POLAROGRAPHY OF 3,4,5-TRIHYDROXYBENZOIC ACID

František POSPÍŠIL

Institute of Experimental Botany,
Czechoslovak Academy of Sciences, 160 00 Prague 6

Polarographic oxidation of 3,4,5-trihydroxybenzoic acid on mercury and platinum electrodes is described. The electrode process is influenced by dissociation of the acid and adsorption of the oxidation products on the electrodes. The anodic peak height in differential pulse polarography is not a linear function of the concentration, which limits the determination of the acid concentration. A linear dependence of the anodic current on the concentration was found on a rotating platinum disc electrode at pH 6·5 or 2·0, which is of advantage in determining the acid in physiological solutions and eluates from chromatographic columns.

3,4,5-Trihydroxybenzoic acid is formed in a number of biosynthetic ways during degradation of lignin by hydroxylation of 3,4-dihydroxybenzoic acid, demethoxylation and hydroxylation of 3,5-methoxy-4-hydroxycinnamic acid[1-3]. It is oxidized by air or tyrosinase to 3-hydroxy--1,2-benzoquinonecarbonic acid and finally to purpurogallic acid[1,2]:
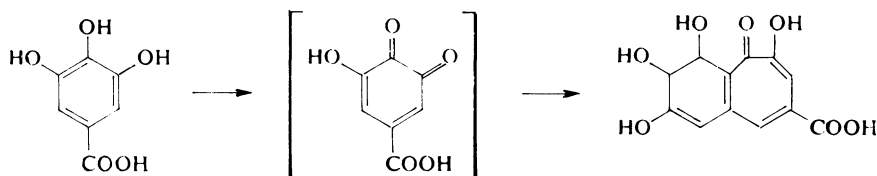
It forms the component of tannic acid[4] in plants, further it is present together with other phenolic compounds in waste waters. Therefore, its determination appears necessary, usually in combination with chromatography, the anodic current on a platinum indicator electrode being recorded[5-8].

3,4,5-Trihydroxybenzoic acid gives two anodic waves in d.c. polarography with a dropping mercury electrode[9,10]. The larger wave is diffusion-limited and proportional to the acid concentration. The more negative wave is formed in acidic medium up to pH 6·0 and its height depends on the concentration in the form of an adsorption isotherm[10].

The anodic oxidation of phenolic acids on solid electrodes is influenced by further processes. Phenoxyl radicals combine to dimers or oligomers and all these forms are adsorbed on the electrode surface. These can be further oxidized or reduced[11].

Adsorption often causes inhibition of the electrode process, as manifested by lowering of the anodic current, formation of a prewave[12], or changes in the form of the anodic curve[13,14].

The aim of the present work was to find suitable conditions for polarographic determination of 3,4,5-trihydroxybenzoic acid and to elucidate the mechanism of its anodic oxidation.

EXPERIMENTAL

Polarographic curves were recorded on a PA 3 Polarographic analyser with an X-Y 4103 recorder (Laboratorní přístroje, Prague). A rotating platinum disc electrode was used at 1 100 or 1 650 r.p. min. The reference electrode was 0·1 mol/l mercurous sulphate electrode joined with the polarographic cell by an agar-agar salt bridge (separated by means of fritted glass). A platinum wire served as auxiliary electrode. All potential values are given against the reference indicated.

All chemicals were of reagent grade. The base electrolyte was Britton–Robinson or phosphate buffer in redistilled water; 3,4,5-trihydroxybenzoic acid was product of Koch-Light Laboratories. The rotating platinum electrode was ground with a fine emery paper SIA 1600 and rinsed with redistilled water prior to every measurement. This pretreatment appeared more suitable than other recommended procedures[15].

RESULTS

Differential Pulse Polarography on Dropping Mercury Electrode

A single peak was formed up to a concentration of 10^{-4} mol/l in a phosphate buffer of pH 6·5; its potential E_p was in the range from $-0·250$ to $-0·310$ V. At higher acid concentrations, a second peak was formed at $E_p = -0·230$ V. The curves are shown in Fig. 1, where further details are given.

The dependence of the first peak height, i_{pa} (in mm) on the concentration c (mol/l) is not linear and can be expressed by the empirical formula $i_{pa} = 125 - 642·2\, e^{-3·355c}$.

D.C. Polarography on Rotating Platinum Disc Electrode

The half-wave potential of 3,4,5-trihydroxybenzoic acid in Britton–Robinson buffer solutions is equal to $+0·4$ V at pH 2·8 and $-0·03$ V at pH 11·0; the pH dependence shows two breaks. In the medium of $0·1\text{M-}H_2SO_4$, the curves consist of two waves with $E_{1/2} = +0·190$ and $+0·420$ V (Fig. 2). The height of the latter is proportional to the concentration. In a buffer of pH 2·0, where the anodic wave is highest, its concentration dependence is precisely linear in the range from $4 . 10^{-5}$ to $2 . 10^{-4}$ mol/l and suitable for analytical determination. The wave height decreases with increasing pH.

DISCUSSION

In analogy to d.c. polarography[9,10], the dependence of the DPP anodic peak heights on the concentration is not linear, its character suggests that the second (more positive) anodic peak current is not limited solely by diffusion but apparently an inhibition of the electrode process takes place. Therefore, the DPP method has a limited applicability for the determination of the acid concentration in physiological solutions, the inhibition of the electrode process increasing with the concentration.

The dependence of $E_{1/2}$ on pH on the rotating electrode is related to the structure of the acid and its dissociation constant, $pK_a = 4.4$. Below pH 4.0, none of its functional groups is dissociated. At pH between 4.0 and 9.0, the carboxyl group is dissociated, and above pH 9.0 apparently the phenolic OH group is dissociated in addition. Alkaline solutions of the acid are distinctly brown in colour, the formation of dimers and oxidation with air oxygen taking place[9]. The anodic current is somewhat lower than that in an acidic medium.

In accord with other data[12], the anodic oxidation of the acid is influenced by chemical reactions, especially in the DPP method. However, on the rotating Pt electrode a linear dependence of the current on the concentration was found especially at pH 2.0, which can be made use of in analysis, *e.g.* combining polarography with chromatography.
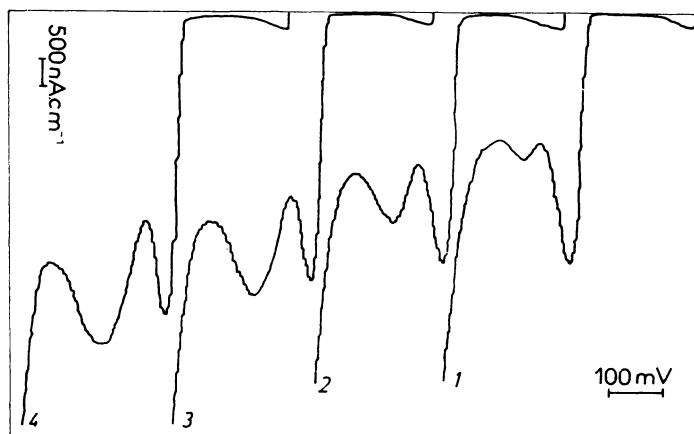

FIG. 1

Cathodic–anodic DPP curves for 3,4,5-trihydroxybenzoic acid in phosphate buffer at pH 6.5. Modulation amplitude 50 mV, scan rate 2 mV/s,$^{-1}$, clock 2, sensitivity 500 nA/cm^{-1}, $m =$ $= 3.6$ mg/s, $h = 45$ cm. Each curve starts at -0.5 V. *1* 0.068; *2* 0.085; *3* 0.119; *4* 0.136 mg of the acid in 3 ml

In view of the application of the polarography of phenolic acids in physiology we followed the anodic oxidation of 3,4,5-trihydroxybenzoic acid at pH 6·0—6·5. Thus, two waves were obtained, the first with $E_{1/2}$ about $-0·180$ V, distinct at higher concentrations, and the second higher with $E_{1/2}$ about $+0·170$ V and with a linear dependence on the concentration.

The character of i–E curves on the rotating Pt electrode suggests that the process is irreversible. The dependence of $\log\left[i/(i_d - i)\right]$ on E has a slope of 0·242 V per decade at pH 6·0 for the more positive wave $(+0·170$ V approximately).

If the electrode is not cleaned after an experiment and is polarized in a pure buffer solution towards negative potentials, a drawn-out cathodic wave is obtained, begin-
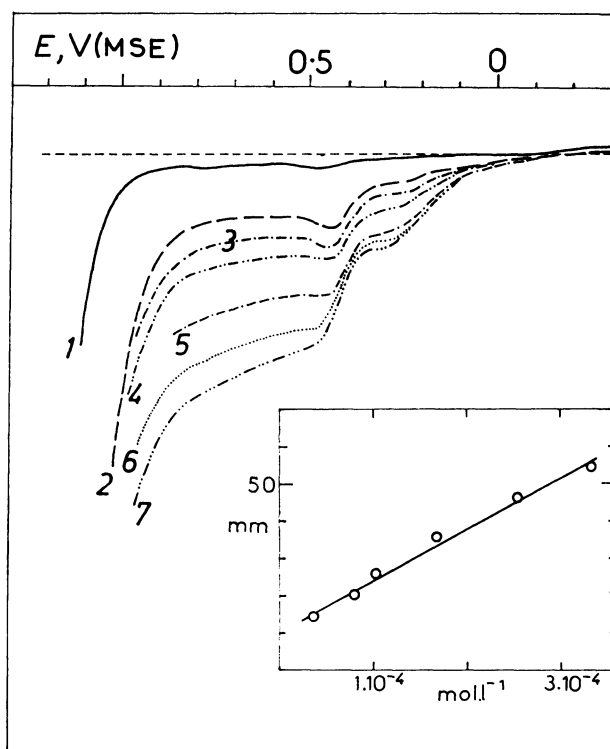

Fig. 2

Cathodic–anodic waves for 3,4,5-trihydroxybenzoic acid in Britton–Robinson buffer at pH 2·0 on rotating Pt disc electrode at 1 650 r.p. min; scan rate 5 mV/s^{-1}, sensitivity 500 nA/cm^{-1}. Each curve starts at $-0·3$ V. Acid concentration: 1 0; 2 3·84 . 10^{-5}; 3 7·40. . 10^{-5}; 4 1·07 . 10^{-4}; 5 1·66 . 10^{-4}; 6 2·46 . 10^{-4}; 7 3·32 . 10^{-4} mol/l

ning at -0.2 V, with E_{pk} about -0.3 V. The peak height increases after enzymatic oxidation of the acid with tyrosinase[3]. The oxidation product is insoluble in organic solvents and can be removed mechanically from the electrode. The oxidation product adsorbed on the solid electrode can be reduced simultaneously with the surface oxides[11,12]; its nature can be judged by analogy with enzymatic oxidations of the acid[3].

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