Electrochemical and radiolytic oxidation of naturally occurring phenols

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The combined use of electrochemical and radiolytic techniques permits elucidation of the mechanism of oxidation of phenols and characterization of the intermediates.

This paper is dedicated to Prof E Haslam on the occasion of his 65th birthday.

Introduction

Phenols are widely involved in plant biology and processing and many of their properties are related to oxidation processes [1–3]. It is therefore a worthwhile goal to try to unravel the mechanism of these oxidations and to determine the kinetic and thermodynamic parameters of the different steps. In this respect, electrochemistry has provided a large body of kinetic and thermodynamic knowledge which coupled with the various available experimental techniques permits the determination of both oxidation and reduction mechanisms [4–7]. Pulse radiolysis allows us to characterize short lived intermediates and to follow their reaction kinetics by recording their spectra in the UV and visible range, starting in the microsecond range [8–13]. These two techniques can be fruitfully coupled to determine oxidation mechanisms of phenols and characterization of intermediates. It is the purpose of this paper to point out, mainly on examples taken from our own work [14–21], the mechanistic information that can be extracted from these data. The oxidation of protonated phenols (ArOH) leads after the transfer of one electron and loss of one proton to a phenoxyl radical. In the case of hindered phenols [7], phenoxonium ions can be obtained corresponding to the transfer of two electrons and the loss of one proton. Phenolates (ArO^-) undergo one electron oxidation to phenoxyl radicals ($ArO^\cdot$) which most often undergo coupling reactions. As phenolate ions are more easily oxidized than their protonated counterparts and as they are in equilibrium with these last species down to media of low acidity, they are the species which are of interest in the oxidation of naturally occurring phenols. We shall therefore mainly describe the oxidation of ArO^-.

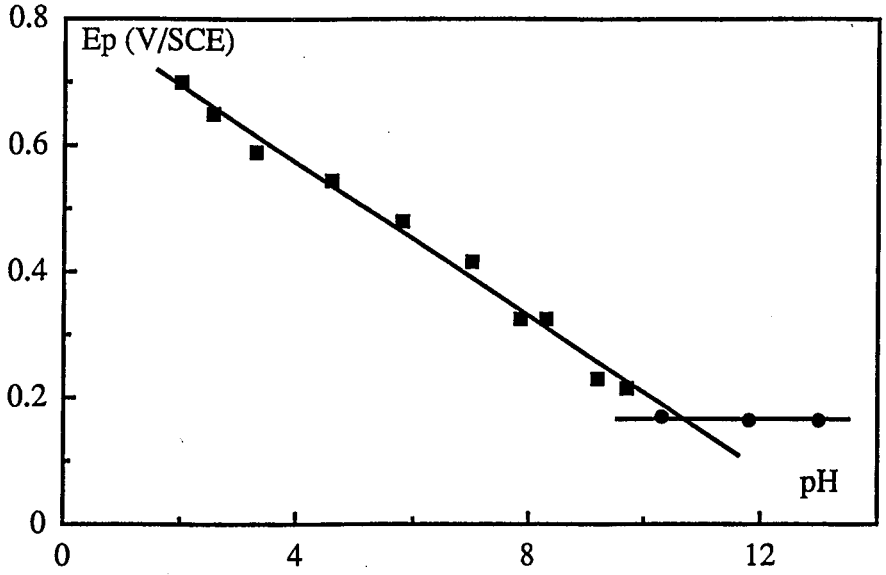


Fig 1. Variation of the anodic peak potential of coniferyl alcohol (c = 1 mM) with the pH in a methanol–water (1:1) buffered solution, 3 mm diameter disk glassy carbon electrode, scan rate $v = 0.2\ V.s^{-1}$).

Determination of the acidity constants

A first parameter which can be determined by electrochemistry, as an alternative method to spectroscopy, is the pK_a of the phenols [22]. If the peak potential in cyclic voltammetry or the half wave potential in polarography is plotted as a function of the pH of the buffered solution one obtains a straight line with a slope of $0.059/n$ (in V per unit pH, n being the number of electrons which are transferred, ie, one in the case of phenolates); at pH higher than the pK_a, the potential does not depend on the pH and a horizontal straight line is obtained. The pK_a is obtained as the intercept of the two straight lines. This is shown in figure 1 on the particular example of coniferyl alcohol for which a pK_a of 10.7 was found, close to the value obtained by spectroscopy. It should, however, be remarked that the use of such a procedure assumes that all the equilibria involved (proton and electron exchange) are fast and that the rates of the different steps do not vary with the pH. A slow step in one of the equilibria may be responsible for the small differences between the pK_a obtained by electrochemistry and by spectroscopy. The pK_a, measured by electrochemistry of different naturally occurring phenols are gathered in table 1 [23].

Table I. pK_a of some phenols measured by electrochemistry.

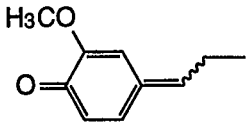
Coumaryl alcohol	10.2
Coniferyl alcohol	10.5
Sinapyl alcohol	11.1
Coumaraldehyde	8.4
Coniferaldehyde	8.8
Sinapaldehyde	9.1
4-Coumarate ion	8.8
4-Ferulate ion	10.1
4-Sinapate ion	10.4

[a] in 50/50 water-methanol solution.

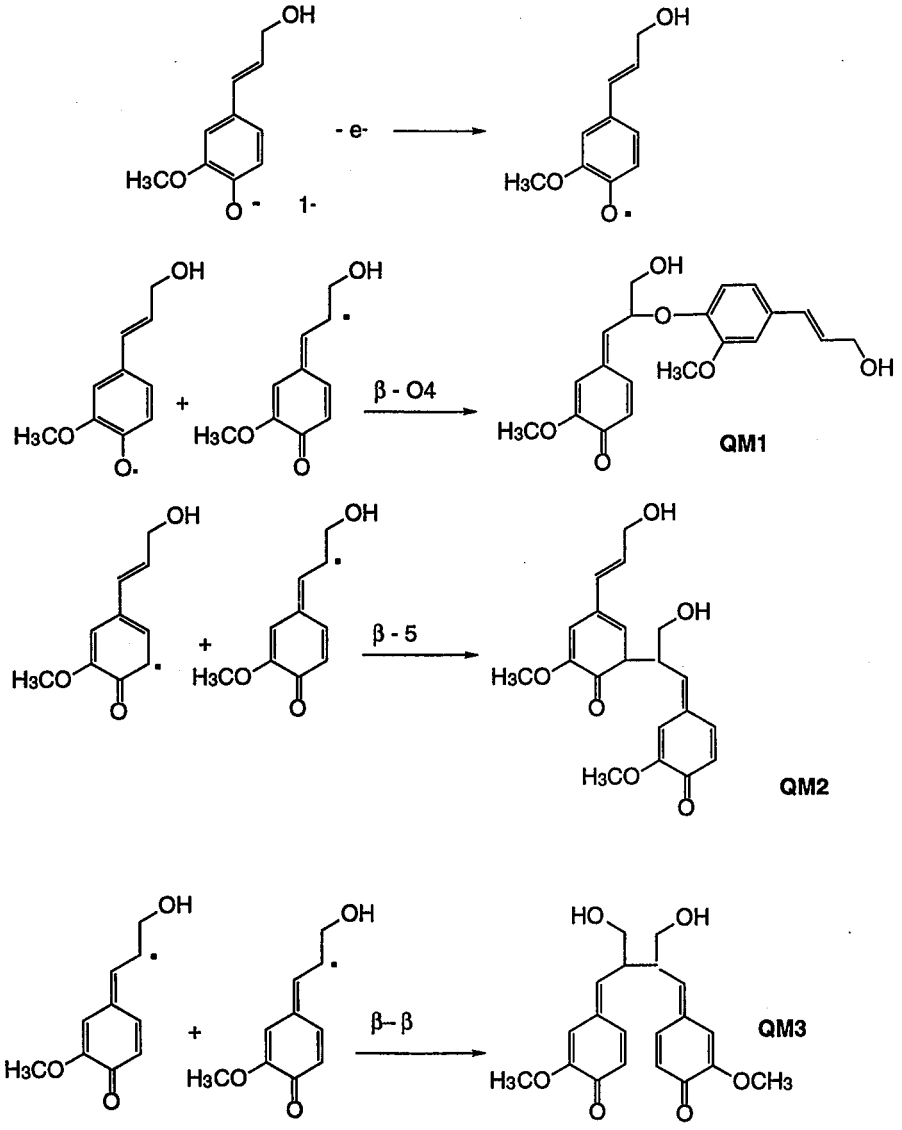
radical anion substrate should be favored. In every case, pulse radiolysis in water pointed to a radical coupling mechanism (second order decay of the spectrum of the phenoxyl radical and independence of the rate constant upon the concentration of coniferyl alcohol). It was therefore possible to propose the following mechanism for the first step of polymerization of coniferyl alcohol (*scheme 2*).

Spectroelectrochemical experiments

In addition to the demonstration that the dimerization takes place through a radical–radical coupling, this mechanism is based on the observation of dimeric quinonemethides by spectroelectrochemistry and radiolysis. By inserting a platinum grid in a UV cell, it is possible to record the UV-visible spectra while oxidizing the phenolate ion of coniferyl alcohol. This method provides the spectra of intermediates on the time scale of the order of one minute. With coniferyl alcohol the observed spectrum was similar to that of a synthesized quinonemethide (*scheme 3*):



scheme 3



Scheme 2

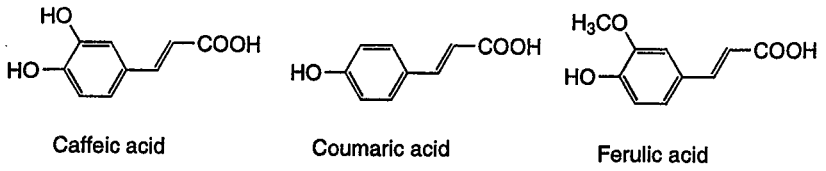
In addition, the quinonemethides QM1, QM2 and QM3 were also observed (fig 4) through their reduction peak (the peaks of the three quinonemethides are located at very close potentials) on the voltammogram of coniferyl alcohol in aqueous, acetonitrile or dimethylsulfoxide solution after the coniferyl alcohol had been oxidized on the anodic scan. Spectroelectrochemical measurements also indicated that upon reduction the quinonemethide dimers partly reverted (depending on the medium) to the starting phenolate. Finally the spectra of the quinonemethides were also observed after radiolytic oxidation of coniferyl alcohol. However, the quinonemethides are not the final dimeric products observed through analysis by thioacidolysis [41, 42] of an electrolyzed solution. The final dimeric products are obtained by addition of water or methanol to the dimeric quinonemethides.

Extension to other biological systems

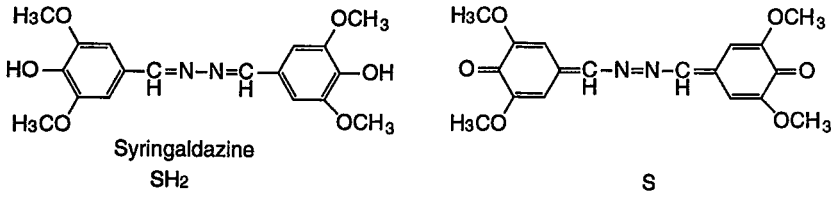
We also examined [21] the oxidation of caffeic acid, an analog of caftaric and coutaric acids, by using the different methods described above. These acids (*scheme 4*) are responsible for the browning of white wines through the oxidative formation of coupling products [43].

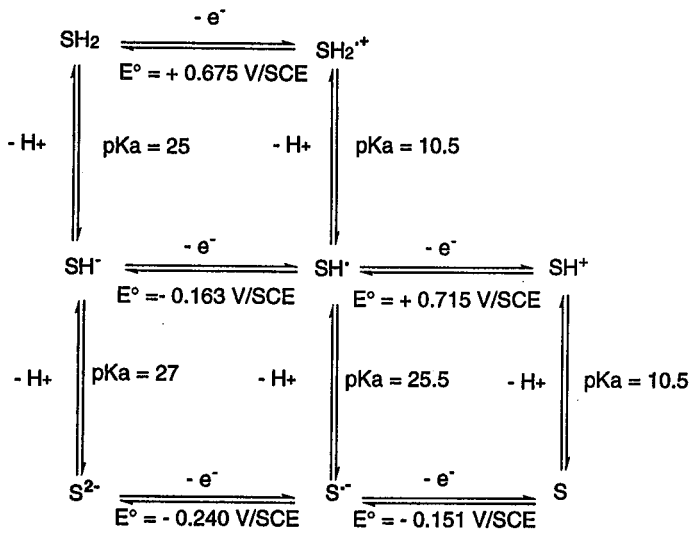
The conjugated base of coumaric and ferulic acid are oxidized to their corresponding phenoxyl radicals as described above; these phenoxyl radicals undergo a fast dimerization (10^8 L.mol^{-1}.s^{-1}). The behavior of caffeic acid is different: in water at pH 4 a reversible two electron wave is observed with the usual characteristics of a hydroquinone/quinone system and in dimethylsulfoxide (after deprotonation of the acidic and the two phenolic functions) two reversible one electron systems are obtained which correspond to the formation of the semiquinone ($E°$ = -0.86 V/SCE) and the quinone. The semiquinone was also characterized by its UV spectrum obtained during a spectroelectrochemical and pulse radiolysis experiment. The quinone is formed by disproportionation of the semiquinone as deduced from the second order decay observed by pulse radiolysis; the rate constant of this disproportionation at pH 5.7 was found equal to $2k = 8.4\ 10^7$ L.mol^{-1}.s^{-1}. The *o*-quinone is not entirely stable and undergoes further reactions to give a black precipitate. These measurements permitted us to rule out alternative mechanisms which had been proposed for the formation of the coupling products: direct dimerization of the semiquinone or comproportionation between the *o*-quinone and caffeic acid.

Syringaldazine (SH_2) is a colorimetric reagent used in wood research for the



Scheme 4





Scheme 5

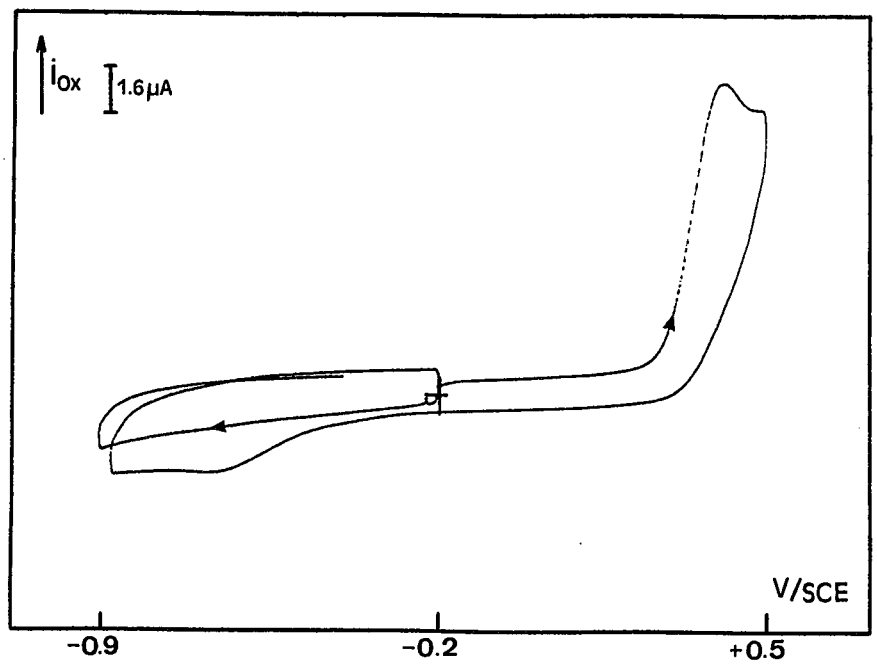


Fig 4. Cyclic voltammogram of coniferyl alcohol in a methanol–pH 6 water (1:1) buffer on a glassy carbon electrode . Reference SCE. Scan rate $v = 0.2\ V.s^{-1}$.

identification of enzymes involved in the polymerization of coniferyl alcohol in lignifying cells. A complete thermodynamic description [16] of its oxidation both by electrochemistry and by pulse radiolysis has been achieved (*scheme 5*).

Conclusion

Through these few examples we have pointed out the kind of information which, in the field of phenol oxidation, can be extracted from the combined use of electrochemical and radiolytic studies: pK_a, standard potentials, rate constant of reactions associated with an electron transfer. But even more interesting than these raw data is the possibility of establishing the oxidation mechanism on a firm basis (or at least, the first steps preceding or following the electron transfer). In the case of coniferyl alcohol, the main monomer involved in the formation of lignin, it was possible to demonstrate the existence of the phenoxyl radical and to obtain some of its electrochemical and spectral characteristics, to establish the radical–radical mechanism of dimerization to the quinonemethides which could be characterized by their UV spectrum and their reduction voltammetric peak. In one case a radical anion substrate has been observed which opens new perspectives on the lignification mechanisms. However the rate constants for the coupling of radicals which have been measured represent an average of the different couplings (β-O 4, β-5, β-β) leading to the formation of the lignin network; it would therefore be interesting to investigate possible correlation of these rate constants with the percentage of the different types of coupling formed in various media. It would also be possible to investigate this sort of problem in cellulosic gels more alike the natural medium of lignin formation.

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Isotopic labelling of proanthocyanidins

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Isotopic labelling is a powerful technique for studying the bioavailability and metabolism of polyphenols. The respective advantages of biolabelling and labelling through organic synthesis are discussed.

This paper is dedicated to Prof E Haslam on the occasion of his 65th birthday.

Introduction

Proanthocyanidins (PAs, *syn* condensed tannins) are among the most abundant polyphenolic compounds in plants and are common constituents of many foods (fruits, vegetables, cereals) and beverages (tea, wine, beer). The daily intake of total polyphenolic compounds is estimated at 1 g and PAs likely form the bulk of them.

Some epidemiology studies and experimental works on both animals and cell cultures suggested a protective effect of polyphenols against cancers and cardio-vascular diseases. For example, PAs with their anti-oxidant properties may inhibit the oxidation of low density lipoproteins [1], and thus prevent the formation of the atheromous plaque. Polyphenolic compounds are thus supposedly absorbed in the gastro-intestinal tract. However, their absorption and metabolic fate is still a matter of debate. Radio-labelled PA polymers prepared by incorporation of a radioactive biosynthetic precursor in plants, were shown not to be absorbed by chickens [2] and sheep [3], while oligomers of low molecular weight (dimers) were metabolised and absorbed by mice [4]. These contradictions may either come from the difference in animal species or from the use of insufficiently characterised PAs, possibly contaminated by other radio-labelled compounds.

We undertook the preparation of labelled PAs, properly purified and characterised that may be used to provide reliable data on their bioavailability. Two routes have been explored: either synthetic or biosynthetic. Their respective advantages are discussed.