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Anodic electrochemical oxidation of cholic acid

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Abstract

Regioselectivity in the anodic electrochemical oxidation of cholic acid with different anodes is described. The oxidation with PbO_2 anode affords the dehydrocholic acid in quantitative yield after 22 h. 3α,12α-Dihydroxy-7-oxo-5β-cholan-24-oic acid (59%) and 3α-hydroxy-7,12-dioxo-5β-cholan-24-oic acid (51%) are obtained stopping the reaction at lower time. The rate of the OH-oxidation is $C_7 > C_{12} > C_3$. The electro-oxidation with platinum foil anode gives selectively the 7-ketocholic acid in 40% yield. On the other hand, the graphite plate anode, varying the reaction conditions, produces selectively the dehydrocholic acid in quantitative yield or the 3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid (96%) while the 3α,7α-dihydroxy-12-oxo-5β-cholan-24-oic acid (34%) is obtained together with the other oxo acids.

Keywords: Electrochemical anodic oxidation; Cholic acid; Regioselectivity; Dehydrocholic acid; 3α,12α-Dihydroxy-7-oxo-5β-cholan-24-oic acid; 3α,7α-Dihydroxy-12-oxo-5β-cholan-24-oic acid; 3α-Hydroxy-7,12-dioxo-5β-cholan-24-oic acid

1. Introduction

Inorganic electrosynthesis has long since found important industrial applications (e.g. chlor-alkali industry). Organic electrosynthesis, on the other hand, is much less widespread and, although successful experiences, like the Monsanto hydrodimerization of acrilonitrile are known [1], has been considered for many years an exotic branch of organic chemistry. Recently electroorganic chemistry has become an important organic synthetic tool and possible interesting developments in this direction have been described [1].

The electrochemical reactions can take place directly at the electrode interface or can be mediated by a primary electron transfer. The most common electrochemical methods allow the reduction or the oxidation of a mediator (electron carrier) able to exchange electrons with a less active organic species at a given electrode. In particular the indirect electrochemical oxidation (Scheme 1) of alcohols is useful because of the inherent high oxidation potential of the hydroxyl group [2]. Even if there are many available methods for alcohol oxidation, there is still a demand for new, versatile and selective oxidation procedures. As electron transfer is involved in any oxidation reaction, electrochemical anodic oxidation of alcohols seems an interesting alternative which may avoid some disadvantages of the existing chemical methods. Above all, by using the electric current as oxidation reagent, low-valent transition metal compounds, which could lead to environmental pollution in chromic acid or permanganate oxidation, are excluded. A variety of indirect procedures have been therefore devised by utilizing appropriate inorganic and also organic chemicals as mediators (Med in Scheme 1) [3].

In this paper we applied the indirect anodic electrochemical oxidation to the synthesis of the various oxo-cholic acids. Cholic acid is the primary bile acid in human bile and is the starting material in the synthesis of ursodeoxycholic acid that has important pharmaceutical applications related to its ability to solubilize cholesterol gallstones. Chemical sequences in the synthesis of ursodeoxycholic acid involve the regioselective oxidation of the C_7-OH and C_{12}-OH [4,5]. The regioselective oxidation of the cholic acid is described both chemically and biochemically. The hydroxyl groups at the different positions in the nucleus show variable reactivity toward oxidizing agents. The order of reactivity is $C_7 > C_{12} > C_3$ [6]. In particular 7-keto derivative is obtained selectively with N-bromosuccinimide [7] and bromine in alkaline methanol afforded the 12-keto derivative [8] while

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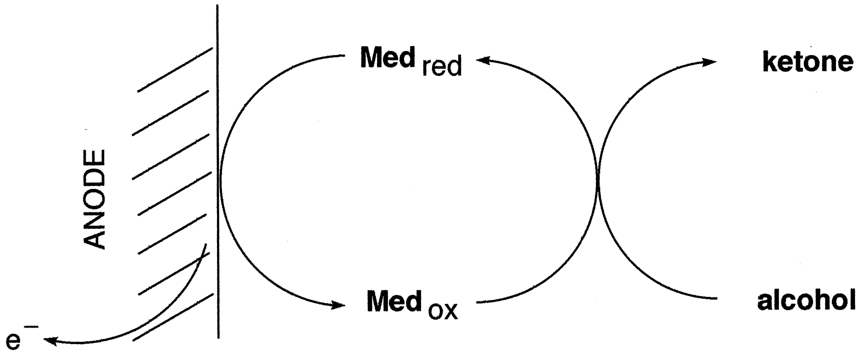


the 7α,12α-dihydroxy-3-oxo-5β-cholan-24-oic acid can be obtained by reduction of the protected dehydrocholic acid [9] or by oxidation with silver carbonate on Celite [10]. Recently the regioselective microbial oxidation of cholic acid produced the 7-keto, 7,12-diketo derivatives together with dehydrocholic acid [11].

The electrochemical oxidations offer numerous advantages over conventional methods: i) mild reaction conditions; ii) independence of a chemical redox reagent; iii) better use of raw materials; iv) easier isolation of products; v) simplification of continuous processing [12]. An example is the selective electrochemical oxidation of various hydroxy steroids at C_3 position using nickel oxide hydroxide electrochemically regenerated at a nickel hydroxide electrode [13].

In this paper we have studied the indirect electrochemical oxidation of cholic acid with PbO_2, platinum and carbon anodes in order to obtain selectively the various keto derivatives.

2. Experimental

Cholic acid **1** and the oxidation products **2–5** (see Scheme) are commercially available (Steraloids Inc., USA). The sodium salt of cholic acid has been supplied by ICE industry.

2.1. GLC analyses

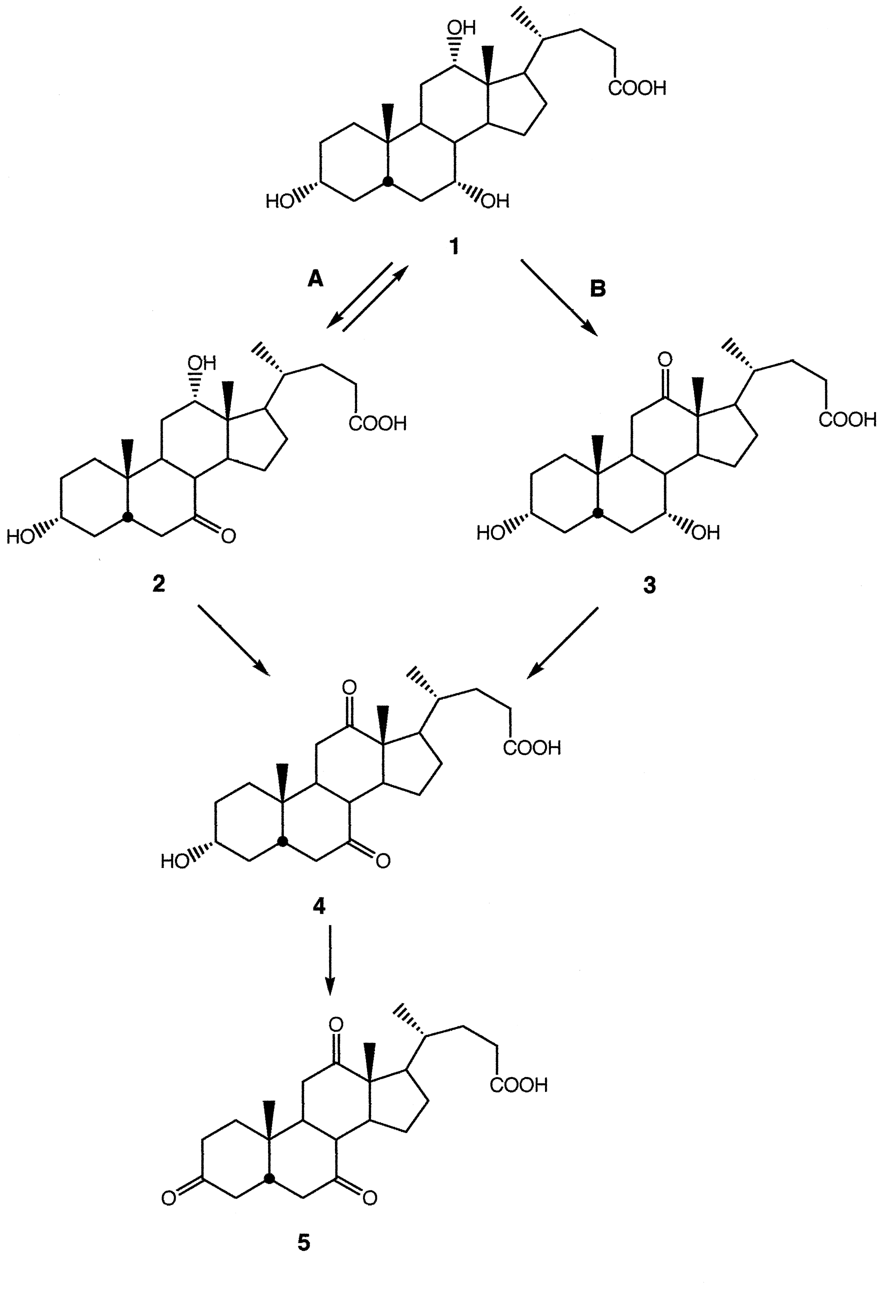
Gas chromatographic analyses were performed on a Carlo Erba HRG 50160. The reaction products, previously derivatized with trifluoroacetic anhydride and hexafluoroisopropanol, are analyzed by GLC on fused silica capillary column SE52 (25 m X 0.32 mm) from Mega s.n.c: helium as carrier gas (0.55 atm); temp. 250°C for 5 min, 250–300°C (5°C/min) and then 300°C for 3 min using as internal standard ursocholic acid (3α,7β,12α-trihydroxy-5β-cholan-24-oic acid). Retention time in min: ursocholic acid, 8.58; cholic acid **1,** 9.27; 3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid **2,** 10.91; 3α,7α-dihydroxy-12-oxo-5β-cholan-24-oic acid **3,** 11.53; 3α-hydroxy-7,12-dioxo-5β-cholan-24-oic acid **4,** 14.01; dehydrocholic acid **5,** 16.17. All the products are compared with authentic samples [14] and confirmed by GC/MS [15].

2.2. Electrolysis apparatus

An single-body cell (250 ml volume) fitted with a gas inlet pipe, a stirring bar and a thermometer was used. Ti/PbO_2 grid (16 cm^2), graphite plate (38 cm^2), and platinum foil (16 cm^2) were used as working electrodes (anodes). In the electrolysis all the anodes were placed between two zirconium plate cathodes (16 cm^2).

2.3. Electrochemical oxidation of cholic acid with lead dioxide electrodes

To a solution of cholic acid (1.66 g, 8.3 g/l) in ethylene glycol dimethyl ether/H_2O 2:1 (200 ml), placed in electrolytic vessel, NaCl (2.83 g, 14.16 g/l) and $NaClO_4$ (28 g, 140.4 g/l) were added (pH 5). Into the solution was immersed the Ti/PbO_2 grid electrode between the two Zr plate electrodes and the mixture was electrolyzed under a constant current of 50 (current density 31.25 A/m^2), 100 (current density 62.5 A/m^2) and 200 mA (current density 125 A/m^2), respectively with a moderate stirring (applied voltage: 3.1, 3.3, and 4 V respectively). During the electrolysis the mixture was maintained at 25°C. Samples (1 ml) were withdrawn periodically, acidified with 5% HCl to pH 3–4, extracted with ethyl acetate, dried over sodium sulfate, analyzed by TLC (silica cyclohexane/ethyl acetate/acetic acid 50/50/1) and after derivatisation with trifluoroacetic anhydride and hexafluoroisopropanol monitored by GLC. The electrochemical oxidation at 200 mA was repeated at 12°C (applied voltage 5 V) and at 50°C (applied voltage 4 V) and varying the concentration of the mediator (NaCl 14.16 or 28.32 g/l) and of the cholic acid (8.3 or 16.6 g/l). The dehydrocholic acid (95%, entry 5) is separated by column chromatography (silica, cyclohexane/ethyl acetate/acetic acid 50/50/1). The results are summarized in Table 1.



1; cholic acid (3α,7α,12α–trihydroxy5β-cholan-24-oic acid)
2; 3α,12α–dihydroxy-7-oxo-5β-cholan-24-oic acid
3; 3α,7α-dihydroxy-12-oxo-5β-cholan-24-oic acid
4; 3α-hydroxy-7,12-dioxo-5β-cholan-24-oic acid
5; dehydrocholic acid (3,7,12-trioxo-5β-cholan-24-oic acid)

2.4. Electrochemical oxidation of cholic acid with platinum foil electrodes

To a solution of sodium cholate (1.74 g, 8.7 g/l) in water (200 ml), placed in electrolytic vessel, NaCl (2.83 g, 14.16 g/l) and $NaClO_4$ (28 g, 140.4 g/l) were added and the pH was adjusted to 12 with 10% NaOH. Into the solution were immersed the platinum foil anodes between the two Zr cathodes and the mixture was electrolyzed under a constant current of 100 (current density 62.5 A/m^2), 200 (current density 125 A/m^2) and 300 mA (current density 185.5 A/m^2), respectively with a moderate stirring (applied voltage: 3.3, 3.3, and 3.8 V respectively). During the electrolysis the mixture was maintained at 25°C. Samples (1 ml) were withdrawn periodically, acidified with 5% HCl to pH 3–4, extracted with ethyl acetate, dried over sodium sulfate, analyzed by TLC (silica cyclohexane/ethyl acetate/acetic acid 50/

Table 1
Indirect electrooxidation of cholic acid with lead dioxide electrode under various conditions

Entry	Cholic acid g/l	Mediator NaCl g/l	Electrolyte (g/l)	Electricity mA (temp)	Time h	Products distribution %[a]				
						1	2	3	4	5
1[b]	8.3	14.16	$NaClO_4$ (140.5)	50 (25)	2	40	60	—	—	—
					4	20	52	—	28	—
					6	2	48	10	40	—
					40	—	—	—	—	100
2[b]	8.3	14.16	$NaClO_4$ (140.5)	100 (25)	2	21	73	—	−6	—
					4	—	80	9	11	—
					6	—	54	—	18	28
					30	—	—	—	—	100
3[b]	8.3	14.16	$NaClO_4$ (140.5)	200 (25)	2	41	59	—	—	
					4	—	60	13	27	
					6	—	24	17	28	31
4[b]	8.3	14.16	$NaClO_4$ (140.5)	200 (12)	2	57	43	—	—	—
					11	—	51	22	27	—
					14	—	7	—	66	27
5[b]	8.3	14.16	$NaClO_4$ (140.5)	200 (25)	2	41	59	—	—	—
					11	—	7	7	51	35
					14	—	1	3	38	58
					22[c]	—	—	—	—	100
6[b]	8.3	14.16	$NaClO_4$ (140.5)	200 (50)	2	—	86	—	14	—
					11	—	10	8	48	34
					14	—	10	6	44	40
7[b]	8.3	28.32	$NaClO_4$ (140.5)	200 (25)	2	76	24	—	—	—
					11	—	51	10	32	7
					14	—	21	12	43	24
8[b]	16.6	14.16	$NaClO_4$ (140.5)	200 (25)	2	76	24	—	—	—
					11	—	51	10	32	7
					14	—	33	10	46	11
9[d]	8.7[e]	14.16	$NaClO_4$ (140.5)	200 (25)	2	94	6	—	—	—
					10	36	43	—	21	—
					20	—	45	22	33	—

[a] The % of the product distribution is determined by GLC using ursocholic acid as internal standard (overall yield 95%). [b] Reactions carried out in ethylene glycol dimethylether/ water 2/1 (pH 5–6). [c] The products are separated by column chromatography. [d] Reaction carried out in water at pH 12. [e] The substrate is sodium cholate.

50/1) and after derivatisation with trifluoroacetic anhydride and hexafluoroisopropanol monitored by GLC. The electrochemical oxidation at 300 mA was repeated at 12°C (applied voltage 5 V) and at 50°C (applied voltage 4.2 V), varying the concentration of the mediator (NaCl 14.16, 28.32 or 40 g/l), and adding Na_2SO_4 (201.3 g/l) as supporting electrolyte. Cholic acid **1** (57%) and the 7-oxo derivative **2** (38%) of the entry 5 are separated by column chromatography (silica, cyclohexane/ethyl acetate/acetic acid 50/50/1). The results are summarized in Table 2.

2.5. Electrochemical oxidation of cholic acid with graphite electrodes

To a solution of cholic acid (1.66 g, 8.3 g/l) in ethylene glycol dimethyl ether/H_2O 2:1 (200 ml), placed in electrolytic vessel, NaCl (2.83 g, 14.16 g/l) was added (pH 5). Into the solution was immersed the graphite plate anode between the two Zr plate cathodes and the mixture was electrolyzed under a constant current of 100 (current density 26.02 A/m^2), 200 (current density 54.04 A/m^2), 400 (current density 104.07 A/m^2), 700 (current density 182.14 A/m^2), and 800 mA (current density 208.17 A/m^2) with a moderate stirring (applied voltage: 4.3, 5.6, 8.7, 10, and 12 V respectively). During the electrolysis the mixture was maintained at 25°C. Samples (1 ml) were withdrawn periodically, acidified with 5% HCl to pH 3–4, extracted with ethyl acetate, dried over sodium sulfate, analyzed by TLC (silica cyclohexane/ethyl acetate/acetic acid 50/50/1) and after derivatisation with trifluoroacetic anhydride and hexafluoroisopropanol monitored by GLC. The electrochemical oxidation at 700 mA was repeated at 12°C (applied voltage 11 V) and at 50°C (applied voltage 8 V), varying the concentration of the mediator (NaCl 14.16 or 28.32 g/l) and of the cholic acid (8.3 or 24.9 g/l) and adding $NaClO_4$ (28 g, 140.4 g/l) as supporting electrolyte. The final products of the entry 3 (dehydrocholic acid **5,** 88%; 12-oxo derivative **3,** 4.5% and 7,12-oxo derivative **4,** 2%) and 8 (dehydrocholic acid **5,** 95%) are separated by column chromatography (silica, cyclohexane/ethyl acetate/acetic acid 50/50/1). The results are summarized in Table 3.

Table 2
Indirect electrooxidation of cholic acid with platinum foil electrode under various conditions.

Entry	Sodium cholate[a] (g/l)	Med NaCl (g/l)	Electrolyte (g/l)	Electricity mA (temp)	Time h	Products distribution %[b] 1	2
1	8.7	14.16	$NaClO_4$ (140.5)	100 (25)	14	94	6
					20	92	8
2	8.7	14.16	$NaClO_4$ (140.5)	200 (25)	10	89	11
					20	80	20
3	8.7	14.16	$NaClO_4$ (140.5)	300 (25)	14	71	29
					20	65	40
4	8.7	14.16	$NaClO_4$ (140.5)	300 (12)	2	93	7
					7	96	4
					10	90	10
5	8.7	14.16	$NaClO_4$ (140.5)	300 (25)	2	93	7
					7	84	16
					10	75	25
					20	60	40
6	8.7	14.16	$NaClO_4$ (140.5)	300 (50)	2	100	
					7	95	5
					10	94	6
7	8.7	28.32	$NaClO_4$ (140.5)	100 (25)	3	99	1
					10	94	6
8	8.7	28.32	$NaClO_4$ (140.5)	300 (25)	3	90	10
					10	81	19
9	8.7	40	$NaClO_4$ (140.5)	100 (25)	3	98	2
					10	92	8
10	8.7	40	$NaClO_4$ (140.5)	300 (25)	3	85	15
					10	75	25
11	8.7	14.16	Na_2SO_4 (203.1)[c]	200 (25)	10	92	8
					20	84	16

[a] Reactions carried out in water at pH 12. [b] The % of the products distribution is determined by GLC using ursocholic acid as internal standard (overall yield 95%). [c] With a saturated solution of $NaHCO_3$ at pH 8.

3. Results and discussion

Preliminary tests were made in order to find the optimal conditions for the electrochemical oxidation of the cholic acid: solvent, mediator, supporting electrolyte and the appropriate concentrations. Since cholic acid is water–soluble at pH 12 and water-insoluble at pH 4–5, various water–soluble solvent (acetone, DMF, dioxane, alcohols, ethylene glycol dimethyl ether) were tested with an electrode formed by Sn 40%, Ti 30% and Ru 30%, and ethylene glycol dimethyl ether/H_2O (2:1 ratio) was chosen as solvent. The oxidation reactions were carried out with NaCl or NaBr as mediators and no products were obtained with NaBr. Regarding the electrolytes usually used in this reaction (e.g. Na_2SO_4, $NaClO_4$, NaOH), $NaClO_4$ was utilized because it is the only salt soluble at $pH < 7$.

The cholic acid **1** is oxidized to dehydrocholic acid **5,** but also the 7-keto and 12-keto derivatives **2** and **3** together with the 3α-hydroxy-7,12-dioxo-5β-cholan-24-oic acid **4** can be obtained in satisfactory yields on depending of the anode and of the time of reaction (Scheme 2).

The results of the anodic oxidation of cholic acid **1** with lead dioxide electrodes are summarized in Table 1.

The dehydrocholic acid **5** is achieved in quantitative yield after 22 h at room temperature (entry 5), but with a poor faradaic yield (15%) [16] using a current intensity of 200 mA (current density 125 A/m^2). As confirmed by the entries 1, 2 and 3, initially the 7-OH function is regioselectively oxidized to 7-oxo derivative **2** (59–86% after 2 h), also varying the current intensity (50, 100 and 200 mA), then, after 6 h, the 7,12-dioxo derivative **4** is obtained in 18–40% (pathway A) and it increased until 51% (11 h) to afford finally the dehydrocholic acid **5.**

The reaction tested without mediator, both at 100 and 300 mA, does not afford oxidation products. On the other hand the increasing of the concentration of the mediator (entry 7) and of the cholic acid (entry 8), the use of the sodium cholate at pH 12 (entry 9) and the reactions carried out at 12°C (entry 4) and at 50°C (entry 6) decrease the rate of oxidation. Moreover varying the current intensity from 50 to 200 mA the quantitative yield of dehydrocholic acid **5** are obtained in 40, 30 and 22 h respectively (entries 1, 2, and 5) but the faradaic yield are progressively lower (33, 22 and 15%).

While the anodic oxidation of the cholic acid with lead dioxide electrodes affords the OH-oxidation products in the order $C_7 > C_{12} > C_3$, the reactions carried out with platinum foil electrode are much slower and stop selectively at the 7-oxo derivative **2** (Table 2).

Table 3
Indirect electrooxidation of cholic acid with graphite plate electrode under various contitions

Entry	Cholic acid[a] (g/l)	Med NaCl (g/l)	Electrolyte (g/l)	Electricity mA (temp)	Time h	Products distribution %[b]				
						1	2	3	4	5
1	8.3	14.16	$NaClO_4$ (140.5)	200 (25)[c]	2	90	1	7	—	1
					14	36	14	33	11	6
					23	25	—	40	18	17
1	8.3	14.16	—	200 (25)[c]	1	15	85	—	—	—
					10	—	28	31	24	17
					19	—	—	18	10	72
3	8.3	14.16	—	100 (25)[c]	1	68	32	—	—	—
					10	—	44	29	20	7
					19[d]	—	—	5	2	93
4	8.3	14.16	—	400 (25)[c]	1	81	12	7	—	—
					10	—	29	34	22	15
					19	—	3	18	21	58
5	8.3	14.16	—	700 (25)[c]	1	—	60	20	16	4
					6			7	23	70
					10	—	—	2	2	96
					19	—	—	—	—	100
6	8.3	14.16	—	800 (25)[c]	1	—	96	3	1	—
					10	—	23	23	22	32
					19	—	6	19	15	60
7	8.3	14.16	—	700 (12)	1	—	39	12	45	4
					6	—	8	5	48	39
8	8.3	14.16	—	700 (50)	1	—	32	12	33	21
					6[d]	—	—	—	—	100
9	24.9	14.16	—	700 (25)	2	42	44	4	7	3
					7	—	51	9	28	12
					10	—	45	10	32	13
10	8.3	28.32	—	700 (25)	1	—	1	1	34	64
					4	—	—	—	6	94
					6	—	—	—	—	100

[a] Reactions carried out in ethylene glycol dimethylether/ water 2/1 (pH 5–6). [b] The % of the products distribution is determined by GLC using ursocholic acid as internal standard (overall yield 95%). [c] After 10 h the temperature was raised to 35°C. [d] The products are separated by column chromatography.

All the reactions were carried out in water at pH 12 with sodium cholate because the conditions chosen for the lead dioxide electrodes does not yield any oxidation product with platinum foil. The rate of oxidation increases with increasing the current intensity from 100 to 300 mA (entries 1–3) and decreases deviating from room temperature (entries 4 and 6), increasing the concentration of the mediator (entries 7–10) and changing the supporting electrolyte (entry 11). A maximum yield of 40% **2** can be achieved (entry 5).

The results of electrochemical oxidation of cholic acid **1** with a graphite plate electrode are summarized in Table 3.

The reaction carried out at 200 mA (entry 1) in the same conditions used for the lead dioxide electrodes (Table 1, entry 5) produces, after 23 h, the 12-oxo derivative **3** (40%) and, together with the 3α-hydroxy-7,12-dioxo-5β-cholan-24-oic acid **4,** the dehydrocholic acid **5,** following preferentially pathway B as indicated by the low yield of the 7-oxo derivative **2.** Much faster is the reaction carried out without supporting electrolyte (entry 2) that affords selectively the 7-oxocholic acid **2** (85% after 1 h) and the dehydrocholic acid **5** (72% after 19 h).

In this case, the high yield of **2** after 1 h indicates a pathway A, while the presence of the 12-oxo derivative **3** in 31% yield after 10 h supports a pathway B. Therefore we can reasonably suggest that, together with the anodic oxidation of the cholic acid to give **2,** the cathode reduction of **2** to **1** takes place, that can be slowly oxidized to **3.** Also with the graphite plate electrodes various current intensities were tested, e.g. 100, 300, 400, 700 800 mA (entries 3, 4, 5, and 6, respectively) in order to optimize the reaction conditions.

3α,12α-Dihydroxy-7-oxo-5β-cholan-24-oic acid (96%) is obtained after 1 h at 800 mA (entry 6) while 19 h at 700 mA are enough to achieve quantitatively dehydrocholic acid (entry 5), the faradaic yield being 7%. Best results for **5** are obtained at 100 mA (entry 3) which allows 93% yield after 19 h (100% after 33 h) with 20% faradaic yield. The reaction rate decreases with increasing concentration of cholic acid (entry 9) and lowering the temperature (entry 7), while the reaction is faster with increasing concentration of NaCl (entry 10) and the temperature (100% dehydrocholic after 6 h, entry 8). In all the reactions no chlorine or organic chlorinated compounds were detected.

In conclusion the lead dioxide and graphite plate electrodes are able to oxidize the OH-functions of cholic acid to give the dehydrocholic acid in quantitative yield. If the reactions are stopped at the appropriate time, the intermediates 3α,12α-dihydroxy-7-oxo- and 3α-hydroxy-7,12-dioxo-5β-cholan-24-oic acids are achieved following the oxidation rate $C_7 > C_{12} > C_3$. Interesting is the behavior of the graphite anode where the reversible oxido-reduction of the C_7-OH takes place, thus the 3α,7α-dihydroxy-12-oxo-5β-cholan-24-oic acid is slowly formed. On the other hand, the platinum foil anode slowly oxidizes only the C_7-OH function.

The results obtained show that the alcoholic functions of cholic acid can be converted to keto-groups by anodic oxidation only in the presence of chloride ions. The direct oxidation does not seem to take place at commonly used anodic materials like galvanic platinum, graphite, lead dioxide. Occurrence of an oxidative attack of the substrate with increasing the anodic current density cannot be excluded, at least at PbO_2 electrodes. However, the high reactivity of adsorbed hydroxyl radicals formed at the electrode surface under these conditions, would lead to non-selective oxidation and, eventually, to mineralization of the organic substrate [17]. In the presence of chloride ions the anodic oxidation of cholic acid to dehydrocholic acid can be carried out with reasonable yields. A few interesting points should be considered in this connection. First, the faradaic yield of the mediated electro-oxidation is still dependent on the nature of the electrode material. The second important aspect is that the chemical attack by hypochlorite/hypochlorous acid does not lead to the formation of significant amounts of dehydrocholic acid. Further, no chlorination seems to take place as a side reaction of the main oxidative process. From the point of view of the reaction mechanism, on the basis of the above results, we can conclude that the mediated electrochemical oxidation of cholic acid to the dehydro-derivative is essentially a surface reaction. The absence of organic chloro-derivatives can be also taken as an indication that the role played by volume reactions involving ClO^- and/or HClO is quite secondary.

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