

ELECTROCHEMICAL OXIDATION OF PROBUCOL IN ANHYDROUS ACETONITRILE

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Electrochemical oxidation of probucol in anhydrous acetonitrile was studied as a model of the metabolic oxidation of the substance. The study was performed by DC voltammetry, potentiostatic coulometry, cyclic voltammetry and preparative electrolysis. Probucol gives a single anodic wave $E_{1/2} = 0.92$ V. Cyclic voltammetry shows that its electrooxidation proceeds by formation of probucol radical. 2,6-Di-*tert*-butyl-4-(isopropylsulfanyl)phenol, 2,6-di-*tert*-butyl-4-sulfanylphenol, 2,6-di-*tert*-butyl-4-methylphenol and 2,6-di-*tert*-butylphenol were isolated as products of electrochemical oxidation.

Key words: Metabolic oxidation; Radicals; Probucol; Voltammetry; Potentiostatic coulometry; Preparative electrolysis; Electrochemistry.

Probucol, 4,4'-[isopropylidenebis(sulfonediyl)]bis(2,6-di-*tert*-butylphenol) (Fig. 1), is the main component of several antihyperlipoproteinemic drugs (Lorelco®, Lurselle®) used in treatment of heart and blood-vessel diseases. Probucol was synthesized by Neuworth¹ in 1969, who also described its bio-

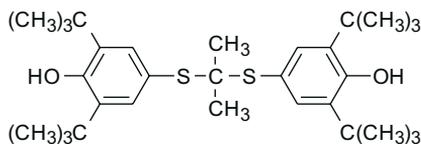


FIG. 1

Chemical structure of probucol

logical properties^{2,3}. Pharmacological studies of probucol were published by Barnhart *et al.*⁴ and Drake *et al.*⁵. Heel *et al.*⁶ reported a review of its pharmacologic and therapeutic use.

Probucol decreases cholesterol concentration in blood, reducing both LDL- and HDL-cholesterol. It has also an antioxidative effect on LDL particles, and decreases depositing LDL in macrophages and blood-vessel walls. The mechanism of its effect is not known in detail, but it is clear that probucol is accumulated in lipid tissues, where its concentration is 100 times higher than in blood. Probucol is metabolized in liver yielding a dipehnoquinone and spiroquinone, which are pharmacologically ineffective.

Electrochemical methods are useful tools for investigation of electron-transfer reactions and can be used for simulations of biological redox reactions⁷. The electrochemical behaviour and a good accordance between biological and electrochemical oxidation of pharmacologically important substances has been widely studied and reviewed⁸⁻¹¹.

The electrochemical oxidation of probucol has not yet been studied; therefore, the aim of this study was to describe electrochemical oxidation of the compound and to identify its products. Probucol is known to be accumulated in lipid tissue, therefore, acetonitrile was used as a medium for the oxidation of compound. It has been reported, that only monomolecular layer of water on the molecule of enzyme is required for enzymatic reactions, which can be performed also in organic media¹²⁻¹⁴. Thus, the oxidation in organic solvents may bring useful information on the behaviour of compound in lipid tissues. Also, in aprotic medium reactions of electron transfer proceed mostly by gradual transfer of one electron, similar to living organisms.

EXPERIMENTAL

Chemicals

Probucol was obtained from Chemische Fabrik Berg, GmbH (Germany). The purity of the substance was checked by the determination of its melting point, by the mass spectrometry and by HPLC.

Acetonitrile for HPLC (Sigma, Germany) purified by a method proposed by Hofmanová and Angelis¹⁵ was used as a reaction medium. Its purity was tested by gas chromatography and water content below $3 \cdot 10^{-3}$ vol.% was found. Sodium perchlorate was prepared by the Biedermann procedure¹⁶. Other chemicals were of analytical grade: AgNO_3 (Kovohutě Vestec, Czech Republic), diethyl ether (Lachema, Czech Republic), Na_2CO_3 (Lachema, Czech Republic), HClO_4 70% (Carlo Erba, Italy) and trifluoromethanesulfonic acid 98% (Fluka, Switzerland).

DC Voltammetry

The voltammetric measurements were performed with a three-electrode system using Eko-Tribo Polarograph (Polaro-Sensors, Czech Republic). A platinum rotating disk electrode (surface area of 0.111 cm^2) was used with constant rotation speed of 1 226 rpm. The reference electrode consisted of silver foil immersed in solution of 0.01 M AgNO_3 and 1 M NaClO_4 in anhydrous acetonitrile. Platinum wire 500 mm long and 0.5 mm in diameter served as a counterelectrode. All measurements were performed in a special three-compartment polarographic vessel¹⁷.

Potentiostatic Coulometry

A polarographic analyzer model PA 3 (Laboratorní přístroje, Czech Republic) was used for potentiostatic coulometry. The current *versus* time dependences were recorded on a chart recorder model TZ 4200 (Laboratorní přístroje, Czech Republic), with Fischer's platinum sieve electrode as a working electrode and large-area silver electrode separated by two frits as a counterelectrode. Silver rod immersed in solution of 0.01 M AgNO_3 and 1 M NaClO_4 in anhydrous acetonitrile, served as a reference electrode, was placed in a glass tube separated from the measured solution by two frits.

Cyclic Voltammetry

The CV measurements were performed on Universal modular polarograph E-310 (Bruker, Belgium) with data collection by computer card. A stationary platinum disk with active surface area of 0.159 cm^2 was used as the working electrode, the silver chloride electrode (Crytur, Czech Republic) as a reference and the above-mentioned platinum wire as an auxiliary electrode.

Preparative Electrolysis

Electrochemically generated oxidation products were prepared in a three-electrode system. Platinum wire 600 mm long, 0.5 mm in diameter and platinum plate (area 4 cm^2) were used as the working electrode. Reference electrode consisted of the silver foil immersed in solution of 0.01 M AgNO_3 and 1 M NaClO_4 in anhydrous acetonitrile. Platinum sieve electrode was used as an auxiliary electrode. Polarographic analyzer LP 7 (Laboratorní přístroje, Czech Republic) served as voltage source while the potential of the working electrode was measured by multimeter Keithley 168 (Germany) and maintained manually. The current was registered by a milliamperemeter (Metra Blansko, Czech Republic).

UV Spectrophotometry

The UV spectra were measured by SP-800 spectrometer using 1-cm quartz cells (both Pye Unicam, Great Britain).

High-Performance Liquid Chromatography

Probucol and its oxidation products were separated by a modification of previously described method¹⁸. Measurements were performed using a liquid chromatograph HP 1090 L with a diode array detector (both Hewlett-Packard) working at 230 nm, reverse phase C-18 $250 \times 4 \text{ mm}$ column (RP Select B C-18, Merck, Germany) as the stationary

phase and acetonitrile–water mixture (95 : 5) as the mobile phase. The flow rate was set to 0.5 ml min^{-1} and temperature to $40 \text{ }^\circ\text{C}$.

Mass Spectrometry

A Finnigan MAT LCQ (Finnigan) with atmospheric pressure chemical ionization (with acetonitrile as reaction gas) was used for the mass spectrometry measurements.

RESULTS AND DISCUSSION

DC Voltammetry

Probucol yielded a single, elongated anodic wave in 0.1 M NaClO_4 in anhydrous acetonitrile with half-wave potential $E_{1/2} = 0.92 \text{ V}$. The concentration plot and the plot of i_1 vs $\omega^{1/2}$ were linear in the range of $5 \cdot 10^{-5}$ – $1 \cdot 10^{-3} \text{ mol dm}^{-3}$.

In the presence of growing concentration of perchloric acid (Table I, Figs 2a and 2b), the half-wave potential and irreversibility decreased, but from 1 M HClO_4 up the split of anodic wave was observed and the limiting diffu-

TABLE I

Effect of perchloric acid on voltammetric characteristics of probucol in 0.1 M NaClO_4 in anhydrous acetonitrile (probucol concentration $2.5 \cdot 10^{-4} \text{ mol dm}^{-3}$, electrode area 0.111 cm^2 , $v = 0.05 \text{ V s}^{-1}$, 1 226 rpm , $T = 20 \text{ }^\circ\text{C}$)

[HClO ₄] mol dm ⁻³	Wave I			Wave II		
	$i_1 \cdot 10^6$ A	$E_{1/2}$ V	k^{-1} V	$i_1 \cdot 10^6$ A	$E_{1/2}$ V	k^{-1} V
0	40.2	0.920	0.113	–	–	–
$1.0 \cdot 10^{-4}$	34.7	0.890	0.112	–	–	–
$1.0 \cdot 10^{-3}$	34.9	0.820	0.100	–	–	–
$1.0 \cdot 10^{-2}$	29.7	0.810	0.081	–	–	–
$1.0 \cdot 10^{-1}$	27.6	0.805	0.093	–	–	–
$5.0 \cdot 10^{-1}$	23.3	0.810	0.092	–	–	–
1.0	23.8	0.830	0.106	23.8	1.470	0.132
1.5	16.1	0.825	0.059	20.6	1.415	0.108
2.0	16.2	0.815	0.060	17.1	1.400	0.124

sion current decreased with increasing acidity. From Figs 2a and 2b it is evident, that addition of HClO_4 resulted in lower energy required for probucol oxidation, due to specific solvation of probucol. Addition of solvation proton on free electron pair located on sulfur atom can be expected already at low concentrations of HClO_4 .

A similar effect was found also for trifluoromethanesulfonic acid, the wave split starting at 0.05 M $\text{CF}_3\text{SO}_3\text{H}$.

As the perchloric acid used was a 70 vol.% aqueous solution, the effect of water on voltammetric characteristics of probucol was studied. In the presence of growing concentration of water (Figs 3a and 3b), the reaction irreversibility steadily increased, the half-wave potential reaches its minima at 0.5 vol.% of water. The limiting diffusion current does not exhibit monotonous behaviour either, reaching its maxima at 0.5 vol.% and minima at 4 vol.%. At water concentration above 7.5 vol.% reliable measurements were not possible. These effects are probably originated in specific solvation of probucol as well.

Number of Exchanged Electrons

Comparison of limiting diffusion current of probucol oxidation with reactions of known number of exchanged electrons (phenothiazines) indicated $n = 3.7$. However, the number of electrons determined by potentiostatic

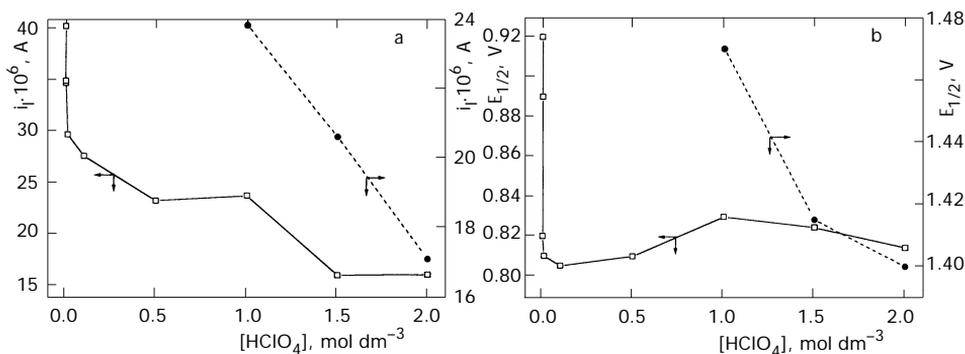


FIG. 2

Effects of perchloric acid on voltammetric characteristics of probucol in 0.1 M NaClO_4 in anhydrous acetonitrile (probucol concentration $2.5 \cdot 10^{-4} \text{ mol dm}^{-3}$, electrode area 0.111 cm^2 , $v = 0.05 \text{ V s}^{-1}$, 1 226 rpm, $T = 20 \text{ }^\circ\text{C}$); ----- wave I, - - - wave II; a effect of perchloric acid on limiting diffusion current of probucol, b effect of perchloric acid on half-wave potential of probucol

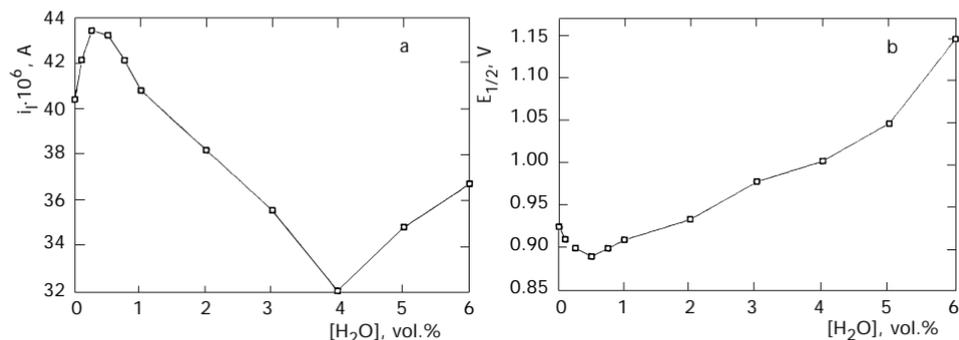


FIG. 3

Effects of water on voltammetric characteristics of probucol in 0.1 M NaClO₄ in anhydrous acetonitrile (probucol concentration $2.5 \cdot 10^{-4}$ mol dm⁻³, electrode area 0.111 cm², $\nu = 0.05$ V s⁻¹, 1 226 rpm, $T = 20$ °C); a effect of water on limiting diffusion current of probucol, b effect of water on half-wave potential of probucol

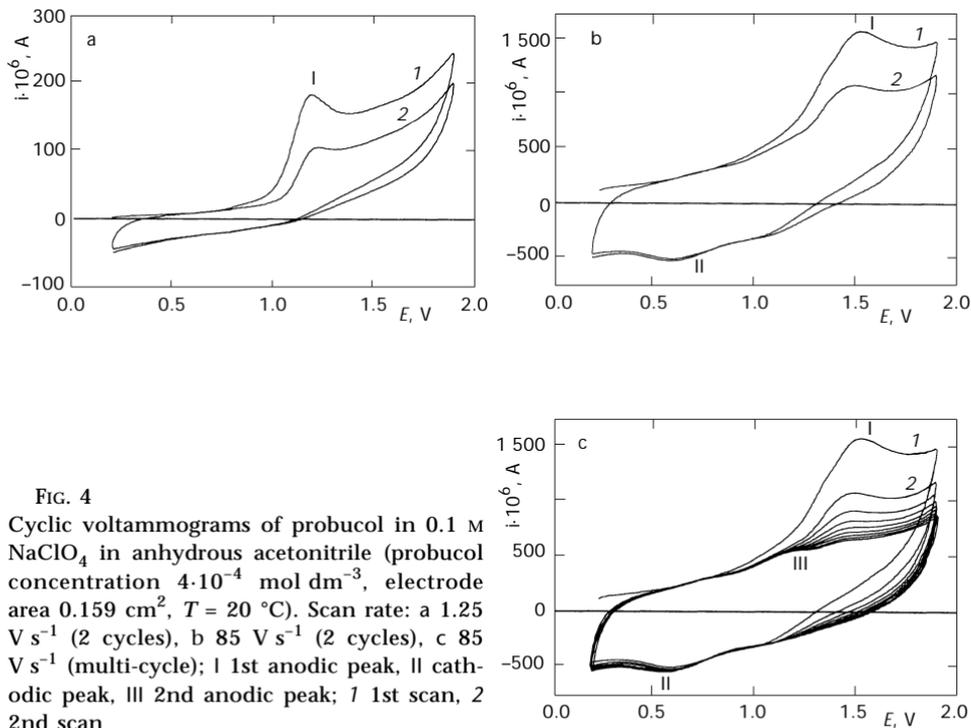


FIG. 4

Cyclic voltammograms of probucol in 0.1 M NaClO₄ in anhydrous acetonitrile (probucol concentration $4 \cdot 10^{-4}$ mol dm⁻³, electrode area 0.159 cm², $T = 20$ °C). Scan rate: a 1.25 V s⁻¹ (2 cycles), b 85 V s⁻¹ (2 cycles), c 85 V s⁻¹ (multi-cycle); I 1st anodic peak, II cathodic peak, III 2nd anodic peak; 1 1st scan, 2 2nd scan

coulometry ranged from 7.17 to 7.65 for 10 measurements in 0.1 M NaClO₄ in anhydrous acetonitrile indicating the complexity of probucol electro-oxidation in anhydrous acetonitrile.

Cyclic Voltammetry

Reversibility of electrochemical oxidation of probucol was studied by cyclic voltammetry. At sweep rates lower than 20 V s⁻¹ (Fig. 4a) one anodic peak (I) was found and the system was totally irreversible. At scan rates higher than 20 V s⁻¹, the cyclic voltammetry yielded cathodic peaks (II) and (I) in two cycles (Fig. 4b). In multi-sweep cyclic voltammetry anodic peak (III) was found (Fig. 4c). The experimental data obtained by cyclic voltammetry are summarized in Table II. Peaks II and III appearing at high sweep rates indicate formation of the probucol radical with a short life-time.

Preparative Electrolysis

An amount of 10 mg of probucol was oxidized in 40 ml of 0.1 M NaClO₄ in anhydrous acetonitrile at potential 1.2 V. The electrochemical oxidation was terminated after 2 h when the current decreased from 7.7 mA to 0.1 mA. The log $i = f(t)$ plot was linear during electrolysis with the number of exchanged electrons about 7. The time course of the oxidation was followed by UV spectrophotometry: 75 μ l of electrolyzed solution was diluted by 3 ml of anhydrous acetonitrile and measured in 1-cm quartz cell against anhydrous acetonitrile (Fig. 5). The voltammetric measurement during electrolysis revealed descended limiting diffusion current and the half-wave potential shifted to lower values.

From the Table I it is evident that the oxidation of probucol in the presence of 1·10⁻² M HClO₄ shows higher degree of reversibility. Therefore in the next experiment, 10 mg of probucol was oxidized in 40 ml of 0.1 M NaClO₄ in anhydrous acetonitrile in the presence of 1·10⁻² M HClO₄ at potential 1.2 V. The electrolytic current decreased from the initial value 6.0 to 0.2 mA, after 2 h when the electrolysis was terminated.

Identification of Products of Electrolysis

An amount of 50 μ l aliquots of the solution taken during electrolysis was frozen in dry ice. Their composition was examined by HPLC (injected amount of 5 μ l). While at the beginning of the electrolysis only one peak of probucol was present (Fig. 6a), a number of peaks appeared in the first

30 min of the experiment (Fig. 6b). A decrease of electrolytic current to about 0.5 mA was accompanied by formation of one main peak (Fig. 6c). From these results, it is evident that during electrolysis of probucol a number of products arised, but some of them ceased to exist during the time

TABLE II

Experimental and calculated data obtained by cyclic voltammetry measurements of probucol in 0.1 M NaClO₄ in anhydrous acetonitrile (probucol concentration 4·10⁻⁴ mol dm⁻³, electrode area 0.159 cm², T = 20 °C); I 1st anodic peak, II cathodic peak, III 2nd anodic peak

ν V s ⁻¹	E_p^I V	$i_p^I \cdot 10^6$ A	E_p^{II} V	$i_p^{II} \cdot 10^6$ A	E_p^{III} V	$i_p^{III} \cdot 10^6$ A	$E_p^{III} - E_p^{II}$ V	i_p^{III} / i_p^{II}
0.20	1.120	69	-	-	-	-	-	-
0.63	1.555	126	-	-	-	-	-	-
1.25	1.185	182	-	-	-	-	-	-
10	1.325	506	-	-	-	-	-	-
20	1.355	704	0.680	67	1.050	43	0.370	0.64
40	1.430	979	0.650	67	1.125	44	0.475	0.66
85	1.505	1 552	0.620	115	1.195	66	0.575	0.57
170	1.650	2 169	0.600	120	1.260	89	0.600	0.74

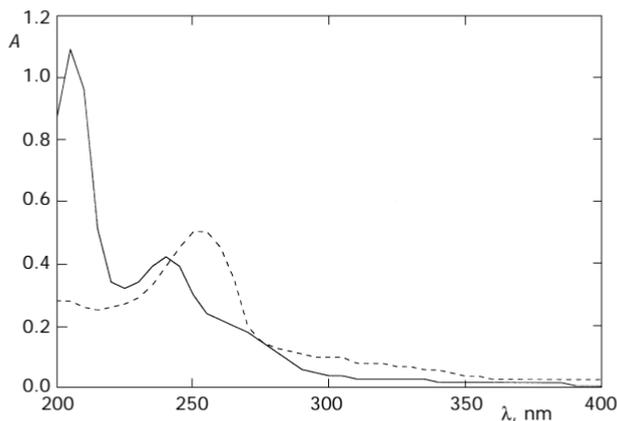


FIG. 5

UV monitoring of electrochemical oxidation of 10 mg of probucol in 40 ml 0.1 M NaClO₄ in anhydrous acetonitrile. Reaction time: ----- 0 min, - - - 120 min

(peaks IV–VII in Fig. 6b). It testified further about complexity of probucol oxidation.

After electrolysis, the solution was evaporated the solid was extracted with diethyl ether and once again evaporated. HPLC analysis showed one main peak. The mass spectrometric analysis, however, found one main

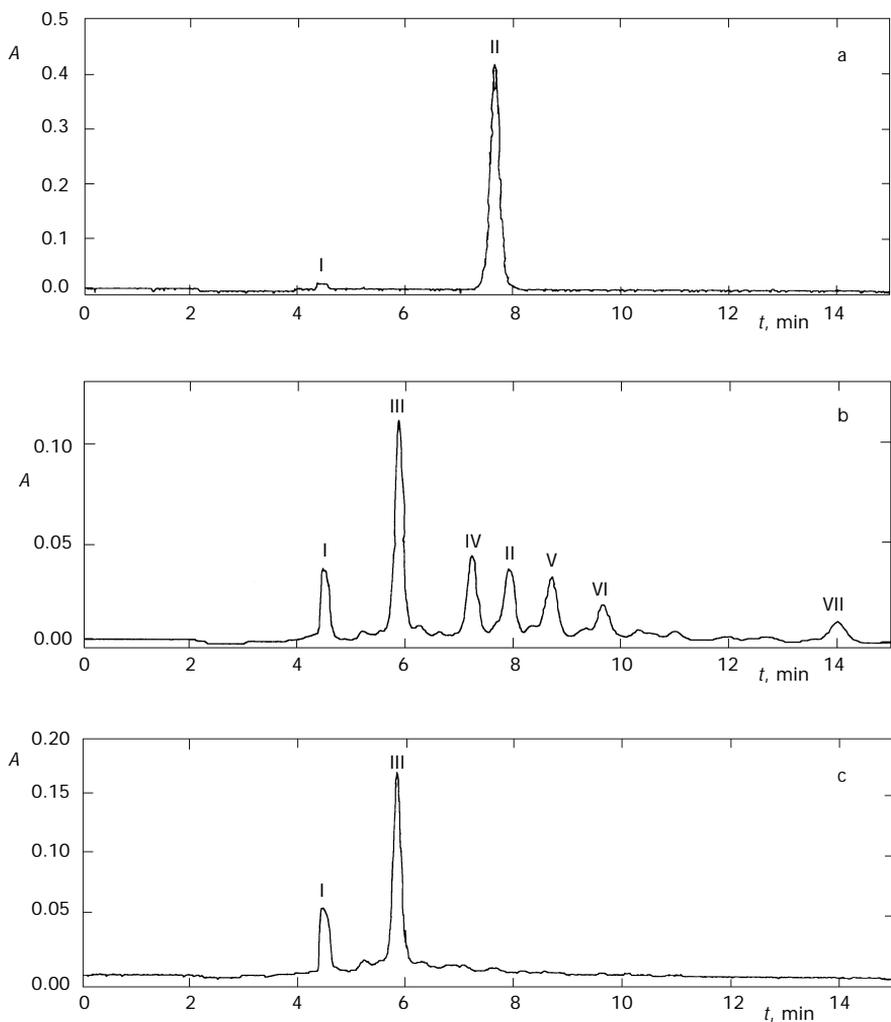


FIG. 6

HPLC monitoring of electrochemical oxidation of 10 mg of probucol in 40 ml 0.1 M NaClO_4 in anhydrous acetonitrile. Reaction time: a 0 min, b 15 min, c 120 min; I inorganics, II probucol, III main peak, IV–VII minor peaks

product 2,6-di-*tert*-butyl-4-(isopropylsulfanyl)phenol, and two minor products 2,6-di-*tert*-butyl-4-sulfanylphenol and 2,6-di-*tert*-butyl-4-methylphenol; percentage of products was not evaluated.

HPLC monitoring of electrolysis of probucol in the presence of perchloric acid yielded similar information about course of electrolysis, but in solution only 2,6-di-*tert*-butylphenol was found by mass spectrometric analysis. It indicated a simpler course of electrolysis of probucol in presence of perchloric acid.

CONCLUSIONS

From our results it is apparent that the oxidation of probucol in non-aqueous medium starts with formation of a probucol radical. In the literature the existence of probucol phenoxyl radical has been proved by ESR (ref.¹⁹). It is formed by loss of hydrogen atom or electron and proton from one of the phenolic groups of probucol²⁰. On the other hand electro-oxidation generated probucol radical has relatively short life-time. The electrochemical oxidation of phenols led mostly to formation of various dimers, but in case of phenols substituted in position 2, 4, 6 the probability of dimer formation is very low^{21,22}. Free electron from phenoxyl radical can be translocated on position 4 as it probably happens at electrochemical oxidation of probucol. The radical formed has a short life-time and decomposes to one or more products (depending on the presence of perchloric acid) by cleavage of S-C bond. The variety of oxidation products shows that mechanism of probucol oxidation is very complex and in the future the attention has to be directed to find its exact reaction pathway.

Our study has shown that the combination of HPLC with mass spectrometry can provide a useful information in the study of electrochemical simulation of biological oxidation.

SYMBOLS

A	absorbance
E	potential, V
E_p	peak potential, V
$E_{1/2}$	half-wave potential, V
i	current, A
i_l	limiting diffusion current, A
i_p	peak current, A
k^{-1}	reciprocal slope of logarithmic analysis of voltammetric wave (<i>i.e.</i> $\log(i/i_l - i)$ vs E plot)
v	scan rate, V s ⁻¹

t	time, min
T	temperature, °C
λ	wavelength, nm
$\omega^{1/2}$	angular speed of rotating disk electrode, $s^{-1/2}$

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