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The study of the oxidation of the natural flavonol fisetin confirmed quercetin oxidation mechanism

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ABSTRACT

Oxidation of the bioactive flavonoid fisetin was studied under inert atmosphere and under ambient conditions. The presence of fast subsequent chemical reactions following the electron transfer was supported by *in situ* spectroelectrochemistry and identification of products by HPLC-DAD and HPLC–ESI-MS/MS. In the absence of oxygen, 2,6-dihydroxy-2-(3′,4′-dihydroxybenzoyl)-benzofuran-3(2*H*)-one was identified as the only oxidation product of fisetin. This product was found also as the main oxidation product in the presence of oxygen. The oxidation pathway leading to formation of a benzofuranone derivative can be considered as common for flavonols containing C2-C3 double bond, C3-OH group and dihydroxy-substituted phenyl moiety in its structure. This product was not stable and decomposed further even in contact with oxygen coming from eluents during chromatography. Two oxidation pathways occur under ambient conditions. DFT calculations support the result.

1. Introduction

Fisetin **1** (2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one) is a bioactive flavonoid present in plants, seeds, fruit and vegetables [1–3]. As other flavonoid compounds, fisetin is important for its antioxidative, anti-carcinogenic, anti-inflammatory and antiviral properties [4–6]. The oxidation mechanism of the flavonol structure was extensively studied in literature for quercetin **2**, which contain one additional hydroxyl group C5-OH [7] Two-electron and two-proton oxidation of the ring B in **2** leads to the formation of an *o*-quinone derivative in acidic and neutral solutions [8,9] A two-electron and two-proton oxidation mechanism of fisetin was reported by Markovic et al. [10], who suggested the participation of C3-OH hydroxyl group in intramolecular rearrangement of resulting *o*-quinone. Hydroxylation or dimerization take place as following chemical reactions [7,11,12]. Jorgensen et al. [13] were the first who isolated and identified 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one as one of the oxidation products of **2**. In our previous works this compound was found as the oxidation product of **2** under inert atmosphere in aqueous and non-aqueous solution [7,14] and its presence was confirmed by complementary techniques [15]. Compound 2,4-dihydroxy-2-(4′-hydroxy-3′-methoxybenzoyl)-6-methoxybenzofuran-3(2*H*)-one was identified as the only oxidation product of the methoxylated flavonol rhamnazin under inert atmosphere [16].

The aim of this work is the determination of the oxidative degradation processes of fisetin **1** in aqueous solutions to confirm the general oxidation mechanism of flavonols. We found that the stability of **1** towards atmospheric oxygen is even lower than in the case of quercetin **2**; strictly inert conditions were required when working with its solutions, particularly in alkaline conditions [17].

2. Experimental

2.1. Reagents

Fisetin was purchased from Sigma Aldrich. The reagents used as supporting electrolytes such as potassium chloride and chemicals for preparation of Britton-Robinson buffers (0.04 mol L^{-1} stock solutions of H_3PO_4, CH_3COOH, H_3BO_3 and 0.2 mol L^{-1} NaOH) were of reagent grade p.a. The Britton-Robinson buffers (pH 4.4–11.2) were prepared at constant ionic strength. The solutions were prepared with ultrapure water (Millipore). Methanol and acetonitrile were HPLC grade (Carlo Erba, Milan, Italy). Standard solution of fisetin for HPLC was prepared in methanol. All reagents and chemicals were used without any further purification.

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2.2. Methods

Electrochemical measurements were done using an electrochemical system for cyclic voltammetry. It consisted of a fast rise-time potentiostat interfaced to a personal computer via an IEEE-interface card (AdvanTech, model PCL-848) and a data acquisition card (PCL–818) using 12-bit precision. Cyclic voltammetry was also conducted using a PGSTAT 12 AUTOLAB potentiostat (Ecochemie, Netherlands). An one compartment three-electrode electrochemical cell was used with an Ag|AgCl|1 mol L^{-1} LiCl reference electrode separated from the test solution by a salt bridge. The volume of measured solution was 5 mL. The working electrodes were glassy carbon electrode (0.7 mm) and platinum electrode (0.8 mm). The auxiliary electrode was cylindrical platinum net. Oxygen was removed from the solution by passing a stream of argon. Cyclic voltammetry was measured in solutions of fisetin in the range of concentrations 0.5×10^{-4}–5×10^{-4} mol L^{-1}. The oxidation products of fisetin were prepared by exhaustive electrolysis of its 2.4×10^{-4}–6.2×10^{-4} mol L^{-1} solutions on carbon paste electrode using a PGSTAT 12 AUTOLAB potentiostat (Ecochemie, Netherlands).

2.3. Spectrophotometry and UV–vis spectroelectrochemistry

Spectroelectrochemistry was performed using an optically transparent thin-layer electrode (OTTLE) cell [18] with a three electrode system (platinum working and auxiliary electrode, silver quasi reference electrode) mounted in a thin layer (thickness 0.18 mm) between optical windows. Sufficiently optically transparent platinum gauze (80 mesh) of the size 5×5 mm served as the working electrode. The response of the cell allows completing electrolysis within time of several tens of seconds (20 s when tested with ferrocene in acetonitrile). The potential scan rate was 5 mV s^{-1}. Spectral changes in the course of the electrolysis were registered using Agilent 8453 diode-array UV–vis spectrometer. The 1.0 cm quartz cuvettes were used for recording the absorption spectra during the bulk electrolysis and when testing the stability of compound when exposed to the air oxygen. The procedure of measurement of fisetin stability in solution was as follow: the first absorption spectrum of solution prepared under inert atmosphere was recorded in a closed cuvette, then, absorption spectra were measured after opening the cuvette and manually shaking of solution in the presence of air.

2.4. High-pressure Liquid Chromatography with photodiode array detector (HPLC-DAD)

An high-pressure liquid chromatography Agilent 1200 Series HPLC Systems equipped with diode array detector (Agilent Technologies) was used. The chromatographic separation was performed on analytical reverse phase C-8 column (HyPurity C8, 150×3 mm, 5 μm, Thermo Scientific, Dubuque, USA) connected to C-18 pre-column (HyPurity C18, 10×3 mm, 5 μm, Thermo Scientific, Dubuque, USA). Column temperature: 20 °C. The gradient elution program used eluents (A): aqueous solution of 0.1% H_3PO_4 and (B): acetonitrile. The gradient was: 0–2 min, 95% A; 2–30 min, linear gradient to 40% A; 30–35 min, linear gradient to 0% A and 100% B; 35–40 min, 100% B. The flow rate was 0.2 mL min^{-1}, the injection volume was 40 μl. Diode array detector acquisition parameters were: acquisition range 190–800 nm, 2 nm step.

2.5. High-pressure liquid chromatography with electro-spray ionisation tandem mass spectrometer (HPLC-ESI-MS/MS)

HPLC-ESI-MS/MS was carried out using a 1200 Infinity HPLC (Agilent Technologies, USA), coupled to a Jet Stream ESI interface (Agilent Technologies) with a Quadrupole-Time of Flight tandem mass spectrometer 6530 Infinity Q-TOF (Agilent Technologies). The chromatographic separation took place at 30 °C and was performed on an analytical reverse phase C-18 column (C18-extended 1.8 μm, 50×2.1 mm, Agilent Technologies, USA)

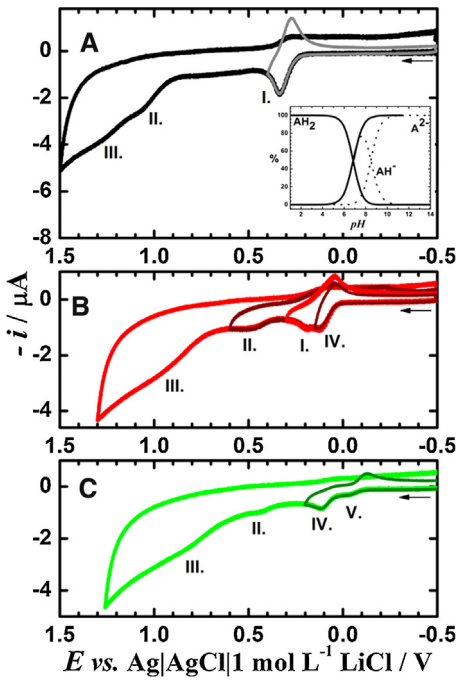

Fig. 1. Cyclic voltammogram of 2×10^{-4} mol L^{-1} **1** in Britton-Robinson buffer and 40% ethanol (*v*/*v*) on glassy carbon electrode at different pH: (A) 5.3, (B) 8.2, (C) 9.4. Scan rate 0.25 V s^{-1}. Inset of Panel 1A: distribution diagram of **1** calculated from $pK_1 = 6.83$ for **1** [19] (solid line) and $pK_2 = 8.48$ for **2** [17] (dotted line).

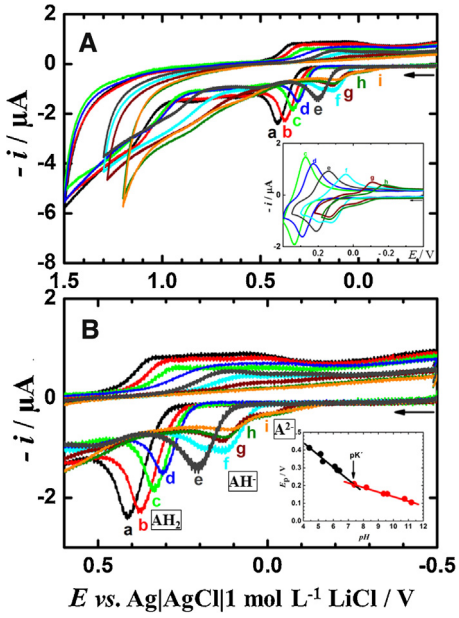

Fig. 2. Cyclic voltammogram of 2×10^{-4} mol L^{-1} **1** in Britton-Robinson buffer and 40% ethanol (*v*/*v*) on glassy carbon electrode at different pH values: (A) (a) 4.4, (b) 4.9, (c) 5.2, (d) 6.2, (e) 7.4, (f) 8.2, (g) 9.4, (h) 10.8, (i) 11.2. Scan rate 0.25 V s^{-1}. Panel B shows CVs in zoomed scale. Inset of Panel A: CVs registered by reversing the scan polarity upon reaching the oxidation wave I. (c)–(e). Inset of Panel B: anodic peak potential dependence on pH.