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Insights in the radical scavenging mechanism of syringaldehyde and generation of its anion

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ABSTRACT

The ability of syringaldehyde, a naturally occurring phenolic antioxidant and medicinally important compound, to scavenge free radicals according different mechanisms was elucidated by computing the respective reaction enthalpies at DFT B3LYP/6-311++G** level. Bond dissociation enthalpy, ionization potentials and proton affinities were calculated in gas phase, benzene, water and DMSO in order to account for different environment (nonpolar lipid membranes and polar physiological liquids) where the antioxidant action in the living organism could take place and various experimental *in vitro* conditions. Molecular and electronic properties influencing the reactivity of syringaldehyde according to the different mechanisms were discussed in the light of the reported radical scavenging activities in crocin bleaching, oxidation potential of the first anodic peak and DPPH test. According to the calculated reaction enthalpies, in polar environment the syringaldehyde reacts preferably by sequential proton loss electron transfer which is related to the formation of a phenoxy anion. Such phenoxy anion was generated in DMSO solution and the changes in the force field, steric and electronic structure, resulting from the conversion, were described in detail based on the IR spectral data and DFT computations.

1. Introduction

Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde) is a phenolic aldehyde found in fruits [1–3], nuts [4] and plants that synthesize lignin-related compounds [5–9]. It is released as aroma and taste compound in liquors as a result of the lignin hydrolysis while the alcohol ages in wooden barrels [10,11]. Pyrolysis of lignin during food smoking also produces syringaldehyde together with a number of other syringol derivatives, which contribute not only to the smoke aroma [12–14], but also provide antimicrobial and antioxidant effects in the smoked foods [14–17]. Antimicrobial, antifungal and antiparasite properties of syringaldehyde have been studied in a number of publications [8 and references therein]. The antioxidant capacity of syringaldehyde has been evaluated in relation to the oxidative stability of smoked foods and alcoholic beverages by various *in vitro* assays: bleaching of carotenoid crocin [18,19], scavenging of DPPH and ABTS radicas [7,18,19], determination of the oxidation potential [18], Rancimat [19] and liposomes assays [19].

In the human organism, reactive oxygen species (ROS) and oxidative stress are associated to inflammation, aging, cancer, heart disease and other disorders [20]. By interfering with ROS production or scavenging, the antioxidants may protect against oxidative tissue damage in vital organs. In order to estimate the antioxidant effect of syringaldehyde in living cells, its ability to scavenge superoxide anion in a cell-free hypoxanthine/xanthine-oxidase system and to inhibit the ROS production by human neutrophils, induced by opsonized zymosan- or phorbol 12-myristate 13-acetate was studied [21]. The compound was not able to scavenge the superoxide anion, while significantly inhibited the ROS production by activated human neutrophils [21]. Comparison to the inhibitory effects shown by vanillin, vanillic acid and acetovanillone, lacking a second methyl group in vicinity to the phenolic function, indicated that C-5 methoxylation leads to enhancement of the ROS inhibitory activity. The antioxidant activity of syringaldehyde was further tested in HL-60 cells by 2′,7′-dichlorodihydrofluorescein diacetate method, but in contrast to the above, no activity was detected [22]. The production of prostagladines is another factor that plays a major role in the inflammation processes. It is regulated by the enzyme cyclooxigenase (COX-2) and for this reason, inhibitors of COX-2 are used in the treatment of

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inflammatory disorders and cancer [23,24]. Cell-based assay that utilizes the mouse macrophage cell line (RAW 264.7) shows that syringaldehyde possesses moderate inhibitory activity towards COX-2 [22]. Syringaldehyde is able to inhibited prostaglandin synthetase in a dose-dependent way, exerting half of the potency of aspirin [25]. Recently the neuroprotective effect of syrinaldehyde on cerebral ischemia injury in rats was reported [26]. It was demonstrated that the anti-oxidative and anti-apoptic properties of the compound makes it very effective in the prevention of ischemic damage to the brain [26]. Other medicinally significant property of syringaldehyde is its ability to lower plasma glucose in streptozotocin-induced diabetic rats [9]. All these reports convincingly outline the syringaldehyde as naturally-occurring anti-oxidant and medicinally important compound as well as good lead in the development of more effective anti-inflammatory and anti-diabetic agents. As a result of its suitable molecular structure and characteristics is was already used as building block in new larger antioxidant dendrimer molecules [27] and alkaloids [28]. Such modification has lead to dramatic increase of the anti-oxidant capacity of the new derivatives [27]. Having in mind, these promising features of syringaldehyde and the various, even contradicting, data on its antioxidant activity, it is worthy to throw more light on the possible mechanisms of its antioxidant action, molecular and electronic parameters that might influence its ability to scavenge free radical through to the different mechanisms and interact with enzymes involved in the redox processes in the organisms. The formation of other active forms such as phenoxy anion is essentially related to the concerned mechanisms hence the characterization of the anion species will provide helpful information. Thus the aim of the present study is to determine the preferred mechanism of antioxidant action in nonpolar and polar environment, describe the molecular geometry and electronic structure of the syringaldehyde and convert it into phenoxy in order to follow the spectral, structural, and electronic changes resulting from the conversion. For this purpose we will relay on experimental IR methods and DFT computations.

2. Materials and methods

2.1. Materials

Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde, 99% putiry) was purchased from Sigma–Aldrich Co and applied without further purification. CD_3OD (99% at. Enrichment) was purchased from Merck and used to obtain CD_3ONa by reacting it with Na. Spectral quality $CDCl_3$ and DMSO-d_6 were purchased from Sigma–Aldrich Co.

2.2. Conversion of syringaldehyde into anion and IR spectra measurements

The corresponding anion was obtained by adding 0.12 mol l^{-1} DMSO-d_6 solution of the parent aldehyde to excess of dry CD_3ONa. The reaction mixture was filtered to remove the remains of solid CD_3ONa and put immediately into a spectroscopic cell to record the IR spectra. The conversion was practically complete (no bands of the parent aldehyde were seen in the spectrum after metalation). The IR spectra were recorded on a Brucker Tensor 27 FT spectrometer at a resolution of 2 cm^{-1} and 64 scans. The following sample cells were used: 0.6 mm NaCl for $CDCl_3$ and 0.129 mm CaF_2 for DMSO-d_6 solutions.

2.3. DFT computations

All theoretical calculations were performed using the Gaussian 09 package [28] of programs. Geometry and vibrational frequencies of species studied were performed by analytical gradient technique without any symmetry constraint. All the results were obtained using the density functional theory (DFT), employing the B3LYP (Becke's three-parameter non-local exchange [Becke's three-parameter non-local exchange [29] and and Lee et al. correlation [30] potentials). To establish the stability order for the neutral, radical and ionic species in solvent we used the Integral Equation Formalism Polarizable Continuum Model (IEF-PCM) [31] on the same level of theory.

In order to find the preferred geometry of the molecule of syringaldehyde, its anion, radical and radical cation, a large number of probable conformers were constructed, taking into account planar and non planar geometries with different mutual orientation of the aldehyde function, methoxy and hydroxy groups. The geometries of all constructed conformers were optimized by application of the UB3LYP functional in conjunction with the 6-311++G** basis set. For every structure, the stationary points found on the molecular potential energy hypersurfaces were characterized using standard analytical harmonic vibrational analysis. The absence of imaginary frequencies, as well as of negative eigenvalues of the second-derivative matrix, confirmed that the stationary points correspond to minima on the potential energy hypersurface. It was found that in all stable conformers the aldehyde group adopts planar conformation, while the hydroxy and methoxy groups have different possible planar and nonplanar orientations. The UB3LYP/6-311++G** optimized geometries of the conformers of syringaldehyde molecule (M1-M4), its anion (A1-A4), radical (R1-R4) and radical cation (RC1-RC4) and their relative stability are depicted in Scheme 1.

Dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) of the most stable conformers were calculated according the equations given by Klein et al. [32].

$$BDE = H(A^{\cdot}) + H((H^{\cdot}) - H(A{-}H)$$

$$IP = H\left(A^{+\cdot}\right) + H(e^{-}) - H(A{-}H)$$

$$PDE = H(A^{\cdot}) + H\left(H^{+}\right) - H\left(A^{+\cdot}\right)$$

$$PA = H(A^{\cdot}) + H(\left(H^{+}\right) - H(A{-}H)$$

$$ETE = H(A^{\cdot}) + H\left(e^{-}\right) - H\left(A^{-}\right)$$

The enthalpy of hydrogen atom, *H*(H), for each solvent were obtained by the same method and basis set. All reaction enthalpies were calculated for 298 K. Solvation enthalpies of proton $H(H^{+})$ and electron $H(e^{-})$, in organic solvents, determined using IEF-PCM DFT/B3LYP/6–311++G** calculations, were taken from the literature [33].

The theoretical vibrational spectra were interpreted by means of potential energy distributions (PEDs) using VEDA 4 program [34]. For a better correspondence between experimental and calculated wave values, we modified the results using the empirical scaling factors, reported by Merrick et al. [35].

3. Results and discussion

3.1. Antioxidant properties of syringaldehyde

It has been found that phenolic compounds inhibit the lipid