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Short communication

Electrosynthesis of dehydrocholic acid from cholic acid

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1. Introduction

Dehydrocholic acid (DHCA) represents an important aid in the dietary supplement industry because of its choleretic activity; it is less toxic to the natural bile acids [1]. It is not a natural component in bile acids and it is generally produced by chemical oxidation of cholic acid (CA) [2]. On an industrial scale, the chemical oxidation of CA is carried out by liquid bromine or calcium hypochlorite. The yield of these processes is satisfactory, but they involve considerable amounts of byproducts, whose separation and disposal may represent a problem. Recently, biotransformations have also been applied to the synthesis of DHCA from cattle bile. Yields were, however, rather low and the process lengthy.

In this context, electrosynthesis may represent an interesting alternative, possibly allowing higher yields, good selectivity and minimal byproducts. In the present work, mediated electrochemical oxidation of the secondary alcoholic functions of CA has been attempted, because of the inherent high oxidation potential of the hydroxyl group [3]. The process has been investigated at different electrode materials, such as galvanic platinum, Ti-supported lead dioxide and graphite, and operating both in alkaline aqueous solution and ethylene glycol dimethyl ether (mono-glyme)/water mixture.

2. Experimental details

Constant current electrolyses were performed using an AMEL model 553 galvanostat. The initial substrate was 20 mM cholic acid in all the processes, except for the case of electrolysis at the platinum electrode in which 20 mM sodium cholate was used. The experimental apparatus was a thermostated (25 °C) single-body cell with a volume of 0.2 dm^3; the anode was symmetrically positioned between two zirconium plate cathodes. Constant stirring was applied during the electrolysis.

Experiments at Ti-supported lead dioxide and graphite were carried out in weakly acidic solution (pH 5) of mono-glyme/water 2:1 mixed solvent. $NaClO_4$ (1 M) was used as supporting electrolyte and NaCl (0.25 M) as mediator of electrooxidation. Preliminary experiments, at galvanic platinum, have shown that, with the above solvent, the substrate reaction was very low. In this case experiments were carried out in alkaline aqueous media (pH ~ 12), in the presence of 0.25 M NaCl. The surface area of Ti/PbO_2 and Ti/Pt electrodes was 16 cm^2, while the graphite anode area was 38 cm^2; applied current densities were in the range 25 to 200 A m^{-2}. During electrolysis, the voltage remained substantially unchanged. Samples were withdrawn hourly.

The first screening of metabolites was performed by thin-layer chromatography (TLC) on silica gel Kiesegel $60F_{254}$. Eluent used was 50:1 ethyl acetate and acetic acid; after evaporation of the solvent the chromatographic plate was treated with a solution of phosphomolibdic acid/sulfuric acid/acetic acid in the ratio 2.5:2.5:50. Heating at 120 °C was necessary to display the spots of the reaction products. Subsequently, a gas-chromatographic analysis was carried out with a Carlo Erba HRG 50160 and a SE (25 m $\times$ 0.32 mm) fused silica column with methyl-phenil-siloxane as stationary phase was adopted. The carrier gas was helium (0.55 atm). A temperature program was applied (first step: 250 °C for 5 min, second step: 250–300 °C with a 5 °C min^{-1} heating rate; third step: 300 °C for 3 min). A flame ionization detector was used. The electrolysis samples (1 ml) were acidified with 5% HCl to pH 3 to 4, extracted with ethyl acetate, dried over sodium sulfate and then derivatized with trifluoroacetic anhydride and hexafluoroisopropanol, prior to the gas–liquid chromatography (GLC) monitoring.

3. Results and discussion

Preliminary tests of the chemical reactivity of secondary alcoholic functions of CA toward oxidation were carried out. Sodium hypochlorite and *N*-chlorosuccinimide were used as oxidizing agents. With the former, regioselective oxidation of the carbon in position 7 was achieved. Not all CA could be converted into the 3,12 dihydroxy-7 oxocholic acid (7-OCA) derivative. In any case, under the applied experimental conditions, no DHCA could be detected. On the other hand, the chemical oxidation with *N*-chlorosuccinimide also led to the regioselective formation of 7-OCA. In this case the conversion was quantitative, but was not followed by further oxidative attack to secondary alcoholic groups in 3 and 12. As far as the electrochemical reactivity is concerned, CA has been found to be substantially unreactive at Pt and PbO_2 anodes, irrespective of the applied current densities.

On the basis of the above results, the chloride-mediated oxidation was tried, in consideration of the enhancement in the anodic oxidation rate of organic substrates sometimes achieved when chloride ions are added to the electrolyte. Experiments at Pt electrodes were carried out in different media. In the aquo-organic media required to ensure the solubility of reactant and products, and in the presence of different amounts of sodium chloride, no significant oxidation of the substrate was observed. Another set of experiments was carried out in aqueous alkaline media, with a NaCl concentration of 0.25 M. In this case, the anodic oxidation of CA to 7-OCA takes place (Figure 1). The chemical and electrochemical yields were 40% and 1.4%, respectively. After 20 h electrolysis at $j = 200\ \mathrm{A\ m^{-2}}$, no other oxidation products were detected. The mediated electrochemical oxidation at Ti/Pt(galvanic) electrodes substantially reproduces the results obtained with the chemical oxidation with sodium hypochlorite.

The observed lack of reactivity in the presence of the organic solvent at Pt electrodes can be tentatively explained on the basis of the well known strong adsorption of mono-glyme at the Pt surface.

Strong adsorption of 7-OCA, less soluble than CA itself, might be the reason for the inhibition of the further oxidation to DHCA in aqueous alkaline media. These results are of essentially preliminary character, the complex behavior of platinum electrodes requiring further investigation.

At Ti/PbO_2 electrodes, in aquo-organic media and in the presence of NaCl, the substrate and its primary oxidation products exhibit a higher reactivity. As shown in Figure 2, a progressive oxidation of CA to DHCA takes place, through 7-OCA, 3,7 dihydroxy-12 oxocholic acid (12-OCA) and 3 dihydroxy-7,12 oxocholic acid (7,12-DOCA). The chemical yield was found to be 100% at all the current densities explored. The faradaic yield, as expectable, was found to be higher at lower current densities: typically about 32% at $j = 30\ \mathrm{A\ m^{-2}}$ and 15% at $125\ \mathrm{A\ m^{-2}}$. The presence of the mediator is essential for achieving anodic oxidation of CA. In consideration of the low concentration of substrate and intermediate species, the effect of current density on the faradaic yield of DHCA production can be attributed to mass transport limitations. This limitation does not apply to the chloride anion, whose concentration in solution is at least ten times greater. Improvement of cell geometry and better control of the hydrodynamics of the process can lead to significant increase of the faradaic yield. It is also important to observe that, in spite of the high oxygen overpotential, typical of PbO_2 electrodes, Cl^- ions still play a decisive role.

The electrooxidation of CA at graphite electrodes was also studied. Results are shown in Figure 3. In this case conversion to DHCA also takes place: after the first hour, CA is totally consumed.

The intermediate products were the same as those observed in the case of PbO_2. The chemical yield was found to depend on current density, lower values being of the order of 90% at $j = 30\ \mathrm{A\ m^{-2}}$, the lowest current density explored. The faradaic yield was found to depend more strongly on *j*. At the highest *j* value ($200\ \mathrm{A\ m^{-2}}$), a faradaic yield as low as 5% against a value of 32% at $30\ \mathrm{A\ m^{-2}}$ was found. Also at graphite anodes the same order of oxidation reaction, C7 > C12 > C3 (Figure 4), as at Ti-supported lead dioxide was observed.

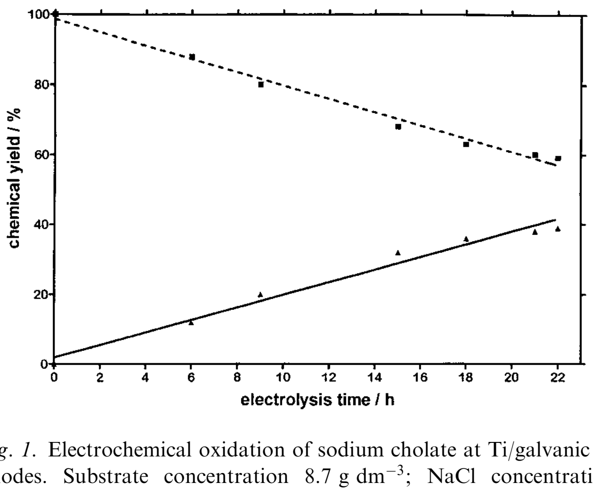

Fig. 1. Electrochemical oxidation of sodium cholate at Ti/galvanic Pt anodes. Substrate concentration $8.7\ \mathrm{g\ dm^{-3}}$; NaCl concentration 0.25 M; background electrolyte 1 M $NaClO_4$ + 0.01 M NaOH. Applied current density $200\ \mathrm{A\ m^{-2}}$. Key: (■) CA and (▲) 7-OCA.

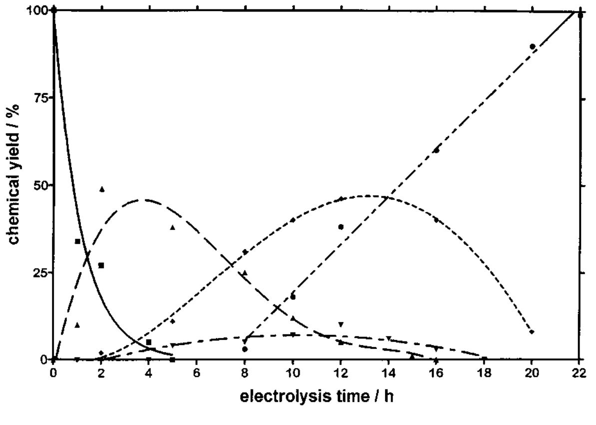

Fig. 2. Electrochemical oxidation of cholic acid at Ti/PbO_2 anodes. Substrate concentration $8.3\ \mathrm{g\ dm^{-3}}$, NaCl concentration 0.25 M, background electrolyte 1 M $NaClO_4$. Applied current density $125\ \mathrm{A\ m^{-2}}$. Key: (■) CA, (▲) 7-OCA, (▼) 12-OCA, (◆) 7,12-OCA and (●) DHCA.

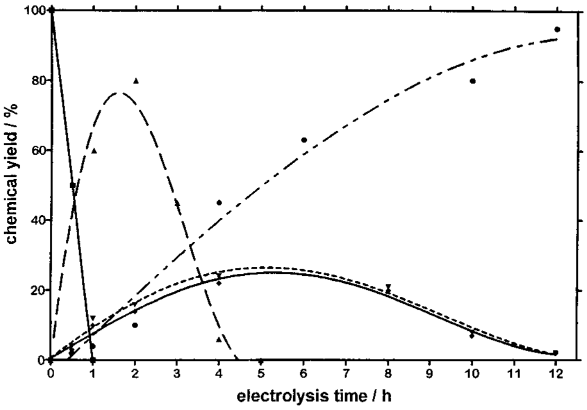

Fig. 3. Electrochemical oxidation of cholic acid at graphite anodes. Substrate concentration 8.3 g dm^{-3}, NaCl concentration 0.25 M, background electrolyte 1 M $NaClO_4$. Applied current density 200 A m^{-2}. Key: (■) CA, (▲) 7-OCA, (▼) 12-OCA, (◆) 7,12-OCA and (●) DHCA.

In the absence of mediator, no oxidation products were detected, independently of the electrode material used. It can be hypothesized that, in the presence of chloride ions, highly reactive chlorine radicals are formed at the electrode surface, together with the hydroxyl radicals. The formation of adsorbed hypochlorous acid is a possible consequence of their interaction.

The oxygen transfer/dehydrogenating action of hypochlorous acid can take place at the electrode surface as well as in the bulk of the solution [4]. However, the electrochemical process seems to be essentially a surface reaction, considering that the chemical attack by hypochlorite/hypochlorous acid does not lead to the formation of significant amounts of dehydrocholic acid. Interestingly, no chlorinated organic species were detected during the chloride-mediated oxidation of CA (the detection limit for chloro-derivatives being 5 ppm). The latter evidence, the lack of reactivity of CA in the aqueous sodium hypochlorite, the scarce reactivity towards other oxidizing media and the influence of the electrode nature on the chloride-mediated oxidation of CA, confirm the above idea. Chloro and oxychloro radicals adsorbed at the electrode surface should play a decisive role. The approach of Comninellis [5] on their reactivity, in terms of interactions with the surface oxide lattice, can be extended to the case of active chlorine mediation. Accordingly, oxidation of the organic substrate could be carried out by adsorbed oxychloro species (Figure 5). The formation of adsorbed •ClO as intermediate in the chlorine evolution reaction in acidic media has been extensively discussed by different authors [6–8].

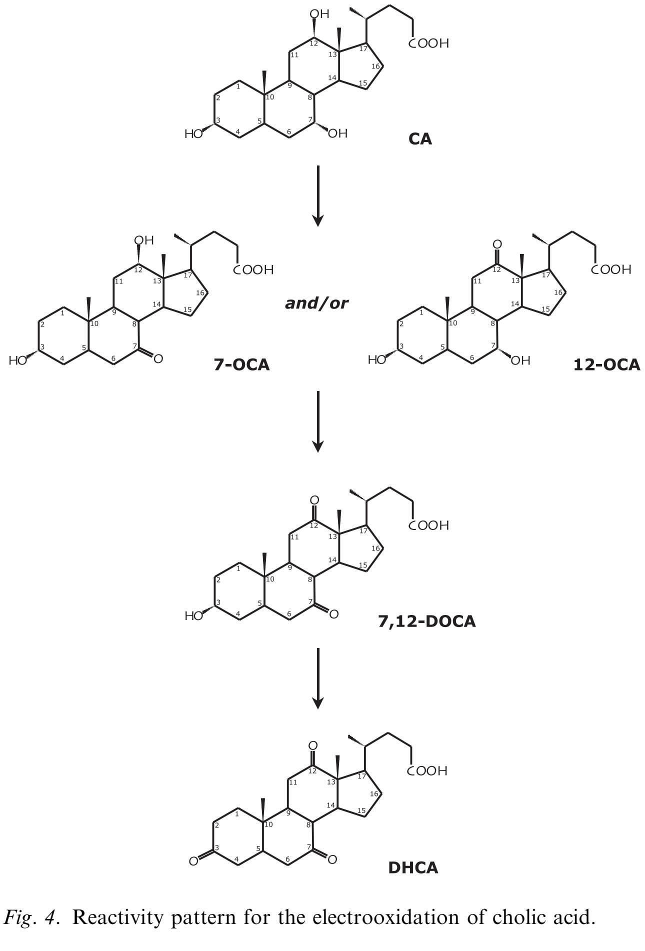

Fig. 4. Reactivity pattern for the electrooxidation of cholic acid.

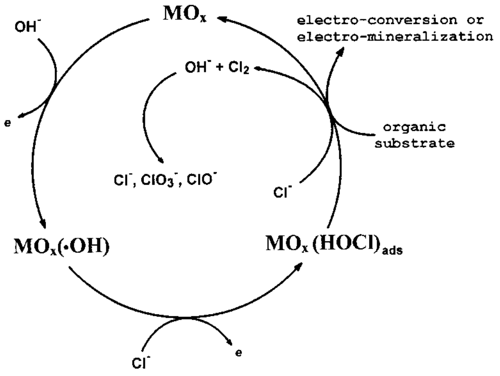

Fig. 5. Extension of the reaction pattern proposed by Comninellis [5] for the electrochemical treatment of organics, to the case of 'active chlorine' mediated electrooxidation.

4. Conclusions

Cholic acid can be oxidized with high chemical yields both at Ti-supported lead dioxide and graphite electrodes. The reaction takes place only by chloride ion mediation. Electrochemical yields were found to depend on electrode material and current density. High-oxygen-overvoltage electrode materials, like lead dioxide and graphite exhibit good performance. At Pt electrodes, the mediated electrochemical oxidation does not occur in aquo-organic media. On the other hand, only partial oxidation of CA can be achieved in aqueous alkaline media. At C (graphite) and Ti/PbO_2 electrodes, the faradaic yield for DHCA electrosynthesis increases with decreasing the anodic current density. If the electrochemical process is stopped at the appropriate time, the 7-OCA and 7,12-DOCA can be obtained as intermediates, the reaction following the oxidation order

C7 > C12 > C3. At Ti/Pt electrodes, only the OH function at C-7 is slowly oxidized. The reactivity of CA in the presence of excess hypochlorite in the same aquo-organic media adopted for the electro-oxidation was found to be very low. The evidence, together with the remarkable dependence of the latter from the nature of the electrode material, suggests a decisive role of the surface reaction. Mediated mineralization of organic substrates also seems to share the same feature [4]. Considering the possible release of lead ions at PbO_2, graphite anodes could be better employed for organic pharmaceutical electrosynthesis. In our experiments, the lead content in solution was found to be around 0.12 ppm. The absence of organic chloro-derivatives is a promising feature for the application of the electrosynthesis of DHCA from CA.

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