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Unusual electrochemical oxidation of cholesterol

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ABSTRACT

It has been found that cholesterol undergoes direct electrochemical oxidation on platinum electrode in dichloromethane. Voltammetric measurements show that the process is controlled by the rate of electron transfer and the height of the oxidation peak is linear *vs.* concentration of cholesterol. Preparative electrolysis with separated cathodic and anodic compartments afforded dicholesteryl ether in a relatively high material yield. Depending on electrolysis conditions (composition of supporting electrolyte and electrolytic cell construction) various by-products with a 3β-chloro, 3β-acetoxy, or 3β-acetylamino group were obtained.

1. Introduction

Cholesterol oxidation products (COPs) have been recently widely studied since they are involved in the pathogenesis of atherosclerosis [1]. Several COPs show various undesirable biological activities [2,3]. Due to the presence of the double bond cholesterol is susceptible to autooxidation in the presence of dioxygen, light, and metal ions via free radical reactions [4]. COPs are also formed during food processing and therefore numerous methods for COPs analysis in food were elaborated [5,6]. A number of papers dealing with electrochemical oxidation of cholesterol has also appeared. Most reports concern indirect oxidation with mediators, such as Mn(III)/IV–porphyrin–O_2, Ti(II)/(III)–hematoporphyrin–O_2, or Br^-/BrO^- [7–9]. The first two systems caused the selective radical oxidation of the cholesterol side chain at C-25 to the corresponding tertiary alcohol. However, the preferred oxidation sites of cholesterol are the C5–C6 double bond, the allylic C-7 position, and the 3β-OH group. The system with hypobromide mediation afforded several products oxidized at the double bond (cholestan-3β,5ξ,6ξ-triol, 5ξ,6ξ-epoxycholestan-3β-ol) or at C-7 (7ξ-hydroxycholesterol and 7-oxocholesterol). Recently described electrochemical system for oxidation of cholesterol acetate induced by dioxygen and iron picolinate complexes afforded mostly 7-hydroxylated products [10]. Direct electrochemical oxidation of cholesterol on platinum electrode in glacial acetic acid led to formation of 7α-acetoxycholesterol and 7β-acetoxycholesterol in the ratio 10:3 [11]. The previous studies on cholesterol electrooxidation were carried out in polar nucleophilic solvents. The proposed mechanism usually includes formation of a cation-radical in the first step. However, it is known that cation-radicals are more stable in dichloromethane, which do not exhibit nucleophilic properties, than in other solvents usually employed in

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electrochemistry [12]. Therefore, this solvent was used in a present study on direct electrochemical oxidation of cholesterol.

2. Experimental

2.1. Equipment

Cyclic-voltammograms were recorded with *iR* compensation at 25 °C using a three-electrode potentiostat (Bioanalytical Systems Model CV-50W). The experiments were conducted in a 15-mL electrochemical cell with an argon-purge system. The working electrode was a Bioanalytical Systems platinum inlay (area, 0.03 cm^2), the auxiliary electrode a platinum mesh (contained in a glass tube with a medium porosity glass-frit) and the reference electrode Ag/0.1 M $AgNO_3$ in acetonitrile. The latter was contained in a Pyrex tube with a cracked soft-glass tip, which was placed inside a Luggin capillary. Before each experiment the working electrode was polished using a Buehler Micropolish Alumina Gamma 3B and Buehler Microcloth polishing cloth and rinsed with glacial acetic acid. The accuracy of potential measurement was equal to 10 mV. All reported values of potentials and currents are means of three independent measurements.

The preparative electrolyses were performed with a potentiostat (Princeton Applied Research Model 273 A) by passing a constant current (10 mA) during 3 h. The working electrode was a platinum plate (area, 7.5 cm^2), the auxiliary electrode a platinum mesh, and the same reference electrode as described above. The low current density applied equal to 1.23 mA cm^{-2} prevented the decomposition of supporting electrolyte and also the occurrence of consecutive reactions. The electrolyses were carried out either in an electrolytic cell, in which the cathode was placed in a glass tube with a medium porosity glass frit (G3) or anode and cathode compartments were connected with an electrolytic bridge filled with 0.4 M solution of tetrabutylammonium tetrafluoroborate ($TBABF_4$) in acetonitrile/dichloromethane (50:50, v/v). All measurements were performed at room temperature. High-purity argon gas was used to deaerate the solutions applied in all electrochemical measurements.

NMR spectra were measured at 200 MHz with a Brucker AC 200F spectrometer using $CDCl_3$ solutions with TMS as internal standard. Column chromatography was performed on Merck silica gel (70–230 mesh). Merck Silica Gel 60, F 256 TLC aluminum sheets were applied for thin layer chromatographic analysis. For visualization of products, a 5% solution of phosphomolybdic acid in ethanol [13] was used. IR spectra were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer as chloroform solutions. Mass spectra were obtained at 70 eV with an ADM-604 spectrometer. Melting points were determined on a Kofler apparatus of the Boetius type.

2.2. Chemicals and reagents

The reagents for the investigations and syntheses were the commercially available highest purity. The solvent for all electrochemical experiments was dichloromethane (Aldrich), which was purified by the procedure described elsewhere [14]. Cholesterol and cholesta-3,5-diene were purchased from Sigma and was used without further purification. Other solvents were purchased from POCh (Gliwice, Poland), dried and distilled before use.

2.3. Electrooxidation of cholesterol—procedures and analysis of products

In all electrochemical measurements, the supporting electrolyte was the solution containing 0.1 M tetrabutylammonium tetrafluoroborate in dichloromethane.

Electrochemical oxidation of cholesterol (3× 200 mg) was initially carried out in an electrolytic cell, in which the cathode was placed in a glass tube with a medium porosity glass frit, with $TBABF_4$ in dichloromethane at 0 °C for 3 h. The progress of reaction was monitored by TLC on silica gel plates developed with hexane–ethyl acetate 8:2. TLC analysis showed formation of a complex mixture of compounds. The dry residue obtained by evaporation of the solvent *in vacuo* from the combined reaction mixtures was subjected to the silica gel column chromatography. Five major fractions were collected; one of them being the unreacted cholesterol **1** (200 mg), eluted with hexane–ethyl acetate 95:5 (fraction 4). Other fractions were subjected to further chromatographic separation. Fraction 1 contained a mixture of nonpolar products (23 mg), mostly isomeric dienes, which could not be separated, and a trace amount of dicholesteryl ether (**6**). Repeated chromatography of fraction 2 (elution with hexane–ethyl acetate 98:2) afforded 5β,6α-dichlorocholestan-3β-ol (**2**; 30 mg, yield 9%). From fraction 3 (eluted with hexane–ethyl acetate 97:3) 5α,6β-dichlorocholestan-3β-ol (**3**; 50 mg, yield 14%) was isolated [15,16]. Further chromatographic purification of fraction 5 (elution with hexane–ethyl acetate 90:10) afforded 6α-chlorocholestane-3β,5β-diol (**4**; 20 mg, yield 6%) [17].

In the case of prolonged cholesterol electrooxidation, 3β-chlorocholest-5-ene (**5**) was additionally isolated. The reaction carried out in dichloromethane with $TBABF_4$ at 0 °C for 7 h afforded 4% of compound **5** (eluted with hexane), in addition to other products.

- 5β,6α-Dichlorocholestan-3β-ol (**2**):
  m.p. 137–138 °C (methanol). ^{1}H NMR δ (ppm): 4.49 (dd, J = 12.4, 5.3, 1H, 6β-H); 4.15 (m, 1H, 3α-H); 1.15 (s, 3H, 19-H); 0.90 (d, J = 6.6 Hz, 3H, 21-H), 0.87 (d, J = 6.7 Hz, 6H, 26,27-H); 0.66 (s, 3H, 18-H). ^{13}C NMR δ (ppm): 83.0 (C), 67.9 (CH), 66.0 (CH), 56.0 (CH), 56.0 (CH), 44.4 (C), 42.5 (C), 42.4 (CH), 40.2 (CH_2), 39.6 (CH_2), 39.4 (CH_2), 36.1 (CH_2), 35.7 (CH), 35.1 (CH), 34.1 (CH_2), 28.1 (CH_2), 28.0 (CH), 27.0 (CH_2), 26.7 (CH_2), 24.0 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 22.0 (CH_2), 19.1 (CH_3), 18.6 (CH_3), 11.8 (CH_3). IR ν (cm^{-1}): 1467, 1283, 1086, 1061, 1023, 1000, 964. MS *m*/*z* (%): 458 (16), 456 (23, M^+), 423 (38), 421 (100), 386 (24), 384 (62), 368 (18), 366 (26).
- 5α,6β-Dichlorocholestan-3β-ol (**3**):
  m.p. 135–139 °C (methanol), literature [16] m.p. 143–144 °C (ethanol). ^{1}H NMR δ (ppm): 4.35 (dd, J = 4.2, 1.8 Hz, 1H, 6α-H); 4.31 (m, 1H, 3α-H); 2.48 (dd, J = 10.4, 3.3 Hz, 1H, 4α-H), 2.33 (m, 1H, 7β-H); 1.37 (s, 3H, 19-H); 0.90 (d, J = 7.9 Hz, 3H, 21-H), 0.87 (d, J = 6.6 Hz, 6H, 26,27-H); 0.71 (s, 3H, 18-H). ^{13}C NMR δ (ppm): 85.4 (C), 67.9 (CH), 63.8 (CH), 56.1 (CH), 55.2 (CH), 45.9 (CH), 43.0 (CH_2), 42.7 (C), 40.7 (C), 39.7 (CH_2), 39.5 (CH_2),

36.1 (CH_2), 35.7 (CH), 35.4 (CH_2), 34.5 (CH_2), 30.3 (CH), 30.3 (CH_2), 28.2 (CH_2), 28.0 (CH), 24.0 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 21.2 (CH_2), 19.7 (CH_3), 18.6 (CH_3), 12.2 (CH_3). IR ν (cm^{-1}): 1467, 1382, 1154, 1085, 1023, 1000, 964. MS *m*/*z* (%): 458 (12), 456 (20, M^+), 386 (28), 384 (25), 368 (21), 301 (21).

- 6α-Chlorocholestane-3β,5β-diol (**4**):
  m.p. 139–142 °C (dichloromethane), literature [17] m.p. 140–142.5 °C. ^{1}H NMR δ (ppm): 4.31 (m, 1H, 6β-H); 4.17 (m, 1H, 3α-H); 0.99 (s, 3H, 19-H); 0.90 (d, J = 6.9 Hz, 3H, 21-H), 0.87 (d, J = 6.8 Hz, 6H, 26,27-H); 0.65 (s, 3H, 18-H). ^{13}C NMR δ (ppm): 77.2 (C), 67.8 (CH), 67.3 (CH), 56.1 (CH), 56.1 (CH), 42.8 (C), 42.8 (CH), 42.7 (C), 39.7 (CH_2), 39.5 (CH_2), 38.4 (CH_2), 36.1 (CH_2), 35.7 (CH), 35.7 (CH); 31.5 (CH_2), 28.1 (CH_2), 28.0 (CH), 27.6 (CH_2), 25.7 (CH_2), 24.0 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.4 (CH_2), 18.6 (CH_3), 16.7 (CH_3), 12.0 (CH_3). IR ν (cm^{-1}): 1467, 1264, 1094. MS *m*/*z* (%): 440 (2), 438 (6, M^+), 402 (24), 384 (29), 368 (36), 366 (100), 351 (4).
- 3β-Chlorocholest-5-ene (**5**):
  m.p. 89–91 °C (dichloromethane), literature (Steraloids catalogue) m.p. 95–96 °C. ^{1}H NMR δ (ppm): 5.38 (m, 1H, 6-H); 3.78 (m, 1H, 3α-H); 1.04 (s, 3H, 19-H); 0.92 (d, J = 6.5 Hz, 3H, 21-H), 0.87 (d, J = 7.5Hz, 6H, 26,27-H); 0.69 (s, 3H, 18-H). ^{13}C NMR δ (ppm): 140.8 (C), 122.5 (CH), 60.3 (CH), 56.7 (CH), 56.1 (CH), 50.1 (CH), 43.4 (CH_2), 42.3 (C), 39.7 (CH_2), 39.5 (CH_2), 39.1 (CH_2), 36.4 (C), 36.2 (CH_2), 35.8 (CH); 31.8 (CH_2), 31.8 (CH), 31.1 (CH_2), 28.2 (CH_2), 28.0 (CH), 24.3 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.0 (CH_2), 19.2 (CH_3), 18.7 (CH_3), 11.8 (CH_3). IR ν (cm^{-1}): 1467, 1377, 869. MS *m*/*z* (%): 406 (35), 404 (100, M^+), 389 (42), 368 (14), 353 (9).

In the typical preparative electrolysis with separated electrodes, 15 cm^3 of the supporting electrolyte (0.1 M tetrabutylammonium tetrafluoroborate in dichloromethane) containing 900 mg (2.33 mmol) of cholesterol was placed into the anodic compartment of electrolyser. The cathodic compartment contained the supporting electrolyte with approximately 2% of acetic acid. Anode and cathode compartments were connected with an electrolytic bridge filled with 0.4 M solution of $TBABF_4$ in 50:50 (v/v) acetonitrile and dichloromethane. The total volume of the solution in the electrolytic bridge was equal to 4 cm^2. The approximate concentration (due to a volume contraction effect) of acetonitrile in the bridge was about 9 M.

The dry residue obtained by evaporation *in vacuo* of the solvent from the crude reaction mixture was subjected to silica gel column chromatography. The reaction products were eluted consecutively with hexane–ethyl acetate mixtures. The non-polar 3β-chlorocholest-5-ene (**5**; 25 mg, yield 3%) was eluted with hexane. Further elution with hexane–ethyl acetate (1–5%) afforded dicholesteryl ether (**6**; 247 mg, yield 28%) followed by cholesterol acetate (**7**; 35 mg, yield 4%) and an unidentified cholestenone (M 384; 7 mg). The starting cholesterol was then eluted with 5–6% ethyl acetate–hexane (257 mg, 29%). The most polar product, *N*-acetyl-cholest-5-en-3β-amine (**8**; 38 mg, yield 4%), was eluted with hexane–ethyl acetate (7:3).

- Dicholest-5-en-3β-yl ether (dicholesteryl ether; **6**):
  m.p. 202–203 °C (dichloromethane), literature [18] m.p. 205–207 °C (benzene–ethanol). ^{1}H NMR δ (ppm): 5.34 (m, 1H, 6-H); 3.29 (m, 1H, 3α-H); 1.00 (s, 3H, 19-H); 0.92 (d, J = 6.5 Hz, 3H, 21-H), 0.87 (d, J = 6.6 Hz, 6H, 26,27-H); 0.68 (s, 3H, 18-H). ^{13}C NMR δ (ppm): 141,4 (C), 121.53 (CH), 76.4 (CH), 56.8 (CH), 56.2 (CH), 50.3 (CH), 42.3 (C), 40.6 (CH_2), 39.8 (CH_2), 39.5 (CH_2), 37.4 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH); 32.0 (CH_2), 31.9 (CH), 29.4 (CH_2), 28.2 (CH_2), 28.0 (CH), 24.3 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3). IR ν (cm^{-1}): 1467, 1381, 1366, 1079. MS *m*/*z* (%): 754 (2, M^+), 739 (1) 384 (31), 369 (100), 368 (70), 355 (11). Elemental analysis calcd for $C_{54}H_{90}O$: C, 85.87; H, 12.01. Found: C, 85.60; H, 11.93.
- Cholest-5-en-3β-yl acetate (cholesteryl acetate; **7**):
  ^{1}H NMR δ (ppm): 5.38 (m, 1H, 6-H); 4.61 (m, 1H, 3(-H); 2.04 (s, 3H, Ac), 1.03 (s, 3H, 19-H); 0.94 (d, J = 6.5 Hz, 3H, 21-H), 0.87 (d, J = 6.5 Hz, 6H, 26, 27-H); 0.68 (s, 3H, 18-H).
- *N*-Acetyl-cholest-5-en-3β-amine (**8**):
  m.p. 238–240 °C (dichloromethane), literature [19] m.p. 239–241 °C (methanol). ^{1}H NMR δ (ppm): 5.37 (m, 1H, 6-H); 5.29 (bd, J = 7.4Hz, 1H, NH), 3.70 (m, 1H, 3α-H); 1.98 (s, 3H, Ac), 1.00 (s, 3H, 19-H); 0.92 (d, J = 6.5 Hz, 3H, 21-H), 0.87 (d, J = 6.5 Hz, 6H, 26,27-H); 0.68 (s, 3H, 18-H). ^{13}C NMR δ (ppm): 169.1 (C), 140.1 (C), 122.0 (CH), 56.7 (CH), 56.1 (CH), 50.1 (CH), 49.7 (CH), 42.3 (C), 39.7 (CH_2), 39.5 (CH_2), 39.3 (CH_2), 37.8 (CH_2), 36.5 (C), 36.2 (CH_2), 35.8 (CH); 31.8 (CH_2), 31.8 (CH), 29.2 (CH_2), 28.2 (CH_2), 28.0 (CH), 24.3 (CH_2), 23.8 (CH_2), 23.6 (CH_3), 22.8 (CH_3), 22.5 (CH_3), 21.0 (CH_2), 19.3 (CH_3), 18.7 (CH_3), 11.8 (CH_3). IR ν (cm^{-1}): 1637, 1560, 1466, 1383, 1368, 1112, 1040. MS *m*/*z* (%): 384 (1), 369 (31), 368 (100); MS ESI *m*/*z*: 450.4 (100, MNa^+).

3. Results and discussion

3.1. *Voltammetric measurements*

Cyclic-voltammogram of cholesterol in dichloromethane (Fig. 1, curve a) shows in the first anodic scan the broad anodic oxidation peak in the region from +1.55 to +1.85 V (*vs.* Ag/0.1 M $AgNO_3$ in acetonitrile) and at scan rate 0.1 V s^{-1} the peak potential was estimated as +1.75 ± 0.02 V. In the subsequent cathodic scan three reduction peaks appear at +0.50, −0.25, and −0.53 V. In the second anodic scan an oxidation peak at +0.15 V in addition to cholesterol oxidation peak (+1.75 V) is present. The height of the peak of cholesterol oxidation depends linearly on cholesterol concentration within the investigated range 0.1–5 mM. This peak is rather broad and therefore a half-peak potential ($E_{p/2}$) was used to analyze its dependence on logarithm of scan rate. The plot is presented in Fig. 2. The half peak potential is shifted towards more positive values during the increase of scan rate and the dependence is linear. This indicates that the process is controlled by electron transfer and value of αn_α equal to 0.48 ± 04 was calculated [20].

Fig. 1 presents also a cyclic-voltammogram of cholesta-3,5-diene (curve b). Cholesta-3,5-diene is oxidized at less positive potential (+1.1 V) than cholesterol (+1.75 V) but in the subsequent cathodic scan and the following second anodic scan similar peaks as on cyclic voltammogram of cholesterol are present. A cyclic voltammogram registered for a mixture of cholesterol and cholesta-3,5-diene (curve 3) confirms the observation. The resemblance of the curves may

suggest that cholesta-3,5-diene is formed during reaction in dichloromethane as an intermediate and its further reaction in the presence of cholesterol may be partly responsible for dicholesteryl ether (**6**) formation. However, it is not very likely that it is a dominating pathway leading to compound **6**. Analysis of the reaction products strongly support an alternative mechanism of compound **6** formation (*vide infra*). An electrochemical oxidation of cholesta-3,5-diene is being under investigation.

3.2. *Preparative electrolyses*

The initial experiments were performed using a divided cell, in which the cathode was placed in a glass tube with a glass frit. The reaction products were separated by column chromatography and carefully analyzed (Scheme 1). The major products appeared to be cholesterol dichlorides **2** and **3** differing in configurations at chiral centers. The major product **3** showed in its ^{1}H NMR spectrum a broad ($w/2 = 23.5$ Hz) multiplet at δ 4.31 ppm coming from an axial proton at C-3. This proved that rings A and B in this compound are trans fused and the chlorine atom at ring junction occupies an α position. Contrary to that in the ^{1}H NMR spectrum of compound **2** a narrow signal of 3α-proton ($w/2 = 15.6$ Hz) was present at 4.15 indicating a trans fusion of the ring system. The configurations at C-6 in dichlorides **2** and **3** were established by analyzing coupling constants and NOE effects of a proton at C-6. Another electrooxidation product was chlorohydrine **4**. The structure of compound **4** was established by spectroscopic methods and confirmed by its acetylation with Ac_2O in pyridine. 3-Monoacetate was obtained proving that the second hydroxyl group is placed at the tertiary carbon atom (C-5). In the case of prolonged cholesterol electrooxidation, 3β-chlorocholest-5-ene (**5**) was additionally isolated. The nonpolar fraction of products was not abounded and appeared to be a mixture of hydrocarbons (dienes).

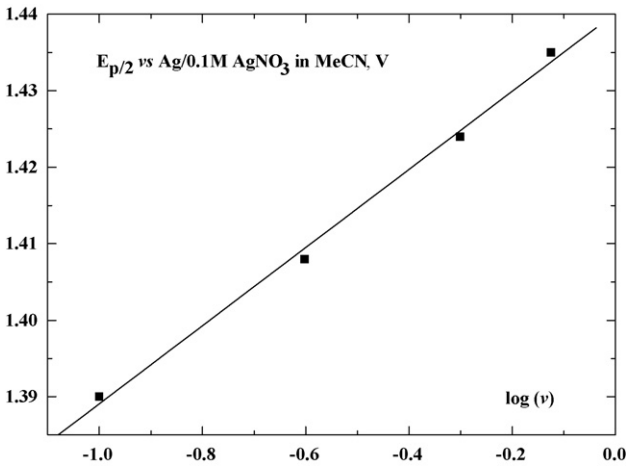


Fig. 2 – Dependence of the half-peak potential ($E_{p/2}$) of cholesterol (1 mM) oxidation process *vs.* log(*v*) (*v* is the scan rate).

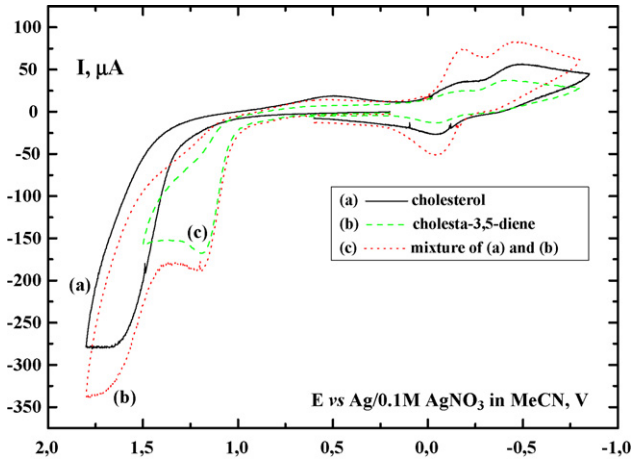
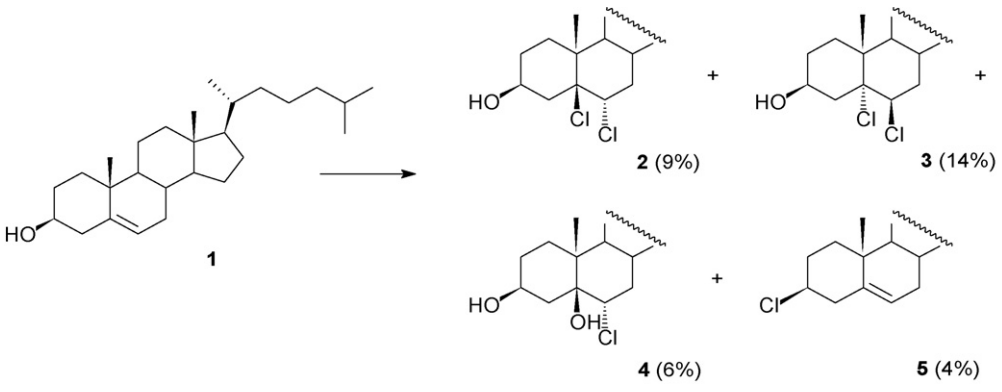
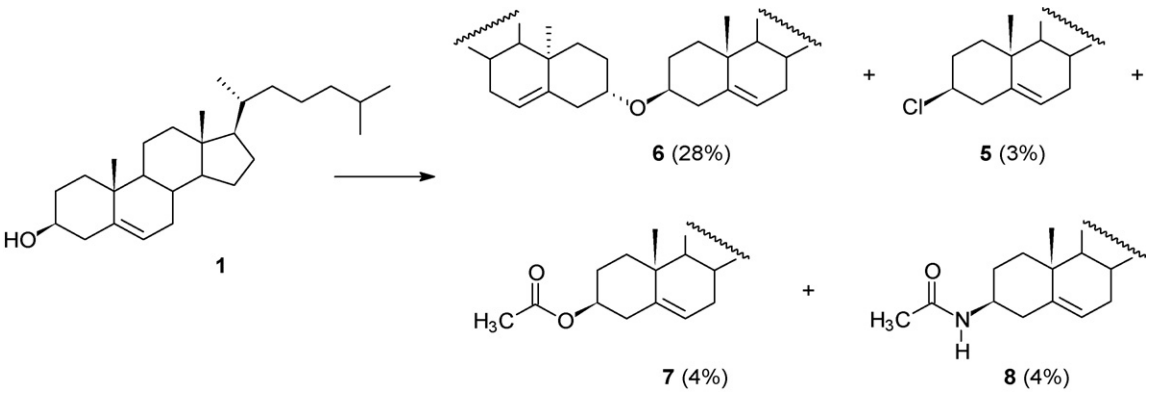


Fig. 1 – Cyclic-voltammograms registered in 0.1 M tetrabutylammonium tetrafluoroborate in dichloromethane on platinum electrode (area, 0.03 cm^2) of (a) 0.5 mM cholesterol, (b) 0.25 mM cholesta-3,5-diene, and (c) the mixture of 0.5 mM cholesterol with 0.25 mM cholesta-3,5-diene. Scan rate 0.5 V s^{-1}, potentials measured *vs.* Ag/0.1 M $AgNO_3$ electrode in acetonitrile.

The results obtained were similar to those described by Japanese authors [8]. They have also obtained products of the double bond chlorination by electrolysis of cholesterol in dichloromethane with metal chloride additives (e.g. $FeCl_3$). The reaction was then stereoselective and only 5α,6β-dichloride **3** was formed in addition to chlorohydrine **4**. It is clear that dichloromethane was a source of chlorine in our experiments since no additives were used. Its cathodic



Scheme 1



Scheme 2

reduction to chloride ions, their diffusion to anodic compartment and electrooxidation in presence of cholesterol causes the formation of chloro-derivatives of cholesterol [12]. To avoid the process, cathodic and anodic compartments were separated and connected with electrolytic bridge, which contained 0.4 M solution of $TBABF_4$ in the mixture of acetonitrile and dichloromethane (50:50, v/v) to increase the conductance. Moreover, glacial acetic acid was added to cathodic compartment of the electrolyser to prevent the electrochemical reduction of dichloromethane [12]. This however, as shown below, resulted in the appearance of 3β-acetylamino and 3β-acetyl derivatives of cholesterol among the products.

The main product of preparative electrolysis in this conditions was dicholesteryl ether **6** (Scheme 2), with material yield equal to about 37%. This naturally occurring compound is formed from cholesteryl sulfates or sulfonates [18]. The formation of **6** as an unwanted side product was observed during synthesis of steroid glycosides by the orthoester method [21] or other methods [22]. The TLC analysis of nonpolar reaction products showed presence of an intensive UV active spot (presumably from cholestadienes) but separation of electrolysis products by column chromatography proved that cholestadienes are formed only in trace amounts.

Apart from the main product some minor products were also formed. One of them was cholesteryl chloride indicating that the separation of anodic and cathodic compartments of electrolyser did not fully prevent the migration of chlorine ions. The addition of glacial acetic acid resulted in the appearance of cholesterol acetate (**7**) among the products (due to diffusion of acetic acid to anodic compartment). The presence of acetonitrile in electrolytic bridge caused the formation of 3β-acetylamino derivative of cholesterol (**8**). These results indicate that it is very difficult to prevent leakage of unwanted species to anodic compartment during the electrolysis process.

3.3. *Tentative reaction mechanism*

The tentative mechanism of electrochemical oxidation of cholesterol is drawn in Fig. 3. During preparative electrolyses the color of the analyte turns light-blue after approximately 15 min and was stable for the extended period of time. This indicates that organic cation-radicals of cholesterol are formed [23].

It seems that the first step of electrooxidation is one-electron transfer from cholesterol to anode. The cation-radical centered at the oxygen atom is formed since the heteroatom lone pair electrons are easily removable. The next step is heterolysis of the C3—O bond leading to the homoallylic carbocation. The cleavage of this bond is assisted by the π electrons of the neighboring double bond and the mesomerically stabilized nonclassical carbocation is formed [24]. Since electrolyses were performed in dichloromethane, which does not show nucleophilic properties, the only partner for this carbocation in the reaction medium was the oxygen atom of the second molecule of cholesterol **1** and dicholesteryl ether **6** was formed. However, leakage of nucleophilic ions (Cl^-) or molecules (acetic acid, acetonitrile) from the cathodic compartment or from the electrolytic bridge caused the unwanted side reactions. The identification of reaction by-products, cholesteryl 3β-chloride (**5**), 3β-acetate (**7**), and 3β-acetamide (**8**), unequivocally confirmed the reaction mechanism.

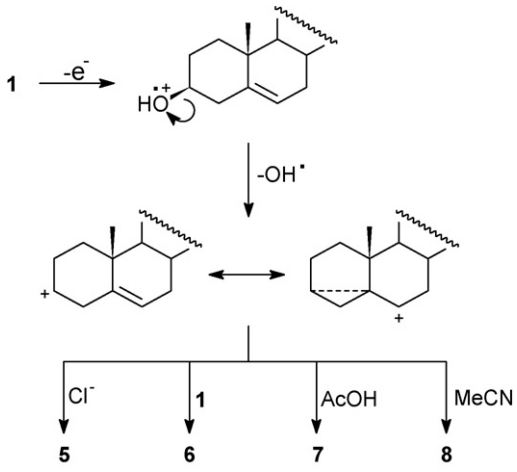


Fig. 3 – The tentative mechanism of electrochemical oxidation of cholesterol.

4. Conclusion

In the previous studies on cholesterol electrooxidation various products were formed oxidized at the C5—C6 double bond or at the allylic C-7 position. Electrooxidation of cholesterol in dichloromethane with tetrabutylammonium tetrafluoroborate as a supporting electrolyte led to the nonstereoselective chlorination of the C5—C6 double bond. However, the reaction carried out in separated cathodic and anodic compartments unexpectedly afforded dicholesteryl ether (**6**) in a relatively high yield. This reaction is rather surprising since secondary alcohols are usually oxidized to ketones [25–27]. The most likely reason for such unusual course of reaction is presence of the C5—C6 double bond. It seems to be clear that during electrooxidation a cation-radical centered at the cholesterol oxygen atom is initially formed. The heterolytic cleavage of the C3—O bond assisted by the π electrons of the double bond afforded the homoallylic cation which reacted with the second molecule of cholesterol (or any other nucleophile present in the reaction mixture) yielding the corresponding product.

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