)] and i.r. spectrum by comparison with the authentic material.

The filtrate from the latter separation was freeze-dried to give a brown residue (59 mg) which was passed through a Sephadex G-10 column and eluted with 0.1 *M* acetic acid. In the first *ca.* 108 ml of eluate, after freeze-drying, a small amount (4 mg) of a pale brown unidentified solid was obtained. Subsequent eluent after freeze drying gave a pale yellow powder (40 mg, 0.213 mmole, 14.2%). On the basis of its u.v. (λ_{max} 231 nm in water), i.r. and mass spectrum [(70 eV, 170°C, *m/e*): 170 (64, M^+)] and comparison with the authentic material this product was identified as dimethylalloxan monohydrate.

Oxidation of 5-chlorobarbituric acid in chloride buffer pH 1.0

5-Chlorobarbituric acid (244 mg, 1.500 mmole) was electrolyzed at 1.00 V in chloride buffer pH 1.0 under nitrogen for 27 h. The resultant precipitate was filtered and washed with water and then dissolved in boiling methanol (100 ml). After filtration and evaporation a white solid was obtained (85 mg, 0.237 mmole, 31.6%) which was identified (m.p., i.r. and mass spectra) as 5,5′-dichlorohydurilic acid. The filtrate and washings were freeze-dried, and eluted with methanol (800 ml) through a column of methanol washed Dowex 50W-X8 (H^+-form). Evaporation of the eluate gave a pink-orange powder (181 mg). This was passed through a Sephadex G-10 column and eluted with 0.1 *M* acetic acid. After passing 140 ml of solvent the first component appeared in the next 24 ml of eluent which on freeze-drying gave 20 mg of an unidentified orange solid (m.p. 70°C(d)). The second component to appear was eluted in the next 40 ml and, after freeze-drying, gave a white powder (66 mg, 0.413 mmole, 27.5%) of alloxan monohydrate (by comparison of m.p., i.r. and mass spectra with the authentic material). The final component was eluted in the next 180 ml of eluent which, after freeze-drying , gave a white solid (41 mg, 0.208 mmole, 13.9%) of 5,5-dichlorobarbituric acid.

Oxidation of 1,3-diethylbarbituric acid in chloride buffer pH 1.0

1,3-Diethylbarbituric acid (500 mg, 2.714 mmole) was electrolyzed at 1.00 V in chloride buffer pH 1.0 (150 ml) under nitrogen. The electrolysis proceeded very slowly, apparently because of very strong adsorption of product(s) at the electrode surface, and took several days to reach completion. A white precipitate was formed and was separated by filtration, washed with water, dried and dissolved in boiling methanol which on evaporation gave a white solid (89 mg, 0.205 mmole, 7.5%). This was recrystallized from methanol to give white crystals, m.p. 220–224°C. Analysis calculated for $C_{16}H_{20}N_4O_6Cl_2$ (435.30): C 44.14%, H 4.64%, N 12.87%;

found: C 42.86, H 5.00, N 13.46. Mass spectrum (70 eV, 150°C, *m/e*): 436(16,M^+), 434(21.5, M^+), 401(17.5), 399(46.5), 366(40.5), 363(27.5), 336(27.5), 335(74), 295(20.5), 259(47), 257(100), 250(32), 186(35), 87(36), 80(36), 80(30.5), 70(65), 69(26), 56(56.5), 53(23), 44(37.5), 42(40.5), 29(66.5), 27(35). The above analysis, melting point and mass spectrum agreed with those expected for 5,5′-dichloro-1,1′,3,3′-tetraethylhydurilic acid.

RESULTS AND DISCUSSION

Voltammetry of barbituric acids

In chloride buffer *ca.* pH 1.0 barbituric acid, 1-methylbarbituric acid and 1,3-dimethylbarbituric acid show a single well-defined voltammetric oxidation peak at the pyrolytic graphite electrode (PGE) (Table 1). No reduction peaks are observed unless the oxidation peak is first scanned. Above pH 2.2 all of the barbituric acids show additional peaks at more positive potentials. Many of the peaks overlap each other resulting in very complex and ill-defined voltammograms and electrochemical behavior. The details of the processes responsible for the voltammetric peaks will be presented in a subsequent report[3]. Methylation of barbituric acids at the N_1 and/or N_3 positions has essentially no effect on the voltammetric peak potential ($E_p = 1.02 \pm 0.02$ at pH 1.3) or on the peak current, *i.e.*, the peak current function ($i_p/Acv^{\frac{1}{2}}$) for all three compounds at pH 1.3 was 2078 ± 86 μA cm^{-2} mM 1 V$^{-\frac{1}{2}}$ s$^{\frac{1}{2}}$ at a sweep rate of 0.005 V s^{-1}. The peak potential for the first peak of the barbituric acids is strongly pH dependent[3] (for barbituric acid $E_p = 1.156$–0.079 pH between pH 1–5) indicating the involvement of protons in the potential-controlling reactions. Barbituric acid and its methylated derivatives are electrochemically oxidized in, overall, totally irreversible processes as evidenced by cyclic voltammetry where no reversible reduction peak is observed (*vide infra*). In addition, the slope of the peak ($E_p - E_{p/2}$) is larger than expected for a reversible process, and the peak potentials shift more positive with increasing sweep rate and concentration.

The apparent voltammetric n value was calculated from the measured peak current, peak and half-peak potentials and hence αn_a, using the well known equation for an irreversible peak voltammogram[16] (see Table 1). Determination of αn_a values for a system which includes a follow-up chemical reaction (see later discussion) can often lead to errors in estimation of n-values since α can be influenced by the solution reaction. However, for the systems studied here the voltammetric n-values calculated using experimental αn_a values (Table 1) lie between 3 and 4 and agree very closely with coulometric values (*vide infra*). Accordingly, it was assumed that for these processes α was essentially unaffected by the solution reactions.

The relative complexity of the voltammetric reactions is illustrated in the cyclic voltammograms of the barbituric acids (Fig. 1). Thus, at a sweep rate of 20 mV s^{-1}, after having swept through the single initial voltammetric oxidation peak of barbituric acid or its N-methyl derivatives (peak I_a, Fig. 1, A–C), at least two pronounced reduction peaks are observed on the subsequent sweep towards negative potentials. The first pronounced peak (peak III_c, Fig. 1A–C), is rather broad with $E_p = -0.25$ to -0.35 V. The second, sharper peak (peak IV_c) occurs at $E_p = -0.60$ to -0.70 V. In addition, generally at least one more reduction peak is observed which is

TABLE 1

VOLTAMMETRIC DATA OBTAINED ON ELECTROCHEMICAL OXIDATION OF BARBITURIC ACIDS AND RELATED COMPOUNDS AT THE PGE[a] IN CHLORIDE BUFFER pH 1.3[b]

Compound	*Concentration /mM*	*Peak current*[c] $i_p/\mu A$	*Peak potential*[c] E_p/V *vs. SCE*	$(E_p - E_{p/2})$[c,d] /V	αn_a[e]	*Calculated voltammetric*[f] *n-value*
Barbituric acid	0.940	16.83	1.020	0.096	0.500	3.42
1-Methyl-barbituric acid	1.149	21.05	1.025	0.109	0.440	3.73
1,3-Dimethyl-barbituric acid	1.214	23.30	1.030	0.116	0.414	4.02
5-Chloro-barbituric acid	1.147	8.00	0.750	0.050	0.960	0.98
5,5-Dichloro-barbituric acid	1.103	N.O[g]	N.O[g]			
Hydurilic acid	1.022		(I)[h] 0.50 (II) 1.00			

[a] Area: 0.1257 cm^2.

[b] See Experimental for buffer composition, actual pH was 1.3.

[c] Mean of at least three replicate measurements. Voltage sweep rate 0.005 V s^{-1} in all cases. Repeatability of E_p values was about ± 20 mV.

[d] $E_{p/2}$ = Half peak potential.

[e] Determined from the expression[15] $E_p - E_{p/2} = 0.048/\alpha n_a$.

[f] Calculated from the equation[16] $n = i_p/2.98 \times 10^5\, n(\alpha n_a)^{\frac{1}{2}}\, AD^{\frac{1}{2}}\, v^{\frac{1}{2}}\, c$ where all terms have their usual electrochemical significance. The value of D was taken as 7.86×10^{-6} cm^2 s^{-1} obtained for 5,6-diaminouracil under similar solution conditions[17] using the potentiostatic method of Shain and Martin[18].

[g] Not oxidized.

[h] This compound exhibits two peaks.

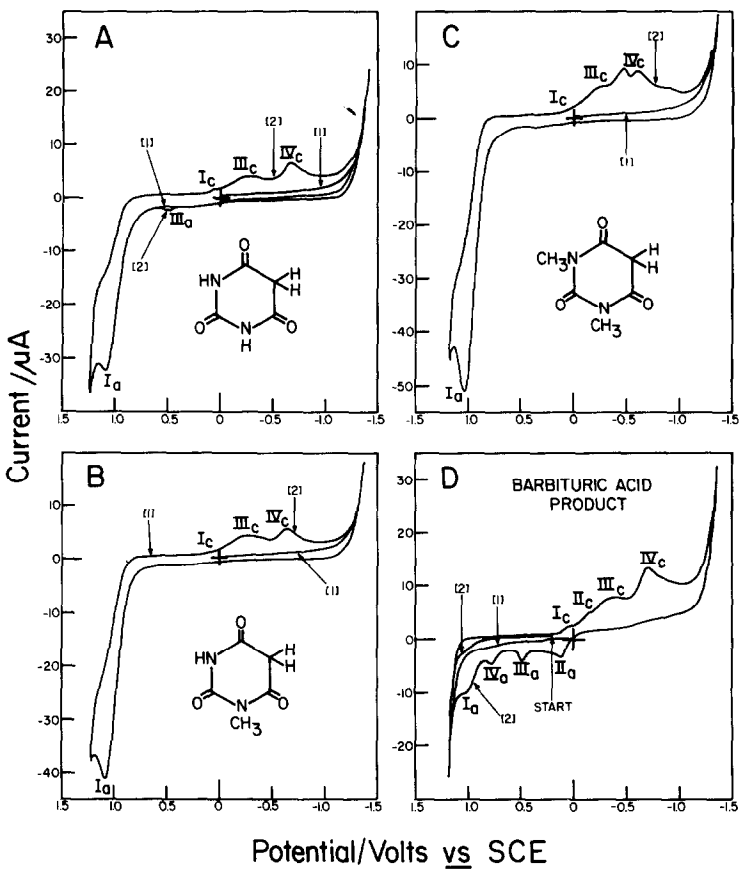

Fig. 1. Cyclic voltammograms at a sweep rate of 20 mV s^{-1} at the PGE (0.126 cm^2) in chloride buffer pH 1.3 of (A) 0.94 m*M* barbituric acid, (B) 1.149 m*M* 1-methylbarbituric acid, (C) 1.214 m*M* 1,3-dimethylbarbituric acid. Sweep pattern: 0.00 V→−1.40 V→1.25 V→−1.40 V→0.00 V. (D) Product obtained after electrolysis of 1.43 m*M* barbituric acid at 1.00 V; sweep pattern: 0.20 V→1.20 V→−1.40 V→1.20 V→0.20 V. Numbers in square brackets refer to cycle number.

small, drawn out and has a peak potential at $E_p \approx 0.1$ V (peak I_c, Fig. 1A,C). On the subsequent second sweep towards positive potentials occasionally, at slow sweep rates, one or more oxidation peaks are observed prior to the principal barbituric acid oxidation peak. For example, in Fig. 1A a small oxidation peak at $E_p = 0.5$ V is clearly visible. At much faster sweep rates (*e.g.* > 200 mV s^{-1}) the oxidation peak of the barbituric acids merge with the background and the rather broad, ill-defined reduction peaks shown in Fig. 1A–C, became even less well defined. However, at a sweep rate of 20 V s^{-1}, where it is necessary in effect, to sweep beyond the anodic background discharge potential in order to oxidize barbituric acid, it is possible, after sweeping more negative than peak IV_c (see Fig. 1A), to observe on the second sweep towards positive potentials, an oxidation peak at 0.55 V (equivalent to peak III_a in Fig. 1A) which in turn gives a product almost reversibly reduced ($E_p = 0.50$ V) on the next negative-going sweep. The species oxidized at $E_p = 0.55$ V will be subsequently shown to be hydurilic acid.

After controlled potential electrolysis of *ca.* 1 m*M* barbituric acid at 1.00 V, cyclic voltammetry of the resultant products (Fig. 1D) revealed the presence of four reduction peaks at E_p = *ca.* 0.07 V (peak I_c), *ca.* −0.15 V (peak II_c), *ca.* −0.35 to −0.40 V (peak III_c) and −0.70 V (peak IV_c). Having scanned the latter reduction peaks four oxidation peaks are observed on the next positive-going sweep at E_p = *ca.* 0.1 V (peak II_a), 0.50 V (peak III_a), 0.78 V (peak IV_a) and *ca.* 1.02 V (peak I_a, *i.e.*, the same potential as for the initial oxidation of the barbituric acids). At sweep rates greater than 200 mV s^{-1} a small reduction peak is observed after having scanned peak III_a forming a quasi-reversible couple. Peak II_a is observed after having scanned peak I_c, the two peaks also forming a quasi-reversible couple. Cyclic voltammetry of the products of controlled potential electrooxidation of 1-methyl- and 1,3-dimethylbarbituric acids was essentially identical with that observed for the unsubstituted acid.

Cyclic voltammetry of the pH 1.3 chloride buffer under the same conditions used to obtain the voltammograms in Fig. 1 gave no evidence of electrooxidation of chloride ion to chlorine. Formation of appreciable quantities of chlorine in the above solution is readily observed in cyclic voltammetry by formation of a reduction peak at E_p = −0.9 to −1.1 V. This peak is observed only if the positive-going sweep is continued to about 1.4 V.

Polarography of the products of electrooxidation of barbituric acid and its N-methyl derivatives revealed that a reduction wave was present indicating the presence of alloxan ($E_{\frac{1}{2}}$ *ca.* 0.0 V). In addition a wave having a large maximum at *ca.* −0.6 V was observed. One of the identified products, 5,5-dichlorobarbituric acid gave a similar maximum. The rather severe distortion of the latter wave, erratic droptimes and the large maximum precluded use of the polarographic behavior of the product for analytical purposes.

Coulometry, mass electrolysis and product analysis

Controlled potential electrolysis and coulometry of barbituric acid and its

TABLE 2

TYPICAL COULOMETRIC *n*-VALUES OBSERVED ON CONTROLLED POTENTIAL ELECTROLYSIS OF BARBITURIC ACIDS AT 1.00 V IN CHLORIDE BUFFER pH 1.3 AT THE PGE

Compound	*Concentration /mM*	*n-value*[a]
Barbituric acid	1.430	3.48
	9.507	3.23
1-Methylbarbituric acid	0.943	3.20
	10.000	3.42
1,3-Dimethylbarbituric acid	1.073	2.73
	10.000	3.44
5-Chlorobarbituric acid	1.301	1.16
	10.000	1.26
Hydurilic acid	1.54	4.79
	4.93	4.89

[a] The reproducibility of these *n*-values was always better than ±10% of the values shown.

TABLE 3

PRODUCTS OF ELECTROCHEMICAL OXIDATION OF BARBITURIC ACIDS AND RELATED COMPOUNDS AT 1.00 V AT THE PGE IN CHLORIDE BUFFER pH 1.0

Compound	*Product*	*Yield*[a] %	*Exptl.*[b] *n-value*	*Calculated*[c] *n-value*
Barbituric acid	5,5′-Dichlorohydurilic acid	38.7	3.23	3.22
	Alloxan monohydrate	21.1		
	5,5-Dichlorobarbituric acid	30.6		
1-Methyl-barbituric acid	5,5′Dichloro-1,1′-dimethylhydurilic acid	27.3	3.42	2.90
	1-Methylalloxan monohydrate	21.1		
	5,5-Dichloro-1-methylbarbituric acid	30.9		
1,3-Dimethyl-barbituric acid	5,5′-Dichloro-1,1′,3,3′-tetra-methylhydurilic acid	54.9	3.44	3.16
	1,3-Dimethylalloxan monohydrate	14.2		
	5,5-Dichloro-1,3-dimethylbarbituric acid	23.4		
5-Chloro-barbituric acid	5,5′-Dichlorohydurilic acid	31.6	1.26	1.14
	Alloxan monohydrate	27.5		
	5,5-Dichlorobarbituric acid	13.9		
Hydurilic acid	5,5′-Dichlorohydurilic acid	13.6	4.89	4.40
	Alloxan monohydrate	37.8		
	5,5-Dichlorobarbituric acid	26.4		

[a] Moles of product per mole of reactant × 100.
[b] Measured at *ca.* 10 m*M* concentration level.
[c] Calculated on the basis of the yields of products actually found.

N-methylated derivatives at potentials close to the oxidation peak gave faradaic *n*-values of 3.2–3.4 (Table 2). The observed *n*-values were independent of concentration (Table 2). Electrolysis of solutions between *ca.* 1 m*M* in the barbituric acids and greater than 10 m*M* always resulted in formation of a white precipitate in the solution.

At the controlled potentials employed for coulometry, electrolysis of the background gave only a small current that rapidly decayed to a very low level (*ca.* 20–40 μA at electrodes having an area of *ca.* 110 cm^2).

Product isolation and quantitation was best done after electrolysis of relatively concentrated barbituric acid solutions (*i.e.*, 150 ml of *ca.* 10 m*M*; see Experimental). The products and their yields are shown in Table 3 where it is clear that in general the major product formed on electrooxidation of barbituric acid or its N-methyl derivatives is the corresponding 5,5′-dichlorohydurilic acid. The yield of the appropriate 5,5-dichlorobarbituric acid is normally slightly lower and that of the appropriate alloxan derivative the lowest. The products obtained account for at least 80% of the barbituric acids oxidized. In fact, calculation of the expected *n*-value based on the observed products and their yields, is in good agreement with the observed experimental *n*-value (Table 3). The slightly lower values of the calculated *n*-values are no doubt related to the small (generally $< 10\%$) amounts of unidentified products noted (see Experimental). It is also of interest to note that the *n*-value calculated from

the height and shape of the peak voltammogram of the barbituric acids (Table 1) are in excellent agreement with the coulometric (and concentration independent) *n*-values (Tables 2 and 3). This could be regarded as reasonable evidence that the processes occurring under voltammetric conditions are essentially identical to those occurring under conditions of prolonged, constant potential electrolysis.

Voltammetry of 5-chloro- and 5,5-dichlorobarbituric acid and hydurilic acid

The identity of the products obtained upon electrooxidation of barbituric acids (Table 3) suggested that 5-chlorobarbituric acid or hydurilic acid might be intermediates in the overall electrode reaction of the former compounds (*vide infra*). Accordingly, the voltammetry of the latter compounds in chloride pH 1.0 was examined. 5-Chlorobarbituric acid is electrochemically oxidized by way of a single voltammetric peak ($E_p = 0.75$ V, Table 1). 5,5-Dichlorobarbituric acid however is not oxidizable at pH 1. Hydurilic acid, on the other hand, exhibits two oxidation peaks, the first at $E_p = 0.50$ V and the second at essentially the same peak potential as for the single peak of barbituric acid (*i.e.*, $E_p \approx 1.00$ V). 1,1′-Dimethylhydurilic acid and 1,1′,3,3′-tetramethylhydurilic acid are oxidized at identical potentials as unsubstituted hydurilic acid at pH 1.0. The detailed electrochemistry of hydurilic acids will be reported shortly[19]. 5-Chlorobarbituric acid exhibits a single voltammetric reduction peak at $E_p = -0.70$ V while 5,5-dichlorobarbituric acid exhibits two reduction peaks at $E_p = -0.30$ V and -0.70 V (all E_p values quoted are in chloride buffer pH 1.3 at a sweep rate of 5 mV s^{-1} at *ca.* 1 m*M* concentration). Hydurilic acid is not electrochemically reducible*.

Cyclic voltammetry of chlorobarbituric acids and hydurilic acid reveal considerable information regarding the overall electrode reactions (Fig. 2A–D). Thus, 5-chlorobarbituric acid at a clean PGE exhibits a single oxidation peak (peak IV_a, Fig. 2B) and a single reduction peak (peak IV_c, Fig. 2A). After having scanned reduction peak IV_c, an additional oxidation peak is observed on the subsequent positive-going sweep at $E_p = 1.05$ V (peak I_a, Fig. 2A). This suggests that 5-chlorobarbituric acid is electrochemically reduced at peak IV_c to barbituric acid which gives rise to peak I_a.

If the single, initial voltammetric oxidation peak of 5-chlorobarbituric acid (peak IV_a, Fig. 2B) is scanned at a clean PGE, then on the subsequent negative going sweep a broad reduction peak (peak III_c, Fig. 2B) is observed at less negative potentials than the primary reduction peak (peak IV_c, Fig. 2B). The broad peak III_c (Fig. 2B) is probably due to reduction of two species, 5,5-dichlorobarbituric acid and 5,5′-dichlorohydurilic acid since, as will be shown shortly, these are major products of oxidation of 5-chlorobarbituric acid (see also Experimental). That part of the process involved in the peak III_c reduction (Fig. 2B) is due to reduction of 5,5′-dichlorohydurilic acid is also supported by the fact that a further oxidation peak (peak III_a, Fig. 2B) can be observed after having scanned peak III_c. Peak III_a corresponds to the oxidation of hydurilic acid (see Fig. 2D). Work already completed has shown that 5,5′-dichlorohydurilic acid is quantitatively reduced electrochemically

* 5,5′-Dichlorohydurilic acids are reducible and give voltammetric peaks and polarographic waves[17] at pH $\geqslant$ *ca.* 6. However, their extreme insolubility at low pH precluded any voltammetric or polarographic studies at *ca.* pH 1.0.

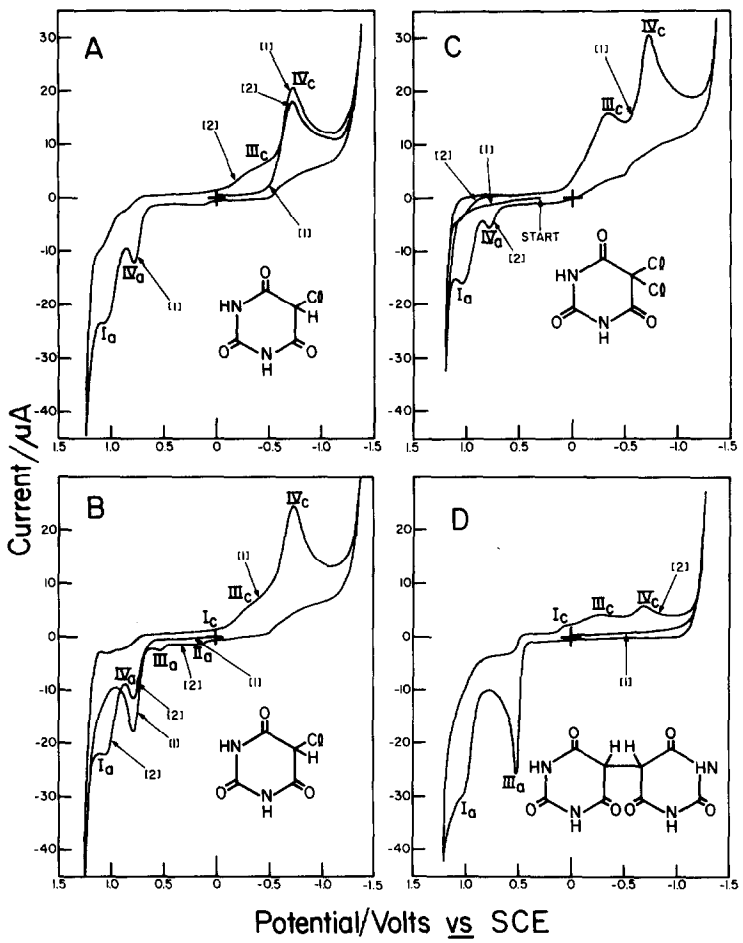

Fig. 2. Cyclic voltammograms in chloride buffer pH 1.3 at the PGE (0.126 cm^2) at a sweep rate of 20 mV s^{-1} of (A) 1.145 m*M* 5-chlorobarbituric acid; sweep pattern: 0.00 V→−1.40 V→1.25 V→−1.45 V; (B) 1.145 m*M* 5-chlorobarbituric acid, sweep pattern: 0.00 V→1.25 V→−1.40 V→1.25 V→0.00 V; (C) 1.103 m*M* 5,5-dichlorobarbituric acid, sweep pattern: 0.30 V→1.20 V→−1.40 V→1.25 V→0.00 V and (D) 1.022 m*M* hydurilic acid, sweep pattern: 0.00 V→−1.30 V→1.20 V→−1.30 V. Numbers in square brackets refer to cycle number.

to hydurilic acid[17]. It will also be noted that following oxidation of 5-chlorobarbituric acid (Fig. 2B) then on the subsequent negative going sweep a very small, drawn-out reduction peak occurs at *ca.* 0.1 V (peak I_c) which appears to form a small quasi-reversible couple with peak II_a (Fig. 2B) on the next positive going sweep. Peak I_c is due to the kinetically controlled reduction of alloxan (electrooxidation product of 5-chlorobarbituric acid, *vide infra*) to dialuric acid[19] which is reoxidized to alloxan (peak II_a) on the next sweep towards positive potentials.

5,5-Dichlorobarbituric acid is clearly sequentially reduced first to 5-chlorobarbituric acid at peak III_c (Fig. 2C) since clipping the voltammogram at potentials just beyond the first reduction peak of 5,5-dichlorobarbituric acid (peak III_c, Fig. 2C) gives rise to a single oxidation peak (peak IV_a, Fig. 2C) on the subsequent sweep

towards positive potentials which corresponds exactly to the single oxidation peak of 5-chlorobarbituric acid. The second reduction peak of 5,5-dichlorobarbituric acid (peak IV_c, Fig. 2C) clearly has the same peak potential as the single reduction peak of 5-chlorobarbituric acid (peak IV_c, Fig. 2A) and is due to reduction of the latter species formed in the peak III_c process. Since the peak IV_c reduction produces barbituric acid, the next sweep to positive potentials gives, in addition to the oxidation peak of 5-chlorobarbituric acid (peak IV_a, Fig. 2C), peak I_a due to oxidation of barbituric acid.

Hydurilic acid is not electrochemically reducible but gives rise to two voltammetric oxidation peaks (peaks III_a and I_a, Fig. 2D). If a cyclic voltammogram is run and clipped after peak III_a an almost reversible reduction peak can be observed at sweep rates greater than about 200 mV s^{-1}. No other peaks are observed under the latter conditions. After scanning both peaks III_a and I_a (Fig. 2D) at least three reduction peaks are observed on the negative-going sweep peaks I_c, III_c and IV_c (see Fig. 2D). The potentials of the latter peaks correspond to those expected for reduction of alloxan[20] (peak I_c), and 5,5-dichlorobarbituric acid (peaks III_c and IV_c).

Coulometry, mass electrolysis and product analysis of 5-chlorobarbituric acid and hydurilic acid

Controlled potential electrooxidation and coulometry of 5-chlorobarbituric acid at 1.00 V in chloride buffer at pH *ca.* 1 gave a faradaic *n*-value of about 1.2 to 1.3 (Table 2). The same three products observed upon oxidation of barbituric acid were found, namely 5,5′-dichlorohydurilic acid, alloxan monohydrate and 5,5-dichlorobarbituric acid (see Table 3 and Experimental). As with the barbituric acids, 5,5′-dichlorohydurilic acid was the major product although the yields of the other two products were reversed. The *n*-value calculated on the basis of the observed products and their yields (Table 3) agree very well with the experimental value measured by coulometry and also with the calculated voltammetric *n*-value (Table 1).

Coulometry of hydurilic acid gave a concentration independent *n*-value of 4.8–4.9 (Table 3). Again, the products were identical to those observed on electrooxidation of barbituric acid and 5-chlorobarbituric acid except that, unlike the case of the latter two compounds, 5,5′-dichlorohydurilic acid was the minor product of the process.

Mechanism of electrochemical oxidation of barbituric acids

Summarizing much of the preceding experimental data, barbituric acid and its N-methyl derivatives are electrochemically oxidized in a pH-dependent process that involves about 3.2–3.4 moles of electrons per mole. There are three major products of the electrode reaction, namely the appropriately substituted 5,5′-dichlorohydurilic acids, 5,5-dichlorobarbituric acids and alloxans. Cyclic voltammetry reveals that after the barbituric acid voltammetric oxidation peak has been scanned (peak I_a, Fig. 1A) 5,5-dichlorobarbituric acid is present as a product as evidenced by the peaks III_c and IV_c (see Fig. 1A and compare to the same peaks in Fig. 2C). 5,5′-Dichlorohydurilic acid is also an initial electrode product since, although no distinct reduction peak can be observed for this under cyclic voltammetric conditions because the peak would be masked by those of 5,5-dichlorobarbituric acid and alloxan[17]

(*vide infra*) and would be very small owing to the very low solubility of 5,5′-dichlorohydurilic acid at low pH, the product of its electrochemical reduction, hydurilic acid, can be detected by cyclic voltammetry on the second cycle towards positive potentials. Thus the small peak III_a (Fig. 1A) should be compared to the same peak III_a for authentic hydurilic acid (Fig. 2D). The failure to observe peak III_a on cyclic voltammetry of 1-methyl- and 1,3-dimethylbarbituric acid (Fig. 1B,C) is undoubtedly due to the even lower solubility of the methylated 5,5′-dichlorohydurilic acids (formed in the peak I_a process), such that essentially undetectable amounts of hydurilic acid are formed. Alloxans could not be convincingly detected on cyclic voltammetry of the barbituric acids. These species are very readily reducible at $E_p = ca.$ 0.1 V at pH 1.0[20] but in a kinetically controlled process to give dialuric acid. In other words, alloxan would be expected to give a very small, drawn out reduction peak on cyclic voltammetry at potentials slightly positive of 0.00 V. However, in the typical voltammograms shown in Fig. 1A,B,C, there is some indication for such a peak (peak I_c).

In view of the fact that the coulometric n-value agrees very closely with the calculated voltammetric n-value for the barbituric acids and, since the products observed after controlled potential electrolysis appear to be detectable directly or indirectly by cyclic voltammetry of the barbituric acids, it seems reasonable to conclude that the mechanisms, products and yields obtained under voltammetric and controlled potential electrolysis conditions are very similar if not identical.

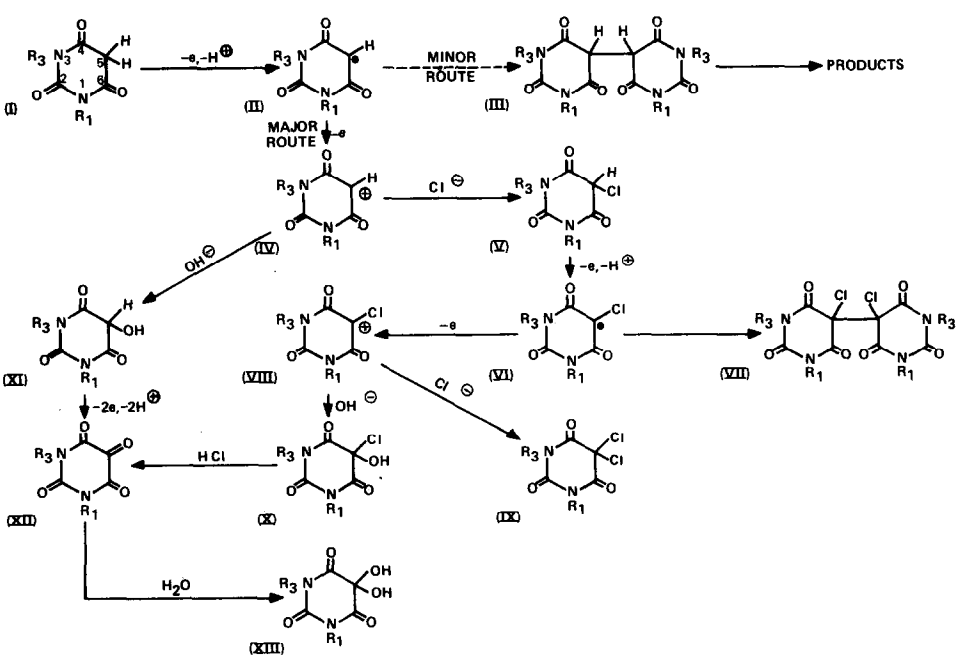

Fig. 3. Proposed mechanism for electrochemical oxidation of barbituric acid ($R_1 = R_3 = H$), 1-methylbarbituric acid ($R_1 = CH_3$, $R_3 = H$) and 1,3-dimethylbarbituric acid ($R_1 = R_3 = CH_3$) in the presence of chloride ion at low pH.

On the basis of this and subsequently discussed information, the mechanism of electrochemical oxidation of barbituric acids in the presence of chloride ion at pH 1 is probably well represented by Fig. 3. The initial step is probably a $1e/1H^+$ oxidation of the barbituric acid (I, Fig. 3) to give a radical species (II, Fig. 3). The evidence for this initial $1e/1H^+$ step is that the measured αn_a value is *ca.* 0.4–0.5 (Table 1). Assuming a typical value of α of 0.5 leads to an n_a value of 1.0. The radical species can then undergo two possible reactions: dimerization to hydurilic acid (III, Fig. 3) or further $1e$ oxidation to the carbonium ion (IV, Fig. 3) and hence to 5-chlorobarbituric acid or 5-hydroxybarbituric acid. Dimerization of the radical (II) to hydurilic acid (III) is proposed to be the less likely route because electrochemical oxidation of the latter, at the same potential and under identical conditions employed for oxidation of barbituric acid, gives the same products as are observed from the latter species but in considerably different yields. Thus 5,5′-dichlorohydurilic acids are the major products from electrooxidation of barbituric acid, while alloxan is the major hydurilic acid electrooxidation product. Indeed 5,5′-dichlorohydurilic acid is the most minor product formed from hydurilic acid (see Table 3). On the other hand, electrooxidation of 5-chlorobarbituric acid (which would be formed from the carbonium ion IV, Fig. 3) gives 5,5′-dichlorohydurilic acid as its major product (Table 3). Thus, on the basis of the product yields obtained on electrooxidation of barbituric acids, hydurilic acid and 5-chlorohydurilic acid, it would appear that the initial radical formed on oxidation of the barbituric acids is preferentially further oxidized to a carbonium ion rather than dimerizing. However, on the basis of the evidence available it is quite possible (indeed likely) that both processes could occur simultaneously.

The barbituric acid carbonium ion (IV, Fig. 3) would, once formed, react with nucleophiles present in the solution, principally chloride ion, to give 5-chlorobarbituric acid (V, Fig. 3), or perhaps water (or hydroxide ion) to give 5-hydroxybarbituric acid (XI, Fig. 3) better known as dialuric acid. It is difficult to compare the relative nucleophilicities of water and chloride ion, particularly in the vicinity of a charged electrode surface[21,22]. Attempts to examine the effect of other nucleophiles (*e.g.*, Br^- and CN^-) on the reaction products and yields failed owing to the preferential electrooxidation of these nucleophiles compared to the barbituric acids. Nevertheless, it would seem reasonable to expect attack of chloride ion on the carbonium ion to predominate. Based on the nature of the products, 5-chlorobarbituric acid, formed by nucleophilic attack of Cl^- on the intermediate barbituric acid carbonium ion, must be further electrochemically oxidized in a $1e/1H^+$ process first to the radical VI (Fig. 3). Again this radical can dimerize to 5,5′-dichlorohydurilic acid (VII, Fig. 3) or be further electrooxidized in a $1e$ process giving the 5-chlorobarbituric acid carbonium ion VIII (Fig. 3). This again would be attacked by nucleophiles in solution (Cl^- and H_2O or OH^-) to give 5,5-dichlorobarbituric acid (IX, Fig. 3) or 5-hydroxy-5-chlorobarbituric acid (X, Fig. 3). The latter species would be expected to readily lose HCl to give alloxan (XII, Fig. 3) and hence alloxan hydrate (XIII).

As mentioned previously, the initial barbituric acid carbonium ion (IV) could be attacked by water to give dialuric acid (XI). This species would be very readily electrooxidized[20] to alloxan in a $2e/2H^+$ process (XII and XIII, Fig. 3).

The mechanism presented in Fig. 3 represents the electrode and related

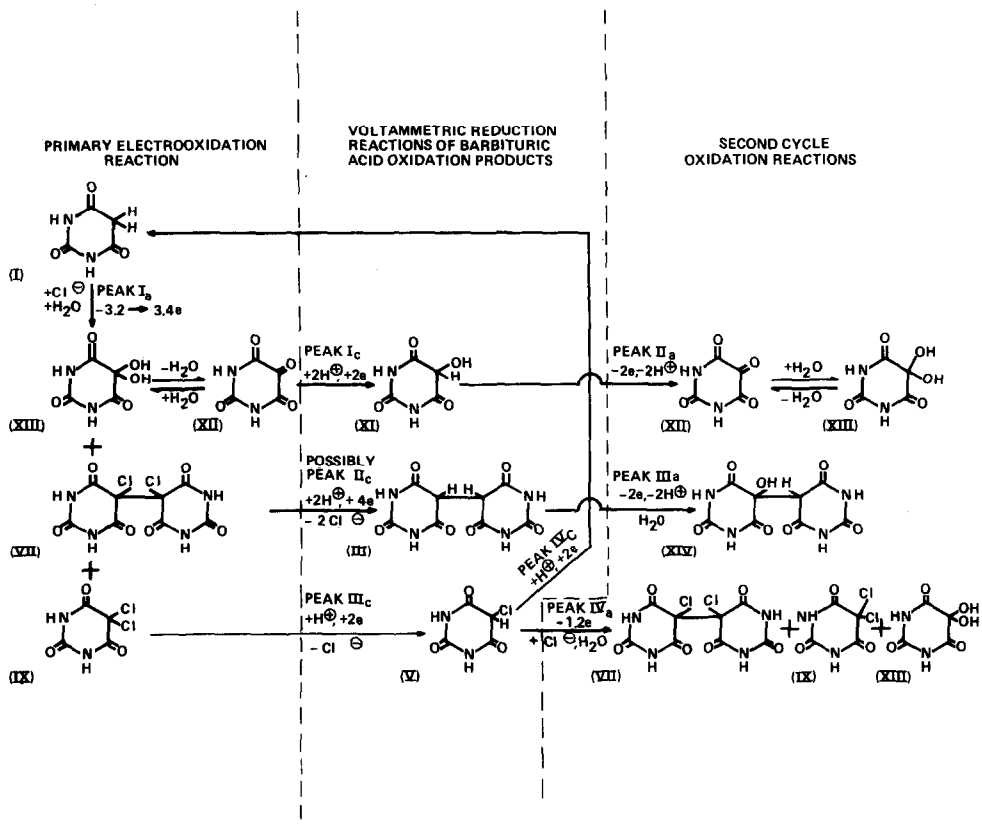

Fig. 4. Reactions associated with the voltammetric oxidation peaks observed on cyclic voltammetry of barbituric acid and its electrooxidation products. Refer to Fig. 1D for peak identification.

chemical processes occurring at the single voltammetric peak (peak I_a, Fig. 1A–C) of barbituric acid and its N-methyl derivatives. Cyclic voltammetry of barbituric acids and particularly of the product of controlled potential electrolysis is very complex (see for example Fig. 1D). On the basis of the information included in this paper and reported earlier in the literature, the basic reaction scheme associated with each peak observed on cyclic voltammetry of oxidized barbituric acid is shown in Fig. 4. Thus, as a result of the primary electrooxidation of barbituric acid (peak I_a) alloxan (XIII, XII, Fig. 4), 5,5′-dichlorohydurilic acid (VII) and 5,5-dichlorobarbituric acid (IX) are formed. Reduction peak I_c corresponds to the kinetically controlled reduction of alloxan (XII) to dialuric acid (XI). Reduction peak II_c may correspond to reduction of 5,5′-dichlorohydurilic acid (VII) to hydurilic acid (III)*. Peaks III_c and IV_c correspond to the stepwise reduction of 5,5-dichlorobarbituric acid (IX) first to 5-chlorobarbituric acid (V) then to barbituric acid (I). On the next sweep towards positive potentials peak II_a is due to reoxidation of the

* Attempts to dissolve sufficient 5,5′-dichlorohydurilic acid at pH 1.0 to give a voltammetric reduction peak were unsuccessful. However, it is possible that electrooxidation of barbituric acid may give local supersaturated solutions of 5,5′-dichlorohydurilic acid close to the electrode which leads to peak II_c.

dialuric acid (XI), formed by reduction of alloxan (XII), back to alloxan. Peak III_a is a 2*e* oxidation of hydurilic acid (III) to 5-hydroxyhydurilic acid (XIV); the mechanism of this reaction will be reported at a later date[19]. Finally, peak IV_a is due to oxidation of 5-chlorobarbituric acid (formed in the peak III_c process) to 5,5′-dichlorohydurilic acid (VII), 5,5-dichlorobarbituric acid (IX) and alloxan (XIII).

ACKNOWLEDGEMENTS

The authors would like to thank the National Science Foundation which supported this work. We would also like to acknowledge the valuable preliminary studies conducted in this laboratory on the electrochemical oxidation of barbituric acid by Dr. Jean Pinson.

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