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Spectroscopic determination of the dissociation constants of 2,4- and 2,5-dihydroxybenzaldehydes and relationships to their antioxidant activities

Rafael Estévez Brito [a], José Miguel Rodríguez Mellado [a, *], Mercedes Ruiz Montoya [b], Alberto Palma [b], Rafael Rodríguez-Amaro [a], Manuel Mayén [c]

[a] Departamento de Química Física y Termodinámica Aplicada, Facultad de Ciencias, CeiA3, Campus Universitario Rabanales, edificio Marie Curie, Universidad de Córdoba, 14014 Córdoba, Spain
[b] Departamento de Ingeniería Química, Química Física y Química Orgánica, Facultad de Ciencias Experimentales, Campus de "El Carmen", Universidad de Huelva, 21071 Huelva, Spain
[c] Departamento de Química Agrícola y Edafología, CeiA3, Campus Universitario Rabanales, edificio Marie Curie, Universidad de Córdoba, 14014 Córdoba, Spain

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ABSTRACT

UV–visible spectra of 2,4- and 2,5-dihydroxy-benzaldehydes (2,4DHB and 2,5DHB) are recorded in a wide range of pH. The dissociation p*K* values obtained from these measurements were 6.94 ± 0.03 and 9.28 ± 0.03 for 2,4DHB and 8.42 ± 0.03 and 10.93 ± 0.03 for 2,5DHB. The results indicate that the pH at which the assays for antioxidant capacity measurements are made is very important in light of the hydroxyl group dissociation, because of the different dissociation constants of the different isomers. The percentage of dissociation of each group is essential, the positions of these groups in the ring appearing as a secondary factor.

1. Introduction

2,5-Dihydroxybenzaldehyde (2,5DHB) is found in olive processing effluents [1] and in *Beta vulgaris* seeds [2]. 2,4-dihy-droxy-benzaldehyde (2,4DHB) is found in the heartwood of *Toxicodendron vernicifluum* [3], in red wines aged in acacia barrels [4] or in the aerial parts of *Cassia grandis* [5]. Both compounds showed bactericidal activities against *Campylobacter jejuni*, *Escherichia coli*, *Listeria mono-cytogenes*, and *Salmonella enteric* [6]. They can also act as sensitizers of antifungal agents [7,8] and as antioxidants [3,9–11].

The antioxidant activity is typically measured far to the physiological conditions. For example, in the DPPH method (scavenging of α,α-diphenyl-β-picrylhydrazyl free radicals) the medium is methanol, and electrochemical measurements using the H_2O_2 oxidation on mercury electrodes are made in the pH range of 10–10.5 due to restrictions of the electrode [9–11].

The dissociation state of phenolic benzaldehydes and gallic acid derivatives [12] is crucial to understand their chemical and bacteriological activities that take place under physiological conditions. However, only one reference in the literature deals with the value of the higher dissociation constant of gentisaldehyde [13]. Only the main maximum wavelengths of the UV spectra in methanol have been reported for both derivatives [14].

The aim of this paper was, first, to obtain the dissociation constants of the 2,4DHB and 2,5DHB to show the relevance of the dissociation of the –OH groups in the interpretation of the antioxidant activities. To reach this

* Corresponding author.
E-mail address: jmrodriguez@uco.es (J.M. Rodríguez Mellado).

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goal, the UV spectra of the aldehydes must be investigated in order to obtain their dissociation constants and antioxidant activities are measured at two pH values.

2. Experimental

2,5DHB and 2,4DHB were from Sigma and the rest were from Merck (both analytical quality). Supporting electrolytes used for the determination of the antioxidant activity were solutions of 0.1 M in both sodium bicarbonate, at pH = 10.5, and 0.1 M phosphoric acid at pH = 12.5. The ionic strength was adjusted to 0.5 M with solid KNO_3 and the *pH* was adjusted with solid NaOH. Antioxidants were dissolved in ethanol and the stock solution concentrations were 5×10^{-3} M. These solutions were stored in darkness at 277 K to avoid decomposition.

UV–vis absorption spectra (190–800 nm) were recorded with a double beam Perkin–Elmer Lambda 750S spectrophotometer with Hanna quartz cuvettes of 1 cm pathlength. Solutions of 0.1 M in both phosphoric and acetic acids for pH < 8.5 and 0.1 M in both sodium bicarbonate and phosphoric acid for pH > 8.5 were used. pH was measured using a Metrohm pH-meter. Alkali error was corrected with the correction curves supplied by the manufacturer. Ultrapure water was obtained with a Millipore-Milli Q system.

A CHI650A electrochemical workstation from IJCambria coupled to an EF-1400 controlled growth mercury electrode from BAS instruments was used in the HMDE mode. The Hg drop area was 6.70×10^{-3} cm^2. The temperature was kept at 298 ± 0.1 K. All potentials were measured against an $Ag|AgCl|KCl_{sat}$ electrode (BAS MF-2052). A platinum counter electrode BAS MW-1034 was used. Differential pulse voltammetry (DPV) experiments were made with a pulse amplitude of 0.05 V, a pulse width of 0.05 s and a pulse period of 0.2 s. The antioxidant activity was determined as reported [10,11].

To ensure the reproducibility of the measurements, the experiments were made repeatedly, and the standard deviations of the data were less than 5%.

3. Results and discussion

The antioxidant activity was obtained from the decrease of the DPV signal corresponding to the H_2O_2 oxidation on a mercury electrode in the presence of antioxidants [10,11]. This assesses the interaction with the radicals produced during the oxidation of H_2O_2, being a measurement of the scavenging ability. The measurement pH must be 10 or higher, because at lower pH values the oxidation of the mercury electrode prevents the appearance of the H_2O_2 signal. The volume that decreases the area of the H_2O_2 oxidation peak in 10% is related to the scavenging activity. Its inverse was selected to express the antioxidant activity because the higher the values of this parameter the higher the scavenging activity [11].

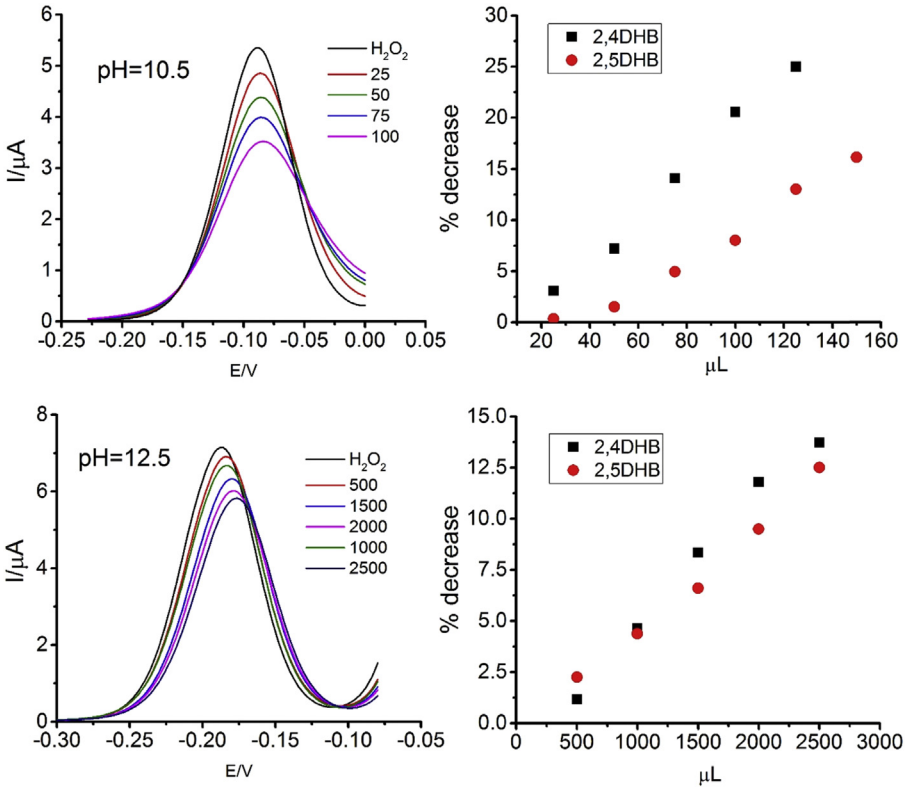

Fig. 1. Left: DPV of 5×10^{-4} M H_2O_2. Numbers correspond to microliters of 5×10^{-3} M 2,4DHB in 10 mL of final volume. Right: Decrease of the DPV peak area with the added amounts of 5×10^{-3} M antioxidant solution at pH: (up) 10.5, (down) 12.5.