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Isoprenylhydroquinone glucoside: a new non-antioxidant inhibitor of peroxynitrite-mediated tyrosine nitration

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Abstract

Three hydroquinone glucosides and four caffeoylquinic esters were examined for their effect on tyrosine nitration, as well as on the oxidation of dihydrorhodamine (DHR) 123 and cytochrome c^{2+} induced by peroxynitrite. All these phenolics, which had previously been characterized as the active principles of the plant *Phagnalon rupestre*, were fairly active in preventing the oxidation of DHR 123, though inefficient in the cytochrome *c* test. While their antioxidant potency is associated with the presence of a caffeoyl moiety, not so an obvious chemical character was correlated to a greater activity against nitration of tyrosine. Here, the highest potency corresponded to 2-isoprenylhydroquinone-1-glucoside. On the basis of the fact that the susceptibility to nitration of given aromatic compound confers to it inhibitory activity of tyrosine nitration, the analysis of ultraviolet and nuclear magnetic resonance spectral shifts provides valuable information for explaining the ability of natural phenolics to interfere with that reaction.

Keywords: Antioxidant; Cinnamates; Cytochrome *c*; Hydroquinone glucosides; Peroxynitrite; Tyrosine nitration

Although many studies have focused on the antioxidant and free radical scavenger activities of natural phenolic compounds [1–3], little is known about their capacity as scavengers of peroxynitrite anion ($ONOO^-$), a cytotoxic species generated from nitrogen monoxide (NO) under oxidative stress. $ONOO^-$, which is capable of oxidizing and nitrating a wide array of biomolecules such as proteins, nucleic acids, and lipids [4,5], along with its activated intermediate $ONOOH^*$ [4] and several of its decomposition products (e.g., hydroxyl and nitrogen dioxide radicals) [6] are recognized as important mediators of the cellular damage and have been implicated in several chronic inflammatory diseases, including rheumatoid arthritis or systemic sclerosis [7]. While there is little information about the effects of $ONOO^-$ in the skin, some authors have examined the mechanisms of generation and loss of cutaneous nitrated proteins in rats treated with $ONOO^-$ [8], as well as the possible roles of this anion in skin diseases, especially in relationship to the induction of poly(ADP–ribose) polymerase [9].

Some catechin polyphenols [10,11] and hydroxycinnamate derivatives [12–15] have been reported as being active against peroxynitrite-induced nitration and oxidation. To extend this research to other kind of natural phenolics, we have experimented with three prenylhydroquinones (compounds **1**–**3**) and four di-*O*-caffeoylquinic acids (compounds **4**–**7**), which we had previously isolated [16,17] from *Phagnalon rupestre* (Asteraceae), a Mediterranean shrub traditionally held to have antimicrobial and skin clearing activities. These two groups of principles differ, not only in their polarity and aromatic hydroxyl substitution pattern, but also in their pharmacological

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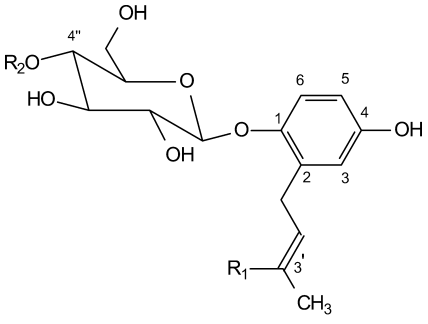
activity, since prenylhydroquinones showed anti-allergic activity in contact hypersensitivity models induced by dinitrofluorobenzene and sheep red blood cells. Moreover, the test compounds were effective both as oxygen radical scavengers and as inhibitors of lipid peroxidation and some pro-inflammatory leukocyte functions [18]. To explore the interaction with peroxynitrite–mediated reactions, the seven above-mentioned principles have been evaluated for their effects on the nitration of tyrosine as well as on the oxidation of dihydrorhodamine (DHR) 123 and cytochrome c^{2+}.

Materials and methods

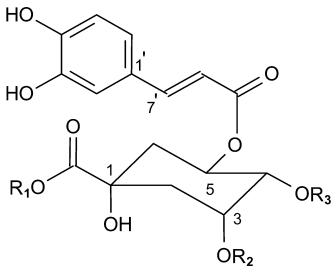
Chemicals

Hydrogen peroxide 3%, sodium nitrite, sodium hydroxide, manganese dioxide, tyrosine, 3-nitrotyrosine, potassium hydrogen phosphate, potassium dihydrogen phosphate, phosphoric acid, dihydrorhodamine (DHR) 123, rhodamine 123, dimethylformamide, diethylenetriaminepentaacetic acid (DTPA), cytochrome *c*, sodium dithionite, epigallocatechin gallate, and pyrogallol were purchased from Sigma Chemical (St. Louis, MO, USA). Hydrochloric acid 36–38% and methanol for HPLC were obtained from Baker (Deventer, The Netherlands), and deuterated methanol (CD_3OD) from Merck (Darmstadt, Germany). Sephadex G-25 columns were purchased from Amersham Biosciences (Uppsala Sweden). Natural test compounds, isolated from *Phagnalon*, were: 1-*O*-β-glucopyranosyl-2-(3′,3′-dimethylallyl) hydroquinone (**1**), 1-*O*-β-glucopyranosyl-2-(3′-hydroxymethyl-3′-methylallyl) hydroquinone (**2**), 1-*O*-(4″-*O*-caffeoyl)-β-glucopyranosyl-2-(3′,3′-dimethylallyl) hydroquinone (**3**) (Fig. 1),



	R_1	R_2
1	CH_3	H
2	CH_2OH	H
3	CH_3	caffeoyl

Fig. 1. Structure of hydroquinone glucosides.



	R_1	R_2	R_3
4	CH_3	caffeoyl	H
5	CH_3	H	caffeoyl
6	H	caffeoyl	H
7	H	H	caffeoyl

Fig. 2. Structure of caffeoylquinic esters.

3,5-di-*O*-caffeoylquinic acid methyl ester (**4**), 4,5-di-*O*-caffeoylquinic acid methyl ester (**5**), 3,5-di-*O*-caffeoylquinic acid (**6**), and 4,5-di-*O*-caffeoylquinic acid (**7**) (Fig. 2).

Peroxynitrite synthesis

Peroxynitrite was synthesized in a quenched flow reactor following the method described by Koppenol et al. [19]. Acidified H_2O_2 (0.6 M in 0.7 M HCl, 20 ml) was mixed with $NaNO_2$ (0.6 M, 20 ml) to form peroxynitrous acid (ONOOH), which is stabilized with NaOH (1.5 M, 40 ml) giving sodium peroxynitrite. The excess of H_2O_2 was removed by mixing with MnO_2. The solution was filtered and frozen at −20 °C for less than two weeks The concentration of $ONOO^-$ was determined by measuring the absorbance at 302 nm ($\varepsilon = 1670\ M^{-1}\ cm^{-1}$).

Tyrosine nitration assay

The peroxynitrite-mediated nitration of tyrosine was performed according to Pannala et al. [10], and the detection of 3-nitrotyrosine by HPLC as described by Whiteman and Halliwell [20], using minor modifications in both methods. The reaction mixture consisted in peroxynitrite (2.5 mM), tyrosine (0.5 mM) in 0.5 M phosphate buffer (pH 8) in the absence or presence of increasing concentrations (12.5–100 μM) of the test compounds in a final volume of 1 ml. After vortexing, and incubating for 5 min, the samples were analyzed by HPLC using a Lichrospher C18 column (250 × 4 mm, 5 μM; Merck) with an auto injector L-7200 (Merck–Hitachi). The mobile phase (250 mM KH_2PO_4–H_3PO_4, pH 3.01, with 6% methanol (v/v))