A Study on the Electrochemical Reduction Mechanism of Rotenone

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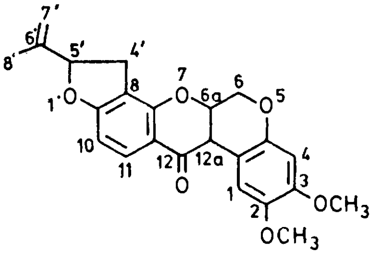
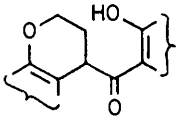
In aqueous alkaline medium the reduction of rotenone leads, by a two electron process, to the almost quantitative formation of rotenol; the electrochemical method is therefore an alternative to the chemical preparation of this compound. In DMF rotenone shows two reduction waves. At the potential of the first rotenone is directly reduced, *via* an e.c.e.c. mechanism; the primary radical anion undergoes a first-order isomerization reaction allowing the cleavage of the 6a,7 ether bond and the formation of a further reducible species. The isomerization reaction is the rate-determining step of the overall process; the value of the rate constant has been evaluated by voltammetric measurements. The final step is a protonation reaction by the depolarizer itself leading to the formation of rotenol anion and 6a,12a-didehydrorotenol anion. The latter gives rise to the second polarographic wave *via* an e.c.e. mechanism.

ROTENONE (I) plays an important role in biological processes;[1] it is in fact a very selective inhibitor of the respiratory chain acting at level of NAD–flavin-linked electron transfer.[1a,b]

Several studies have been devoted to the structural[2] and conformational analysis[3] of rotenone and related compounds. The chemical behaviour of rotenone deserves interest for the variety of oxidation and reduction products obtained according to the conditions.[1b] At present, few data are available[4] on the redox mechanism of formation of these compounds; in this respect it seems interesting to investigate the electrochemical behaviour of rotenone.



(I)



(II)

In this paper the results of a study on the electrochemical reduction of rotenone, in dimethylformamide (DMF) and in alkaline aqueous solution are reported and an electrochemical method for the synthesis of rotenol (II) is proposed.

The behaviour has been investigated by polarography, potential sweep voltammetry (p.s.v.), and macroscale electrolysis followed by analysis of the products.

EXPERIMENTAL

Materials and Apparatus.—Natural 5′β,6aβ,12aβ-rotenone (I) (Ega-Chemie) was purified from a chloroform solution and precipitated by dilution with ethyl alcohol; it was recrystallized from acetonitrile, m.p. 165.5—166 °C (lit.,[3b] 165—166 °C). The *trans*-12a*H*-12-hydroxy-derivative of rotenone, rotenol, 6a,12a-didehydrorotenol, and mutarotenone (a 5′β,6aβ,12aβ, and 5′β,6aα,12aα diastereoisomeric mixture of rotenone) were prepared according to the methods reported by Crombie *et al.*[3b,4]

T.l.c. on silica gel was performed using a 1 : 1 : 1 ethyl ether–light petroleum–chloroform solution. A column of silica gel and the same solution was used for separation.

Commercial grade DMF was dried over K$_2$CO$_3$ and fractionally distilled under reduced pressure; the middle fraction was collected and stored under nitrogen.

Tetrabutylammonium perchlorate (TBAClO$_4$) was prepared by neutralizing a solution of tetrabutylammonium hydroxide (TBAOH) with perchloric acid, crystallized from ethyl alcohol, and vacuum dried.

Polarographic and potential sweep voltammetric measurements were carried out using an AMEL model 448 three electrode apparatus equipped with positive feedback for ohmic compensation. An Ag–AgCl–tetramethylammonium chloride (TMACl) saturated solution in acetonitrile was used as reference electrode, a platinum sheet as counter electrode, and a hanging mercury electrode (h.m.e.) or a dropping mercury electrode (d.m.e.) with mechanical control of the drop time as indicating electrode.

Controlled potential electrolyses and coulometric measurements were performed with an AMEL potentiostat model 557 equipped with an integrator model 558. The cell had a mercury pool of *ca.* 30 cm^2 surface area as cathode and platinum gauze as anode; the anode compartment was separated from the cathode solution by sintered glass. The reference electrode was analogous to that employed in polarography when electrolyses were performed in DMF while in aqueous solution a saturated calomel electrode (s.c.e.) was used.

I.r. spectra were recorded with a Beckmann IR 9 spectrophotometer for solutions in CCl$_4$.

RESULTS

Aprotic Medium.—In DMF the polarographic pattern of rotenone was markedly dependent on temperature and drop time. At 25 °C and 2 s the reduction occurred in two waves of nearly equal height (Figure 1); the relative half-wave potentials $E_{\frac{1}{2}}$ were -1.63 and -1.85 V *versus* Ag–AgCl–TMACl. By decreasing the temperature and/or the time the second wave decreased more rapidly than the first and, at -25 °C; at a drop time of 1 s, only the first was present. If a stoicheiometric amount of TBAOH was added to the solution, the first wave disappears while the second was doubled in height.

The potential sweep voltammetry behaviour (Figure 2) paralleled that shown in polarography: at 25 °C and a low scan rate ($v = 0.15$ V s^{-1}), two cathodic peaks of about the same height were observed. On increasing v, the second peak decreased while, simultaneously, the anodic partner of the first peak appeared. At $v > 100$ V s^{-1}, only the first peak was present; the potential difference ΔE_p between the cathodic and the related anodic peak was *ca.* 60 mV, as expected for a reversible one-electron transfer process.[5]

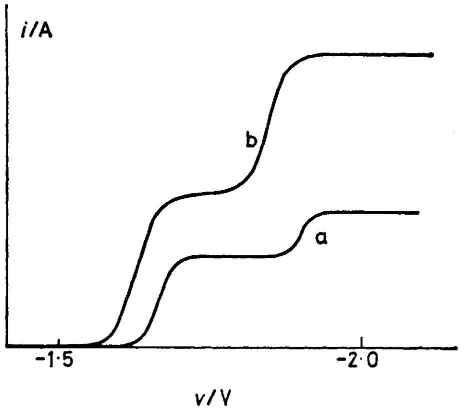


FIGURE 1 Direct current polarograms of 2.00 × 10^{-3}M-rotenone in DMF: drop time 2 s; temperature a, −18 °C; b, 25 °C

The ratio $(i_{ap})_I : (i_{cp})_I$ between the anodic and cathodic peak currents, determined according to ref. 6 at a fixed value of v, was independent of the concentration both of the depolarizer and the supporting electrolyte. The sweep rate dependence of this ratio at three different concentrations of the depolarizer is reported in Figure 3.

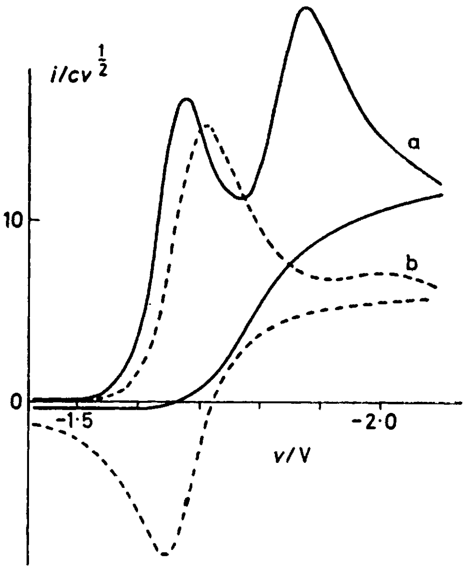


FIGURE 2 Potential sweep voltammetric curves of 2.00 × 10^{-3}M-rotenone at 25.1 °C: a, v 0.15 V s^{-1}; b, v 40 V s^{-1}; $i/cv^{\frac{1}{2}}|10^{-3}$ μAM^{-1} V$^{-\frac{1}{2}}$s$^{\frac{1}{2}}|$

The current function $(i_{cp})_I/v^{\frac{1}{2}}c$ for the first cathodic peak decreases by *ca.* 10% on increasing v (see Figure 4).

The peak potential $(E_{cp})_I$ was independent of the concentration, in the range explored (5 × 10^{-5}—1 × 10^{-3}M), and was also constant with variation in v whenever the corresponding anodic peak was present. However, when, on decreasing v, the anodic component disappeared (pure kinetic conditions), $(E_{cp})_I$ shifted towards less negative values by *ca.* 28 mV per log unit at 25 °C (Figure 4). This voltammetric behaviour is typical of a system with a first-order irreversible chemical reaction following the reversible electron transfer process.[5]

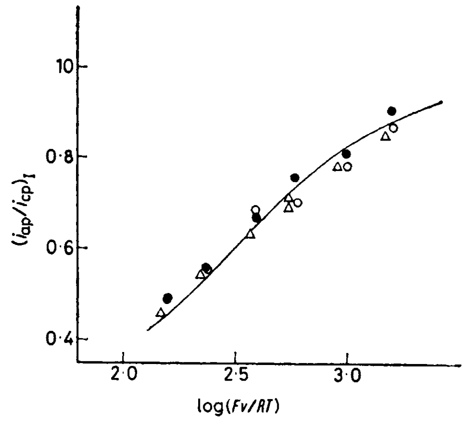


FIGURE 3 Sweep rate dependence of the ratio of the anode to cathode peak current for the first peak of rotenone at 25.1 °C, $nF(E° - E_\lambda)/RT = 4.0$: concentration 4.01 × 10^{-4}M (○); 9.95 × 10^{-4}M (●); 2.00 × 10^{-3}M (△)

In Figure 5 the dependence of $(i_{cp})_{II}$ on v is reported together with the ratio $(i_{cp})_{II} : (i_{cp})_I$. Although the determination of $(i_{cp})_{II}$ is rather approximate since it suffers from the limitation characteristic of subsequent peaks, these results show a behaviour typical of an e.c.e. mechanism.[7]

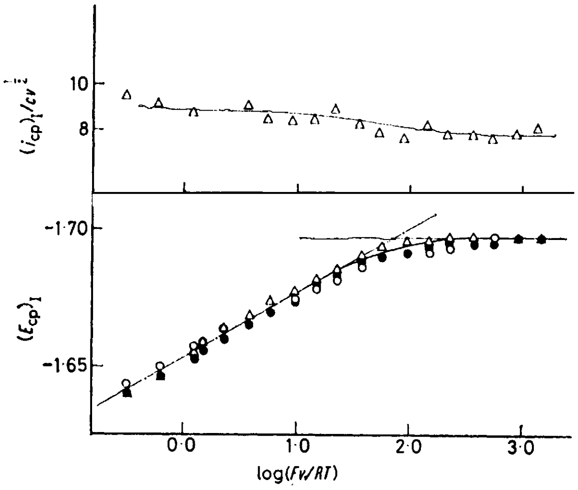


FIGURE 4 Sweep rate dependence of the peak current function $(i_{cp}/cv^{\frac{1}{2}})$ and of the cathode peak I potential $(E_{cp})_I$ of rotenone at 25.1 °C: concentration 4.01 × 10^{-4}M (○); 9.95 × 10^{-4}M (●); 2.00 × 10^{-3}M (△)

Macroscale electrolyses of 5 × 10^{-2}M-rotenone were performed at the potential of the first wave for a DMF (20 ml) solution to which was added an equimolar quantity of TBAClO$_4$ as supporting electrolyte. Polarographic curves, recorded during electrolysis, showed the progressive decrease of the first wave while the second remains unchanged. After exhaustive electrolysis the apparent coulometric value corresponded to 1 F mol^{-1}. From the precipitate obtained by pouring the electrolysed solution into deaerated hydrochloric acid, a product (0.4 g) was recovered after repeated washing to eliminate the supporting electrolyte. T.l.c. analysis showed that the product was a mixture. Three spots were present: that with the highest

R_F corresponds to rotenol while the other two, which are very close to each other, are those expected for mutarotenone. Rotenol and mutarotenone, separated by elution on a silica gel column, were present in roughly equimolar quantities. They were identified from comparison of their i.r. spectra with those of authentic sample and from their m.p.s: rotenol, 119—120 °C; mutarotenone, 145—147 °C (lit.,[3b] 147 °C).

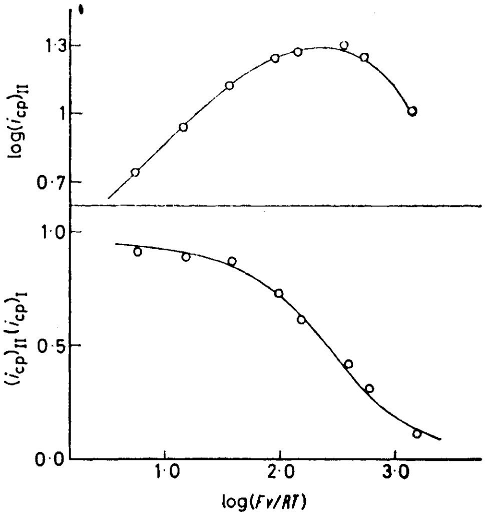


FIGURE 5 log plot of the peak II current against sweep rate, and sweep rate dependence of cathode peak I : cathode peak II current ratio; for 2.00 × 10^{-3}M-rotenone at 25.1 °C

Attempts to record the e.s.r. spectrum of the intermediate radical were made by electrolysing, at the potential of the first polarographic wave, directly in the e.s.r. cavity at low temperature (−30 °C), but no signal was detected.

When electrolysis was performed directly at potentials corresponding to the top of the second wave, the latter started to decrease only after the first wave disappeared. Coulometric measurements after exhaustive electrolysis give *ca.* 2 F mol^{-1}. Work-up in the usual way gives a solid which t.l.c. shows to be a mixture of several products. By elution on a silica gel column the two main products were separated but only rotenol, obtained in a 30% yield was identified from its m.p. and i.r. spectrum. The other compound, obtained in approximately the same yield, is a yellow oil which slowly gives a powder melting between 45 and 55 °C. The substance, as does rotenol, gives a positive iron(III) reaction by treatment with iron(III) chloride indicating the presence of phenolic hydroxy-groups. The i.r. spectrum (CCl_4) shows, at a suitable concentration, strong absorptions at 3 310 and/or 3 425 cm^{-1}; in a 5 × 10^{-5}M solution only the latter is present. This frequency region is characteristic of bonded OH in polymers and in dimers.[8] A band in the CO region, at 1 640 cm^{-1}, is also present.

No attempt was made to identify the other products as they were obtained in low yield.

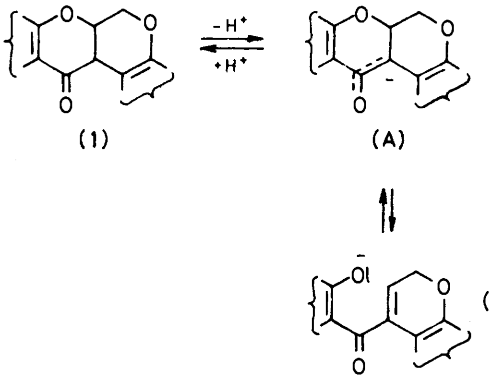
When electrolysis was performed on the single two electron wave, shown by rotenone after neutralization with TBAOH the yield of rotenol drastically decreased, while the number of spots in t.l.c. increased.

Aqueous Alcoholic Medium.—In 1 : 1 aqueous alcoholic solution at pH >10 rotenone was reduced in a single irreversible two-electron polarographic wave; the half-wave potential ($E_{\frac{1}{2}}$) in this medium was pH independent. Electrolyses were performed as follows. Ethyl alcohol (10 ml) and 1N-NaOH in water (10 ml) were poured into the electrolysis cell with rotenone (0.5 g). The saturated solution was reduced at −1.65 V *versus* s.c.e. After exhaustive electrolysis the coulometric *n* value corresponded to 2 F mol^{-1}. The electrolysed solution was neutralized by pouring it into deaerated 1N-hydrochloridric acid and the precipitate thus obtained was filtered off and washed with water. After separation on a silica gel column and crystallization from methyl alcohol, a product was recovered in 70% yield. It was identified as rotenol from its m.p. and i.r. spectrum by comparison with an authentic sample. The yield in rotenol was not affected by the concentration of added base.

Similar polarographic behaviour, with the same product from macroscale electrolysis, was shown by the isomer of rotenone, 6a,12a-didehydrorotenol under these conditions.

DISCUSSION

Aqueous Medium.—The reduction of rotenone in an aqueous alcoholic medium at alkaline pH values leads to the nearly quantitative formation of rotenol through a two-electron process. This result is therefore comparable to that of the chemical synthesis of rotenol but, in the latter case, derritol is obtained as a by-product in a yield which increases with increasing KOH concentration.[9,10] The yield of rotenol in the electrochemical reduction is, on the contrary, independent of the quantity of base added, and higher than that of the chemical reduction. The electrochemical synthesis can therefore be proposed as an alternative to the chemical method.



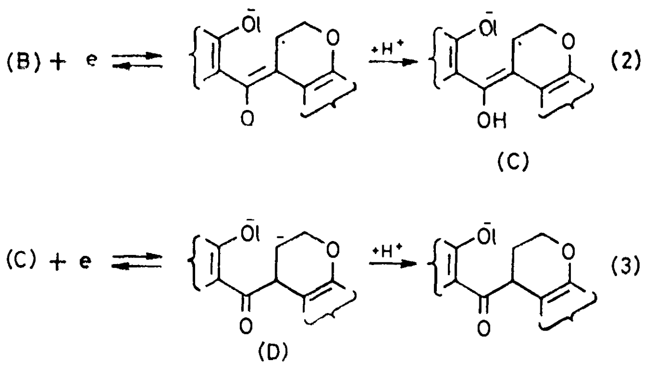
(1) (A)

(B)

As the ether bond, rather than the carbonyl group, appears to be the preferred site of reduction, we assumed that the species undergoing reduction is the anion of 6a,12a-didehydrorotenole (B), the isomer of the rotenone anion (A). It is well known, that species (B) participates in equilibrium (1) [1b,11] which, at pH >10, should be fully displaced towards the electroactive species (B) in agreement with the pH independence of the half-wave potential.

As for the reduction mechanism, since species (B) is an αβ-unsaturated carbonyl compound the activated double bond is first involved.[12] In alkaline medium (pH >10) an e.c.e.c. sequence generally operates for this class of compounds: the first electron uptake precedes the first proton transfer; the resulting neutral radical is reduced at the same potential and the carbanion so formed is protonated to give the final product.[12a]

This mechanism, in the case of rotenone, is shown in the Scheme. It would be interesting to extend the investigation to the acid pH range where equilibrium (1) is displaced towards the left and therefore the reduction would directly involve the carbonyl group with formation of the corresponding alcohol or pinacol.[13] However this was prevented by the low solubility of rotenone in aqueous acidic medium;[14] the investigation has therefore been carried out in DMF.

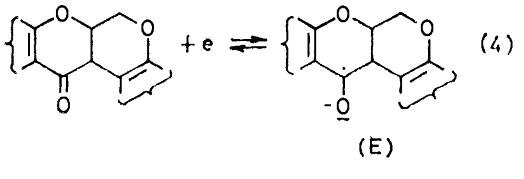


SCHEME

Aprotic Medium.—In aprotic medium, on the basis of the p.s.v. results obtained at low temperatures and/or high scan rates, we suggest that the first step in the electrochemical reduction of rotenone involves the formation of the corresponding radical anion (E). The first peak is, under these conditions, reversible and monoelectronic. The primary radical anion (E), with the unpaired electron probably localized on the carbonyl group, is very reactive: no e.s.r. signals were recorded even when electrolysis was performed *in situ* at low temperature.

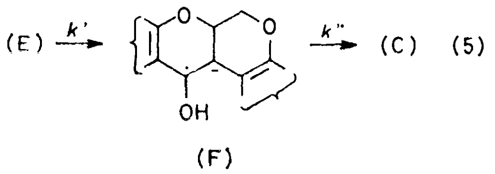
The results of the kinetic analysis carried out on the basis of voltammetric measurements[5] show that the radical decays according to a first-order kinetic law.

Since the reduction product recovered after electrolysis at the first polarographic wave is rotenol, we suggest that an isomerization of the primary radical anion (E) with fission of the 6a,7 bond operates before the second electron transfer takes place. The resulting radical (C) is more stable than the primary radical anion (E) as it contains an allylic structure.



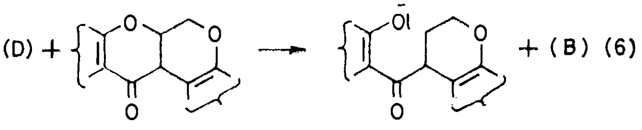
The direct isomerization (E) ⟶ (C) is a rather complex process and some intermediate steps should therefore be involved. It is likely that the fission of the ether bond should be preceded by intramolecular transfer of 12a-H to the carbonyl group.

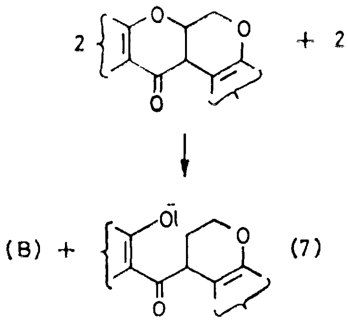
Either of these first-order processes, bond fission or proton transfer, should be the rate-determining step in the decay of the primary radical anion. Support for this hypothesis is provided by the analogous fission of the ether bond in rotenone according to equation (1). This equilibrium is rapidly established in alkaline medium and the rate of formation of the anion (B), which is pH-dependent, can only be measured under very mild conditions.[4,11] It is interesting to recall that by acidification of the anion (B), mutarotenone is obtained.



The formation of the carbanion (A) in water and that of the radical anion (F) in DMF, indicate a similar path for the fission of the ether bond. In both cases, the radical (C) is the intermediate formed by one-electron uptake.

As the reduction product of rotenone in DMF at the first wave potential is rotenol, which requires two electrons per molecule, the radical (C) should be reduced at the potential of formation, as happens in aqueous medium [equation (3) (C) ⟶ (D)]. In aprotic medium, however, the anion (D) is protonated by rotenone itself, to give the corresponding conjugated base and rotenol anion [reaction (6)]. The overall electrode reaction resulting from the steps (4), (5), (3), (C) ⟶ (D), and (6), is then (7). Reaction (7) explains the fact that a two-electron reduction product (rotenol) is obtained in a process requiring the consumption of 1 F mol^{-1}. The anion (B), inactive at the first wave potential, is formed in equimolar amounts. It is then transformed into mutarotenone by acidification.





Rate Constant Measurements.—According to equations (4), (5), (3) (C) ⟶ (D), and (6), reduction at the first polarographic wave proceeds through an e.c.e.c. mechanism in which the rate-determining step is the first-order isomerization (5), while the subsequent chemical reaction is a fast, irreversible, second-order autoprotonation (6).

There is a close analogy between this e.c.e.c. sequence and that indicated by Nadjo and Saveant as an e.P.e.isc.p. mechanism for electrohydrodimerization.[15] In the latter scheme a pseudo-first-order protonation reaction of the radical anion is rate determining while the subsequent chemical reaction is the fast dimerization between the product of the second electrochemical step and the depolarizer itself.

Saveant has pointed out that, in the case of ' pure kinetic ' conditions the behaviour of a system with an e.P.e.isc.p. sequence is equivalent, in linear sweep voltammetry, to an e.c. mechanism but with an apparent rate constant which is four times higher. The factor of four arises because the substrate is involved in the fast chemical step (ion substrate coupling or autoprotonation) and the gradient of the radical anion represents one half of the current.[15]

In our case we can reasonably exclude a pseudo-first-order protonation of the primary radical anion by the solvent, the background electrolyte, or other acidic impurities as the rate-determining step. If this were the case, we should expect a different product, since the resulting neutral ketyl radical would be directly reduced to the 12a*H*-12-hydroxy-derivative or it would dimerize to pinacol.[13]

Therefore also an e.c.e.c. scheme should be equivalent in terms of ' pure kinetic ' conditions to an e.c. scheme with $k_{\text{app.}}$ four times the isomerisation rate constant [reaction (5)]. The experimental behaviour of the voltammetric functions [E_{pc}, $i_{\text{pc}}/v^{\frac{1}{2}}c$, $i_{\text{ap}}/i_{\text{pc}}$), which is typical of an e.c. mechanism, thus finds theoretical support.

According to the procedure derived by Saveant,[15] we can evaluate the overall rate constant from the relation $k_{\text{app.}}RT/v_{\text{i}}\text{F} = 0.518$ where v_{i} is the value of the sweep rate corresponding to the intersection of the oblique linear part (pure kinetic conditions) with the horizontal part (pure diffusion control) of the E_{p}–log v diagram (see Figure 4). For our system, at 25 °C, $k_{\text{app.}} = 40\ \text{s}^{-1}$ and therefore the rate-determining step (5) will have k_1 10 s^{-1}.

Aprotic Medium. Second Wave.—The second polarographic wave can be attributed to the direct reduction of rotenone anion (A) formed according to reaction (6) on the basis on the following observations. (i) By addition of a stoicheiometric amount of TBAOH to a rotenone solution in DMF the first wave disappears while the second is doubled. (ii) When exhaustive electrolysis is performed at the potential of the first wave the second is still present. (iii) In p.s.v., on increasing the scan rate or decreasing the temperature, the second peak decreases progressively while, at the same time, the reversibility of the first one increases until only the first, fully reversible peak is present in the range of explorable potentials. Thus, the second peak shows behaviour typical of an e.c.e. mechanism and is observed only if the scan rate is sufficiently low to permit the formation of the reducible rotenone anion.

The reduction of rotenone at a potential corresponding to the second polarographic wave involves about two electrons and leads to several products among which only rotenol and a yellow substance are formed in appreciable quantities. Rotenol arises from the direct reduction of rotenone while the yellow substance must be derived from the reduction of its conjugated base. In fact, by electrolysing in the presence of TBAOH, rotenol is hardly formed at all.

Since the unreduced fraction of rotenone (50%), recovered after electrolysing at the first wave, is a mixture of two diastereoisomers (mutarotenone), rotenone anion must also be present in the open structure (B) in DMF [equation (1)]. It is well known that racemization at C-6a and -12a takes place under very mild conditions.[11]

However, the large number of different products, separated by chromatography, could be due to the high basicity generated at the double layer when rotenone anion is directly involved in the reduction. In strong basic media chemical decomposition of αβ-unsaturated carbonyl substrates can complicate the electrode process,[12b] and make difficult the elucidation of the reduction mechanism at the second polarographic wave.

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