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Radiation-chemical transformations of coumarins in ethanolic solutions

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HIGHLIGHTS

- Coumarin, 4-hydroxycoumarin and warfarin oxidize α-hydroxyethyl radicals.
- Esculetin and fraxetin predominantly reduce α-hydroxyethyl radicals.
- Coumarins add α-hydroxyethyl radicals to the C=C bonds of the pyrone ring.
- Coumarins display antioxidant activity on radiation-induced oxidation of ethanol.

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ABSTRACT

Effects of coumarin and its derivatives on product formation during radiolysis of deaerated and oxygenated ethanol were investigated. The data obtained in this study indicate that coumarin, 4-hydroxycoumarin and warfarin effectively oxidized α-hydroxyethyl radicals (α-HER), while esculetin and fraxetin predominantly reduced the above named intermediates. Coumarin, esculetin and fraxetin were able to add α-HER to the double carbon–carbon bond of the pyrone ring to form stable products with molecular masses exceeding those of the starting molecules. Coumarin, warfarin, esculetin and fraxetin were shown to display antioxidant activity during radiation-induced oxidation of ethanol.

1. Introduction

Coumarins are naturally occurring compounds of plant origin having the bicyclic benzopyrone moiety as a common structural element (Murray and Mendez, 1982; Keating and O'Kennedy, 1997). These compounds are components of the raw materials traditionally used as natural flavoring additives in food and fragrance industry (Egan et al., 1990).

Polyfunctionality and structural variety of coumarins makes these compounds interesting objects of investigations focused on pharmacological activity associated, as a rule, with moderate toxicity (Hoult and Paya, 1996; Keating and O'Kennedy, 1997; Halliwell and Gutteridge, 2007). To-date, coumarins are known to possess antiviral (Yu et al., 2003), anti-inflammatory (Hoult et al., 1994), immuno-modulating (Berkarda et al., 1983; Egan et al., 1990), antimicrobial (Smyth et al., 2009), anti-tumor (Cullen et al., 2003; Lacy and O'Kennedy, 2004), hepatoprotector (Atmaca et al., 2011; Chen et al., 2013), antiallergic (Matsuda et al., 2002; Choi and Yan, 2009) and neuroprotector (Molina-Jimenez et al., 2004) properties. Coumarins are commonly used in medicine for photochemotherapy of a number of diseases (O'Connor et al., 2009) and as indirect anticoagulants (Thornes et al., 1968; Thumber et al., 2011).

Issues concerning the use of coumarins as antioxidants and radioprotectors are widely discussed in the literature (Grotz et al., 1999; Fylaktakidou et al., 2004), because these compounds are highly reactive towards ROS and can inhibit free-radical oxidation reactions (Hoult and Paya, 1996; Martin-Aragon et al., 1998), thereby protecting biological systems from the oxidative stress. The presence of antioxidant activity in coumarins has been demonstrated in various models in vitro and in animal experiments (Martin-Aragon et al., 1997; Martin-Aragon et al., 1998; Fernandez-Puntero et al., 2001).

It has been established that the radiation damage caused to biologically relevant substances is due to not only oxidation

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reactions being induced in the latter, but also to free-radical fragmentations accompanying these processes (Edimecheva et al., 1997; Shadyro, 1997). The key stage of the fragmentation reactions occurring in hydroxyl-containing organic compounds is decomposition of α-hydroxyl-containing carbon-centered radicals (α-HCR) of the starting compounds. Such reactions take place during homolytic transformations of carbohydrates (Edimecheva et al., 2005), lipids (Shadyro et al., 2002, 2004), amino acids (Shadyro et al., 2003a; Sladkova et al., 2012), peptides (Shadyro et al., 2000, 2003a) and nucleic acids (Petryaev et al., 1988), and cause not only destruction and modification of the starting material but can also result in formation of signaling molecules which regulate cell apoptosis in a living organism (Jayadev et al., 1995; France-Lanord et al., 1997), and a number of other processes (Burdon, 1995; Gelderblom et al., 2001).

Very limited information is available in the literature on the effects of coumarin and its derivatives on free-radical processes involving carbon-centered radicals. In our opinion, studies in this area are necessary for better understanding molecular mechanisms responsible for pharmacological activity manifested by coumarins in treatment of diseases caused by activation of free-radical processes in a living organism.

Information of such kind can be obtained by studying effects of coumarins on the yields of final products being formed during radiolysis of ethanol, since the major intermediates in these processes are α-hydroxyethyl radicals (α-HER), the simplest representatives of α-HCR. α-HER are known to be formed in the liver during biochemical transformations of ethanol and to take part in reactions causing various kinds of damage to intracellular components (Sakurai et al., 2000; Albano, 2006). Therefore, studying reactions of coumarins with α-HER may contribute to elucidation of molecular mechanisms ensuring formation of hepatoprotector properties of these compounds of plant origin.

In this study, effects of coumarin and a number of its derivatives on radiation-chemical transformations of ethanol, deaerated with argon or saturated with oxygen, were investigated using the steady-state radiolysis and mass spectrometry methods.

2. Experimental section

2.1. Chemicals

The following chemicals were used in this study: 2 H-chromen-2-one (coumarin) (**1**), 4-hydroxy-2H-chromen-2-one (4-hydroxycoumarin) (**2**), 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-chromen-2-one (warfarin) (**3**), 4-hydroxy-6-methyl-2H-pyran (**4**), 6,7-dihydroxy-2H-chromen-2-one (esculetin) (**5**), 7,8-dihydroxy-6-methoxy-2H-chromen-2-one (fraxetin) (**6**), acetaldehyde (AA) and (+/−)-meso-2,3-butanediol (2,3-BD) – all from Sigma-Aldrich (Fig. 1). Purity of the chemicals used in this study was at least 97%. Ethanol rectificate (96% v/v) was purified by sorption on Wolfen Zeosorb LA ceolite followed by fractional distillation.

2.2. Preparation of samples for irradiation

Appropriate amounts of the compounds under study, accurately weighed, were placed in densimeters of 10 ml volume, which were then filled with the solvent and stirred on an autoshaker until complete dissolution was achieved. In certain cases, sonication for 2 min on a Bandelin SONOREX RK-52 unit was necessary. The coumarin concentrations in the solutions were 10^{-3} mol/l.

The solutions were irradiated in glass ampoules filled to 70% of volume.

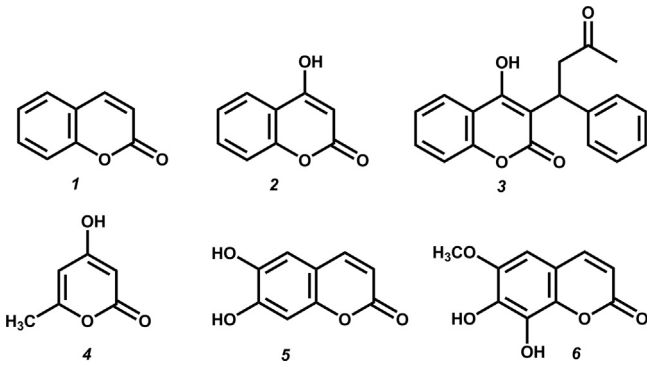

Fig. 1. Structures of the compounds under study.

2.2.1. Deaerated solutions

To remove oxygen, the solutions under study were bubbled through high purity argon for 60 min. Then the volume was made up with deaerated solvent, the solution was stirred and filled into ampoules preliminarily blown through with argon, and the ampoules were sealed.

2.2.2. Solutions saturated with N_2O

Nitrous oxide was used in this study for accepting solvated electrons being formed during γ-irradiation of ethanol. The densimeters containing ethanolic solutions of the test compounds were deaerated with argon for 40 min, then saturated with nitrous oxide for 30 min, and thereafter these solutions were filled into ampoules and the ampoules were sealed.

2.2.3. Solutions saturated with oxygen

The procedure used to obtain oxygenated ethanolic solutions was analogous to that described for deaerated ones, except for saturation of solutions with oxygen for 40 min.

2.3. Irradiation of solutions

The samples prepared as described above were irradiated on a MPX-γ-25M unit equipped with a ^{60}Co source. The absorbed dose rate was 0.32 ± 0.01 Gy s^{-1}. The absorbed dose range was $96 \div 480$ Gy for the samples saturated with oxygen, and $0.19 \div 1.15$ kGy for deaerated and N_2O-saturated samples. A Fricke dosimeter ($G(Fe^{3+}) = 15.6 \times 10^{-7}$ mol J^{-1}) was used to measure the absorbed dose rate (Fricke and Hart, 1966).

2.4. Determination of concentrations of compounds and identification of radiolysis products

GC-FID measurements: AA and 2,3-BD were determined using gas–liquid chromatography on a Shimadzu GC-17A instrument equipped with a flame ionization detector and a capillary column Stabilwax-DA (length 30 m; ID 0.53 mm; stationary phase film thickness 1.0 μm). Chromatographic conditions: carrier gas nitrogen; thermostat program: 40–200 °C heating rate 13 °C/min; both injector and detector temperatures 220 °C; flow rate 2.8 ml/min, linear flow rate 20 cm/s.

GC–MS measurements: Molecular products of interaction of α-HER with coumarin (**1**) were identified using a gas-liquid chromatograph Shimadzu GC–MS-QP2010 Plus equipped with an Equity-5 column (length 30 m, ID 0.25 mm, stationary phase film thickness 0.25 μm) and a mass-spectrometric detector. Chromatographic conditions: carrier gas helium, linear flow rate 1 ml/min; thermostat temperature rising from 60 °C to 270 °C at a rate of 5 °C/min, injector temperature 270 °C, ion source temperature