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Oxidation mechanism of flavanone taxifolin. Electrochemical and spectroelectrochemical investigation

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ABSTRACT

The oxidation of taxifolin on glassy carbon electrode in acetonitrile was studied by cyclic voltammetry, UV–vis and IR spectroelectrochemistry. The oxidation products were identified using HPLC-ESI-MS/MS. The two-electron oxidation mechanism differs from that of flavonols (e.g. quercetin) due to the absence of the double bond between atoms C-2 and C-3. As confirmed by IR spectroelectrochemistry, quinone at ring B is formed as low stable intermediate. The oxidation pathway leads to the formation of hydroxylated derivative of taxifolin 2′,3,3′,4′,5,7-hexahydroxyflavone accompanied by the 2,3-desaturation.

1. Introduction

Flavonoids are naturally occurring compounds found in vascular plants, seeds, fruit, vegetables, red wine, tea. Their anti-inflammatory, anti-carcinogenic properties [1,2,3], anti-atherosclerotic influence and ability to reduce cardiovascular diseases [4,5] have been proved. Flavonoids are also known to inhibit the activity of enzymes [6] and to control the activity of hormones [7]. Many of flavonoids have antibacterial, antiviral and antifungal activity [8].

Flavonoids belong to natural phenolic antioxidants. Their antioxidant activities are related to their chemical structure, mainly with *o*-dihydroxy structure of the B-ring, double bond between carbon atoms 2 and 3 in conjugation with 4-oxo function in the C-ring and 3,5-OH groups [9,10,11,12]. These functional groups are effective for free radical scavenging activity that implies antioxidant abilities.

The flavonoid taxifolin (**1**, 3,5,7,3′,4′-pentahydroxyflavanone, Fig. 1), which occurs in citrus fruits (e.g. grape fruit, orange) belongs to the family of flavanones [9,13]. Although the antioxidant activity of taxifolin is half of that of quercetin (**2**, 3,3′,4′,5,7-pentahydroxyflavone, Fig. 1) [9] due to the lack of double bond between atoms C2 and C3, it still exhibits beneficial properties. Taxifolin has strong antioxidant activity due to the substitution with two OH groups at the position 3′ and 4′ of the B-ring [14]. Taxifolin also produces anti-inflammatory effects by acting as antioxidant and was used as a drug against leukocyte activation [15]. The protective effect of taxifolin against cerebral ischemic reperfusion injury was also shown in the literature [16].

On the one hand, the majority of flavonoid studies have been performed in aqueous solution in order to evaluate their biological activities. Since electron transfer processes are involved in the most common pathway of drug biotransformation, electrochemical methods were often employed as a part of biomimetic modelling of oxidative drug metabolism [17]. On the other hand, biological redox reactions occurring in the lipid double layer of biological membranes could be simulated in non-aqueous solvents [18]. Several studies have investigated the solvation of biomolecules in non-aqueous solvents or co-solvents and its role with respect to their biological activity (e.g. for enzymes [19,20], proteins [21]). The electrochemical oxidation of taxifolin was investigated by Janeiro et al. [22]. The authors found that the first oxidation peak in aqueous buffered solutions is quasireversible, involves one-electron transfer and its oxidation potential is dependent on pH. Le Nest et al. [23] recorded an EPR spectrum of *o*-semiquinone taxifolin radical formed electrochemically only in the presence of Zn(II) acetate. The radical detection in the absence of the Zn(II) acetate was not succesful. The authors suggested the participation of a comproportionation reaction after the electron transfer. The oxidation mechanism of flavonols, which

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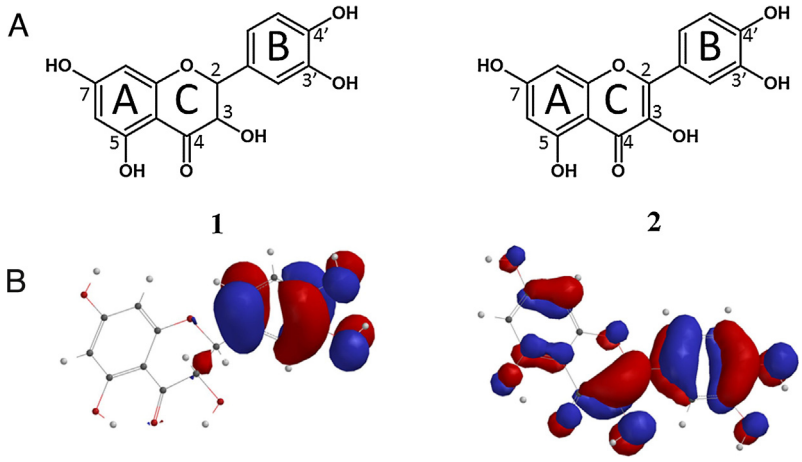

Fig. 1. (A) Chemical structure of taxifolin **1** and quercetin **2**. (B) DFT calculated HOMO distribution of **1** and **2**.

differ from flavanones by the double bond in the ring C (see quercetin **2** in Fig. 1), occurs as following complicated mechanism. Oxidation of quercetin was explained as two-electron process coupled with two-proton transfer and fast hydroxylation caused by traces of water [24]. The oxidation product was identified as a benzofuranone derivative, which is formed after the opening of ring C. Two-electron oxidation with participation of two protons was proved also for quercetin in acidic and neutral solutions, in contrast to one-electron reversible oxidation of its dianion to the anion radical occurring in alkaline solution [25–27].

This paper describes the oxidation mechanism of taxifolin in non-aqueous solution, which has not been elucidated yet. The redox properties of taxifolin are affected by its specific structure, which does not allow the electron delocalization through the whole structure, as can be seen in Fig. 1A.

2. Experimental

2.1. Reagents

The flavonoid taxifolin (3,5,7,3′,4′-pentahydroxyflavanone) was purchased from Fluka. The supporting electrolyte tetrabutylammonium hexafluorophosphate ($TBAPF_6$) was obtained from Sigma–Aldrich and was dried before use. Stock solutions of analyte was prepared in degased anhydrous 99.8% acetonitrile (AN, anhydrous, Sigma–Aldrich, content of water <0.001%) supplied under argon. All reagents and chemicals were used as received without any further purification. Trideuteroacetonitrile (CD_3CN, 99,8 atom % D, anhydrous) for IR spectroelectrochemistry was purchased from Sigma–Aldrich. Trifluoromethanesulfonic acid (triflic acid) was obtained from Sigma–Aldrich.

Eluents for HPLC-DAD analyses were acetonitrile (ChromaSolv for HPLC, 99.8%) from Sigma–Aldrich (Milan, Italy) and bi-distilled water (RPE) from Carlo Erba (Italy). Eluents for HPLC-MS analyses were acetonitrile (LC–MS grade, Sigma–Aldrich, US) and LC–MS grade water (Sigma–Aldrich, US). Formic acid (98% purity, J.T. Baker) was used as mobile phase modifier for both systems.

2.2. Methods

2.2.1. Electrochemical setup

Electrochemical measurements were performed in a three-electrode electrochemical cell. The reference electrode, Ag|AgCl| aqueous 1 $mol \cdot L^{-1}$ LiCl, was separated from the test solution by a salt bridge with double fritted junctions. The working electrode was the glassy carbon electrode with an area of $5.8 \cdot 10^{-3}$ cm^2. The auxiliary electrode was a platinum net. Oxygen was removed from the solution by passing a stream of argon pre-saturated with acetonitrile vapors. Electrochemical measurements were done in 0.1 $mol \cdot L^{-1}$ $TBAPF_6$ in acetonitrile using a home-built system for cyclic voltammetry. It consisted of a fast rise-time potentiostat, which was 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 for A/D and D/A conversions. After each measurement Ferrocene (Fc/Fc^+) was added as an internal standard to check the stability of reference electrode after each measurement.

The exhaustive electrolysis and cyclic voltammetry before and after electrolysis were performed using a PGSTAT 12 AUTOLAB potentiostat (Ecochemie, Netherlands) and the carbon paste electrode as the working electrode.

2.2.2. UV–vis spectroscopy and IR spectroelectrochemistry

Absorption spectra of taxifolin were recorded by Agilent 8453 diode-array UV–visible spectrometer with 10.0 mm quartz cuvettes. $3 \cdot 10^{-5}$ $mol \cdot L^{-1}$ taxifolin solution in acetonitrile was prepared under inert atmosphere of argon and its absorption spectrum was measured in the de-aerated cell. After five hours and two days, the spectrum of each solution after exposure to atmospheric oxygen was recorded, in order to account for possible spectral changes.

Absorption spectra of $1.6 \cdot 10^{-5}$ $mol \cdot L^{-1}$ taxifolin in 0.05 $mol \cdot L^{-1}$ $TBAPF_6$ in acetonitrile were registered before and after the exhaustive electrolysis at 1.5 V.

IR spectroelectrochemistry of taxifolin oxidation in trideuteroacetonitrile (CD_3CN) with $TBAPF_6$ as supporting electrolyte was measured with Nicolet iS50 FT-IR spectrometer. The optically transparent thin-layer electrode (OTTLE) cell [28] 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 was used. Sufficiently optically transparent platinum gauze (80 mesh) of the size 25 mm^2 served as the working electrode. The IR spectra were measured in the OTTLE cell with CaF_2 windows in the range from 4000 cm^{-1} to 1100 cm^{-1}.

2.2.3. HPLC-DAD

The products of bulk electrolysis of taxifolin in 0.05 $mol \cdot L^{-1}$ solution of $TBAPF_6$ in acetonitrile were identified by HPLC-DAD and HPLC-ESI-MS/MS. HPLC-DAD analyses were performed using an HPLC PU-2089 Quaternary Gradient Pump with degasser (Jasco International Co., Tokyo, Japan), equipped with Intelligent sampler AS-950 and coupled to a spectrophotometric diode array detector