

Structural and electronic properties of *Z* isomers of (4 α →6'',2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃ calculated in aqueous solution with PCM solvation model

Rosana Maria Lobayan · Erika N. Bentz ·
Alicia H. Jubert · Alicia B. Pomilio

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Abstract In the search for new antioxidants, flavan structures called our attention, as substructures of many important natural compounds, including catechins (flavan-3-ols), simple and dimeric proanthocyanidins, and condensed tannins. In this work the conformational space of the *Z*-isomers of (4 α →6'', 2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃ was scanned in aqueous solution, simulating the solvent by the polarizable continuum model (PCM). Geometry optimizations were performed at B3LYP/6-31 G** level. Electronic distributions were analyzed at a better calculation level, thus improving the basis set (6-311++G**). A topological study based on Bader's theory (atoms in molecules) and natural bond orbital (NBO) framework was performed. Furthermore, molecular electro-

static potential maps (MEPs) were obtained and thoroughly analyzed. The stereochemistry was discussed, and the effect of the solvent was addressed. Moreover, intrinsic properties were identified, focusing on factors that may be related to their antioxidant properties. Hyperconjugative and inductive effects were described. The coordinated NBO/AIM analysis allowed us to rationalize the changes of MEPs in a polar solvent. To investigate the molecular and structural properties of these compounds in biological media, the polarizabilities and dipolar moments were predicted which were further used to enlighten stability and reactivity properties. All conformers were taken into account. Relevant stereoelectronic aspects were described for understanding the stabilization and antioxidant function of these structures.

Rosana M. Lobayan and Erika N. Bentz contributed equally to this work

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R. M. Lobayan (✉) · E. N. Bentz
Facultad de Ingeniería, Universidad de la Cuenca del Plata,
Lavalle 50,
3400 Corrientes, Argentina
e-mail: rlobayan@ucp.edu.ar

A. H. Jubert
CEQUINOR Facultad de Ciencias Exactas y Facultad de
Ingeniería, Universidad Nacional de La Plata,
CC 962,
1900 La Plata, Argentina

A. B. Pomilio
PRALIB (UBA, CONICET), Facultad de Farmacia y Bioquímica,
Universidad de Buenos Aires,
Junín 956,
C1113AAD Buenos Aires, Argentina

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analysis

Introduction

The role of flavonoids as antioxidants is the subject of intense theoretical and experimental research. The biological and pharmaceutical activities of these compounds have been related to their antioxidant activity as free radical scavengers. Free radicals can damage biomolecules such as lipids, amino acids, proteins, carbohydrates and nucleic acids by the process of oxidative damage resulting in several diseases and aging [1]. They were also associated with deterioration of plant tissues, such as fruits and vegetables. The biological activity of flavonoids is governed by electronic interactions of the biomolecules within the cell [2]. Their antioxidant activity

depends on the capacity to donate protons and electrons [3] and an adequate solubility [4]. Moreover, the ease of deprotonation of the flavonoid determines the intermediates that are formed, and in turn, the stability of the intermediates determines the antioxidant capacity [4, 5].

In the human body flavonoids show a lot of biological properties as antioxidants, antiallergenic, antibacterial, antifungal, antiviral and anticarcinogenic agents. These features provide them useful pharmacological properties to treat diseases ranging from allergies, bacterial and viral infections, to those of greater risk such as heart diseases, cancer and HIV [6].

The study of the electronic and molecular properties of flavonoids is of great importance since it enables a better understanding of the mechanism of their antioxidant activity, which is so far controversial due to the lack of a thorough knowledge of both the molecular structure of these compounds and the structure-property relationship. Therefore, theoretical investigations of the physical and chemical properties, and structural features of flavonoids are very important to disclose the relationship between the structure, properties and performance, and to assist in the design and synthesis of new derivatives with improved properties.

4-Phenylflavans with an additional ethereal bridge comprise an interesting structure, which is included in simple and dimeric A-type proanthocyanidins that have been previously synthesized with various substituents in the aromatic rings [7, 8].

To our knowledge there are no crystallographic reported data of the A-type proanthocyanidins, and there has been no previous study on A-type proanthocyanidins using high-level *ab initio* calculations, except for a few theoretical studies on their electronic, structural, and energetic properties, which we have earlier reported, where the conformational space of unsubstituted and substituted 4-phenylflavans was scanned in gas phase [9, 10]. In addition, intramolecular bonding interactions were thoroughly described and related to their stability and reactivity. The stereochemistry of the molecules and the analysis of the effect of substitution on the behavior of the electron distribution were also studied in our laboratories [10].

Theoretical results on isolated molecules are very useful for determining the intrinsic properties of the system free of any interactions but can only be compared with a few experimental cases, since most experiments are performed in solution. Furthermore, the analysis of the molecular behavior in the presence of water is of great interest owing to the stabilizing function of proanthocyanidins in solution, their ability to form complexes with other biomolecules, and to interfere with biological systems. For these reasons, this paper focuses on the analysis of solvent effects of water on the intrinsic electronic properties and molecular geometry, simulated by a polarizable continuum model.

The ultimate aim of this work is to complete the first step in studying the electronic structure, and the quantification of stereoelectronic and substituent effects to contribute to the study of new antioxidants. Furthermore, this knowledge will be a basis for studying in another step, other substitution patterns and the ability to scavenge free radicals.

Methods

The geometries from simulation by the MD module of the HyperChem [11] package were optimized to an energy gradient less than $0.004 \text{ kJ mol}^{-1} \text{ \AA}^{-1}$ at a semiempirical level (AM1). The lowest-energy conformers were further optimized in gas phase [9, 10] by the density functional theory (DFT), as implemented by the Gaussian 03 package [12]. Geometry optimizations were performed using the Becke three-parameter hybrid functional combined with Lee-Yang-Parr correlation functional [13], giving rise to the well-known B3LYP method. The 6-31 G** basis set was used for all atoms. The optimized geometric structures were confirmed as minimum on the potential energy surface through an analysis of harmonic vibrational frequencies.

The lowest-energy conformers obtained through gas-phase calculations were re-optimized taking into account the solvent effect by the conductor polarizable continuum model (PCM) [14] at the same level of theory. The PCM approach considers that the molecule under study is embedded in a polarizable dielectric representing the solvent, without explicit solvent molecules included. The polarizable dielectric medium was described by the dielectric constant of the solvent ($\epsilon=78.39$ for water). The values of surface area and volume of the cavity were in the range of $333.61\text{--}334.85 \text{ \AA}^2$ and $387.34\text{--}390.57 \text{ \AA}^3$, respectively, for R=OH, and in the range of $333.87\text{--}396.92 \text{ \AA}^2$ and $390.19\text{--}473.65 \text{ \AA}^3$, respectively, for R=OCH₃. This model has proven to be a reliable tool for the description of electrostatic solute-solvent interactions. At the same level of calculation the zero-point energy (ZPE) was obtained, which was used to correct all energy terms.

Maps of electrostatic potential (MEP) were obtained in the van der Waals molecule surfaces using the Gaussian 03 software, and further visualized by MOLEKEL 4.0 [15].

Topological analysis and evaluation of local properties were performed by the PROAIM software [16] using the wave functions calculated at the B3LYP level and the improved 6-311++G** basis set implemented in the G03 program. NBO analysis was carried out at the same level [17]. The B3LYP functional has shown a good correlation with experimental data concerning studies of the activity of phenolic antioxidants [18–20]

Table 1 Energies calculated at B3LYP/6-31 G** level of theory for the (4 α →6'', 2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃^a

Conformer	Vacuum		Solution		Δ^c
	Relative population (%)	ΔE^b (kcal mol ⁻¹)	Relative population (%)	ΔE^b (kcal mol ⁻¹)	
R=OH	$E_0=-696831.64$ kcal mol ⁻¹		$E_0=-696853.90$ kcal mol ⁻¹		
Z1 _{CT}	72.60	0.00	46.99	0.00	-22.26
Z1 _{CC}	19.03	0.79	42.35	0.06	-22.99
Z1 _{TC}	2.23	2.06	1.12	2.21	-22.11
Z1 _{TT}	2.11	2.09	0.46	2.73	-21.62
Z2 _{CT}	3.07	1.87	4.64	1.37	-22.76
Z2 _{CC}	0.79	2.67	4.34	1.41	-23.52
Z2 _{TC}	0.08	4.02	0.05	4.05	-22.23
Z2 _{TT}	0.08	4.02	0.05	4.04	-22.24
R=OCH ₃	$E_0=-746124.30$ kcal mol ⁻¹		$E_0=-746135.48$ kcal mol ⁻¹		
Z1 _{CT}	81.74	0.00	73.40	0.00	-11.18
Z1 _{CC}	17.55	0.91	24.13	0.66	-11.43
Z2 _{CC}	0.71	2.81	2.47	2.01	-11.98
R=H	$E_0=-602433.03$ kcal mol ⁻¹		$E_0=-602433.17$ kcal mol ⁻¹		
Z1	95.93	0.00	95.94	0.00	-0.14
Z2	4.07	1.87	4.06	1.87	-0.14

^a Energies corrected for zero point energy (ZPE)

^b ΔE represents the difference of energy between the most stable conformer (Z1_{CT}) and each of the remaining conformers

^c Δ represents the difference of energy values between the structures in aqueous solvent and gas phase

Through the analysis of (3,-1) CPs (bond critical points or BCs), polar covalent interactions were characterized for C—H bonds, and all C—C bonds of rings A, B, and D in all conformers under study. The C-2—O1 bond of ring C also showed the same typical behavior of a covalent bond. The C-8a—O1 bond of ring C, and the equivalent for ring E, C-1''—O1 and C—O bonds accounting for R=OCH₃ and R=OH, did not behave like C-2—O1, according to values for an intermediate or shared polar covalent bond (see Supplementary material; Tables S2 and S3).

Relevant changes were found in (3,-3) CPs (nuclear critical points or NCPs) in solution, corresponding exclusively to hydrogen atoms. A decrease of 0.7–1.2% of the electron density at NCP of benzenic hydrogen was found in solution. At NCPs of hydrogen of the hydroxy group change was even more relevant; a decrease of 7.3% was observed with respect to the values in gas phase. These changes were remarkable in relation to the features of the maps of electrostatic potential (MEPs) as shown below.

As in gas phase [10], the average value of bond ellipticity (ϵ) in each ring increased in the order of B<A<D. In terms of the orbital model of electronic structure, the ellipticity is a measure of the extend of π -character up to the limit of a “double bond” for which ϵ reaches a maximum [22]. Therefore our results can be rationalized through an enhanced ability of ring D to act on electrophilic aromatic substitution reactions due to the increased availability of

π -electrons also in aqueous phase. In addition, this feature was enhanced with R=OH substitution in solution (see Supplementary material; Tables S2 and S3), which was also found in vacuum [10]. In general, the average ϵ values decreased in solution (changes less than 1.7%). Ring B showed the greatest changes.

In the NBO analysis, the electronic wave functions are interpreted in terms of occupied Lewis-type and unoccupied non-Lewis localized orbitals. Delocalizations of electron density between occupied Lewis-type (bonds or lone pairs) NBO orbitals and formally unoccupied (anti-bonds or Rydberg) non-Lewis NBO orbitals account for stabilizing donor–acceptor interactions.

For each donor NBO (i) and acceptor NBO (j), the stabilization energy ($E^{(2)}$) associated with i/j delocalization is explicitly estimated by the following equation:

$$E^{(2)} = -n_i \frac{F_{ij}^2}{\epsilon_j - \epsilon_i}, \quad (1)$$

where n_i is the *i*th donor orbital occupancy; ϵ_i , ϵ_j , are diagonal elements (orbital energies); and F_{ij} , off-diagonal elements, respectively, associated with the Fock matrix in NBO.

A detailed NBO analysis allowed us to describe the resonance of the lone pairs of O and O1 oxygens with features similar to those found in gas phase [10]. In vacuum

as in solution we characterized two lone pairs for each oxygen atom. One of them, *1n*, is **sp**-type and the other one, *2n*, is **p**-type. Each one played a different role. Also, the substitution in solution led to C-2—C-3 bond more polarized than C-3—C-4, and that polarization increased as R=H<R=OCH₃<R=OH. We also found that the conformational changes were correlated with the relative stability of the structure through the same hyperconjugative mechanisms described in vacuum. Instead of these similarities we found interesting changes in solution.

Tables 2 and 3 show the percentage values of the main changes for second-order stabilization energies (*x*) of relevant $\sigma \rightarrow \sigma^*$ and $1,2n_{O, O1} \rightarrow \sigma^*$, π^* charge transfers ($\% \Delta_{\sigma \rightarrow \sigma^*}$ and $\% \Delta_{1,2n_{O, O1} \rightarrow \sigma^*, \pi^*}$, respectively) calculated from

$$\% \Delta x = \frac{x(\text{solution}) - x(\text{vacuum})}{x(\text{vacuum})} \times 100 \quad (2)$$

in average for all conformers. The *x* (*vacuum*) values are those of the corresponding second-order energies in gas phase.

- i) In solution as in vacuum the $\sigma_{C-3-H} \rightarrow \sigma^*_{C-O}$ transfers were higher than those of $\sigma_{C-3-H} \rightarrow \sigma^*_{C-C}$. Moreover, in solution both kinds of electron delocalizations were enhanced; e.g., transfers to C—O bonding orbital increased 3.4–8.0%, and electron transfers to C—C orbital bonding increased 2.2–5.8%, the effect being improved for R=OH (Table 2).

Table 2 Percentage values of the main changes for second-order stabilization energies, $E^{(2)}$, calculated at B3LYP/6-311++G** level of theory for relevant transferences^{a,b}

Donor	Acceptor	Z1	Z2
σ_{C-3-H}	$\sigma^*_{C-4-C-6''}$	5.8	2.6
σ_{C-3-H}	σ^*_{C-2-O}	6.3	8.0
σ_{C-3-H}	$\sigma^*_{C-4-C-4a}$	2.2	4.8
σ_{C-3-H}	σ^*_{C-2-O1}	4.9	3.4
$\sigma_{O-3''-H}$	$\sigma^*_{C-C}^c$	4.1	4.1
$\sigma_{O-5''-H}$	$\sigma^*_{C-C}^c$	5.0	1.4
$\sigma_{C-2-C-1'}$	$\sigma^*_{C-1'-C-6'}$	0.0	-2.7
$\sigma_{C-2-C-1'}$	$\sigma^*_{C-1'-C-2'}$	2.2	1.4
$\sigma_{C-2-C-1'}$	$\sigma^*_{C-5'-C-6'}$	-0.9	-1.1
$\sigma_{C-2-C-1'}$	$\sigma^*_{C-2'-C-3'}$	-0.7	-0.3
$\sigma_{C-5'-C-6'}$	$\sigma^*_{C-4'-H}$	-2.6	-2.7
$\sigma_{C-2'-C-3'}$	$\sigma^*_{C-4'-H}$	-2.7	-2.9
$\sigma_{C-2-C-3}$	$\sigma^*_{C-1-C-6'}$	7.1	—

^a All values are expressed in kcal mol⁻¹

^b Values in average for all conformers of the ($4\alpha \rightarrow 6''$, $2\alpha \rightarrow O \rightarrow 1''$)-phenylflavans substituted with R=OH and OCH₃

^c C—C bond correspond to bonds of D ring

- ii) The second-order stabilization energies of $\sigma_{O-3''-H} \rightarrow \sigma^*$ and $\sigma_{O-5''-H} \rightarrow \sigma^*$ transfers were increased up to 5.0% in solution (Table 2).
- iii) Charge transfers from O-3'' and O-5'' lone pairs to the D-ring antibonding orbitals increased 2.3–8.6% for R=OH in solution. The same trend was observed for R=OCH₃, but the effect was attenuated (Table 3). Our results showed an increase in the delocalization effects for R=OH in solution, and higher solvent effects for these compounds. Interestingly, the improved donor role of O-3'' and O-5'' lone pairs for R=OH can be explained by their hybridization. In fact, we found that in solution the *p*-character of *1n* bonding orbitals of O-3'' and O-5'' significantly increased for compounds with R=OH. We propose that the enhanced delocalization in connection with O-3'' and O-5'' is based on the solvent effect on the hybridization of lone pairs. The increase of *p*-character of *1n* lone pairs allows a better overlap between them and the C—C antibonding orbitals of ring D (Table 4).

The effects of charge delocalization *i - iii* allowed us to rationalize the enhanced structure stabilization of compounds with R=OH in solution (Table 1).

- iv) The lone pairs of the oxygens O and O1 showed a different behavior. In fact, the O and O1 lone pair transfers decreased 16.1% in solution (Table 3). Second-order stabilization energies for $1n_{O1} \rightarrow \sigma^*_{C-8a-C-8}$ transfers, and their symmetry equivalents ($1n_O \rightarrow \sigma^*_{C-1''-C-2''}$) decreased 5.2–12.1% for R=OH and 1.7–5.4% for R=OCH₃. Our results show a lower donor role of the O and O1 lone pairs in solution, an effect that is enhanced in compounds with R=OH.
- v) In connection with the decrease of hyperconjugative interactions of the oxygen lone pair $1n_{O, O1} \rightarrow \sigma^*_{C-2-C-1'}$ in solution (see Table 3), we found that the $\sigma_{C-2-C-1'} \rightarrow \sigma^*_{C-1'-C-6'}$, $\sigma^*_{C-5'-C-6'}$, $\sigma^*_{C-2'-C-3'}$ transfers also decreased (Table 2). These findings explained the decrease in the average B-ring ellipticity shown above, and allowed us to explain the decrease in the π -character of ring B in terms of a reduction in the effects of charge delocalization related to O and O1 lone pairs in solution (anomeric effect).

Molecular electrostatic potential maps

The molecular electrostatic potential maps have been used extensively to predict the behavior and reactivity of a wide variety of chemical systems [23–25]. The $V(r)$ potentials, created in the space around a molecule by its nuclei and electrons, are a useful tool for the study of molecular reactivity. Unlike other current parameters used as reactivity indices, $V(r)$ is an actual physical property that can be

Table 3 Percentage values of the main changes for second-order stabilization energies, $E^{(2)}$, calculated at B3LYP/6-311++G** level of theory for donation transferences of oxygen lone pairs for Z1 conformers^a

Donor	Acceptor	R=OH				R=OCH ₃	
		Z1 _{CT}	Z1 _{CC}	Z1 _{TC}	Z1 _{TT}	Z1 _{CT}	Z1 _{CC}
1nO1	$\sigma^*_{C-8a-C-8}$	-7.1	-7.1	-12.1	-10.3	-5.4	-1.8
1nO1	$\sigma^*_{C-4a-C-8a}$	-0.8	-1.1	-3.0	-2.6	-1.1	-0.1
2nO1	$\pi^*_{C-4a-C-8a}$	-2.6	-3.2	-5.1	-4.4	-3.3	-2.4
1nO	$\sigma^*_{C-1''-C-6''}$	—	-4.1	-0.7	3.0	-1.1	—
1nO	$\sigma^*_{C-1''-C-2''}$	—	—	-5.2	-6.8	-5.4	-1.7
2nO	$\pi^*_{C-1''-C-2''}$	-6.2	-5.4	-2.6	-3.4	-3.2	-1.7
1nO	$\sigma^*_{C-2-C-1'}$	-2.4	-3.6	-10.3	-9.3	-3.6	-2.4
2nO	$\sigma^*_{C-2-C-1'}$	3.1	1.0	16.2	17.7	-10.3	1.0
1nO1	$\sigma^*_{C-2-C-1'}$	-9.6	-8.5	-2.5	-2.5	-7.4	-2.5
2nO1	$\sigma^*_{C-2-C-1'}$	2.5	3.5	-15.8	-16.1	13.2	0.8
1nO1	$\sigma^*_{C-2-C-3}$	-3.0	-2.7	-4.8	-5.2	-0.2	-1.5
2nO1	$\sigma^*_{C-2-C-3}$	-7.5	-7.4	9.2	9.3	-13.4	-1.3
2nO1	σ^*_{C-2-O}	-4.1	-3.3	0.8	0.5	-2.9	0.1
1n O-3''	$\sigma^*_{C-2''-C-3''}$	4.6	3.8	5.3	5.1	0.7	8.7
2n O-3''	$\pi^*_{C-3''-C-4''}$	2.8	2.3	2.8	3.5	-0.8	-1.1
1n O-5''	$\sigma^*_{C-4''-C-5''}$	7.1	5.3	8.1	7.8	2.2	1.2
2n O-5''	$\pi^*_{C-5''-C-6''}$	7.5	8.6	-10.1	-8.6	4.4	2.4
1n O-3''	$\sigma^*_{C-3a''-H}$	—	—	—	—	-11.2	-7.6
2n O-3''	$\sigma^*_{C-3a''-H}$	—	—	—	—	-5.4	-3.2
2n O-3''	$\sigma^*_{C-3a''-H}$	—	—	—	—	-6.4	-4.2
1n O-5''	$\sigma^*_{C-5''-C-6''}$	—	—	—	—	2.9	—
1n O-5''	$\sigma^*_{C-5a''-H}$	—	—	—	—	-3.0	-1.8
1n O-5''	$\sigma^*_{C-5a''-H}$	—	—	—	—	-13.8	-13.3
1n O-5''	$\sigma^*_{C-5a''-H}$	—	—	—	—	—	3.7
2n O-5''	$\sigma^*_{C-5a''-H}$	—	—	—	—	-5.0	-3.3
2n O-5''	$\sigma^*_{C-5a''-H}$	—	—	—	—	-5.7	-3.2

^aAll values are expressed in kcal mol⁻¹

determined experimentally and by computational methods. The calculation of physicochemical properties on the molecular surface, and its visualization by a color code allows a different view of molecular behavior.

In this paper we used the procedure proposed by Politzer et al. [23] to predict targeted sites for electrophilic attack in the regions of negative $V(r)$ values. Moreover, the study was enriched by a thorough analysis of electrostatic potential maps, in light of the complementarities of the

different theoretical tools used herein, for rationalizing other aspects of their distribution [9, 10].

MEPs in gas phase and in aqueous phase are shown in Fig. 2. In all species there were two very close main sites of attack, associated with the oxygen atoms of rings C and E. The highest negative $V(r)$ values showed that Z1 were more reactive conformers for electrophilic attack. The highest values (positive) showed a 46% increase in solution, and the lowest (negative) showed a 31 %

Table 4 Percentage values of the change of π -character of 1n lone pairs of oxygen atoms for the (4 α -6'',2 α -O \rightarrow 1'')-phenylflavans substituted with R=OH and OCH₃ in aqueous solution

	R=OH								R=OCH ₃		
	Z1 _{CT}	Z1 _{CC}	Z1 _{TC}	Z1 _{TT}	Z2 _{CT}	Z2 _{CC}	Z2 _{TC}	Z2 _{TT}	Z1 _{CT}	Z1 _{CC}	Z2 _{CC}
1nO	0.3	0.2	0.5	0.6	0.3	0.2	1.1	1.1	0.1	0.7	0.2
1nO1	0.2	0.3	0.0	-0.1	0.1	0.2	-0.7	-0.8	0.5	0.9	0.3
1nO3''	2.5	2.6	2.5	2.6	2.5	2.7	2.6	2.6	0.5	1.1	0.5
1nO5''	2.1	2.2	4.7	4.7	2.1	2.2	2.5	2.5	0.2	0.2	0.2

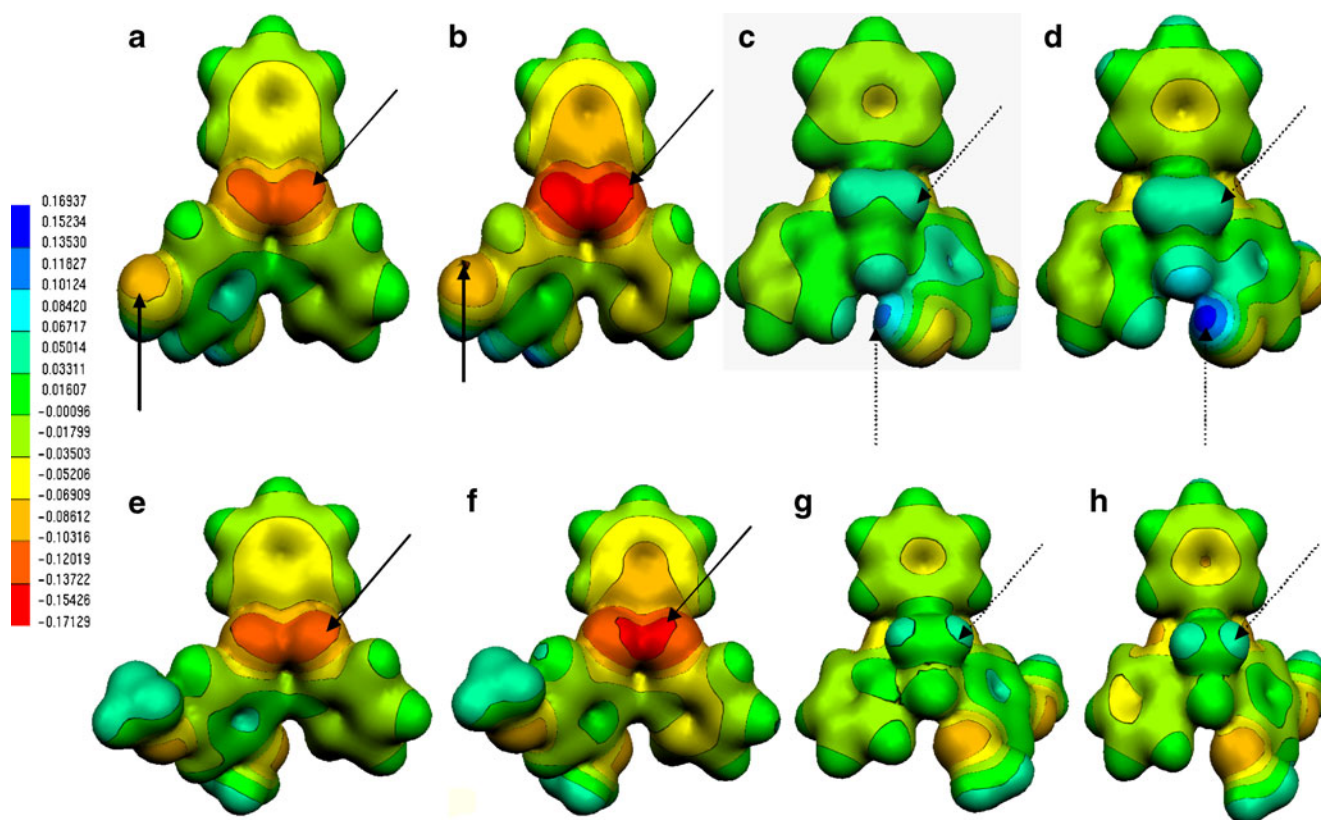


Fig. 2 (a)-(d): Maps of molecular electrostatic potential (MEP) for $Z1_{CC}$ conformer of $(4\alpha\rightarrow6'',2\alpha\rightarrow O\rightarrow1')$ -phenylflavans substituted with $R=OH$ in gas phase (a) and water solution (b), and for $Z1_{TC}$ conformer in gas phase (c) and water solution (d). (e)-(f): MEPs for $Z1_{CT}$ conformer of $(4\alpha\rightarrow6'',2\alpha\rightarrow O\rightarrow1')$ -phenylflavans substituted with $R=OCH_3$ in gas phase (e), (g), and water solution (f), (h). Solid

arrows indicate the change of negative $V(r)$ region over oxygen atoms. Dashed arrows indicate the change of positive $V(r)$ region over hydrogen atoms in both media. The solid oblique arrows indicate the decrease in the lowest values (negative) on the O-O1 region, enhanced in compounds with $R=OH$ than in those with $R=OCH_3$

decrease, thus demonstrating an enhanced reactivity in solution.

We also found other interesting features of MEPs, as follows:

- The most positive region on the hydrogens of CH_2-3 increased in solution, which can be explained by the increased donor role of the $C-3-H$ bonding orbitals in solution, as discussed in item *i*.
- The most positive region on the hydrogens of CH_2-3 was higher for $R=OH$ than for $R=OCH_3$ in vacuum as in solution, which agreed with the enhanced delocalizations found for $R=OH$ in relation with $C-3H_2$ bonding orbitals, as described in item *i*.
- The increase in the highest values (positive) located on the hydrogens of the hydroxyls can be rationalized by charge delocalizations discussed in *ii*. In fact, these results allowed us to propose that the higher donor role of $O-3''-H$ and $O-5''-H$ bonding orbitals in solution was reflected in the increased $V(r)$ values in this region. Interestingly, this finding correlated with the decrease of electron density at NCPs of those hydrogen atoms.

- The decrease in the lowest values (negative) on the O-O1 region can be rationalized by charge delocalizations discussed in *iv*. In fact, these results allowed us to propose that the minor donor role of the O and O1 lone pairs in solution was reflected in the increased reactivity of this region.
- The decrease in the lowest values (negative) on the O-O1 region was enhanced in compounds with $R=OH$. This feature can be explained by charge delocalization as discussed above, because they are lower in compounds with $R=OH$ than in those with $R=OCH_3$ (see *iv*).

Molecular polarizability and dipole moment

The values of dipole moment and the isotropic molecular polarizability are of interest as an indication of the solubility and chemical reactivity of the molecules under study. The isotropic polarizability is a measure of electronic distortion in a molecule, caused by an external electric field, and a good indicator of how the total charge

distribution of the flavonoid molecules is affected by the presence of different substituents at specific positions [26].

Calculation of the polarizability $\langle\alpha\rangle$ (see Table 5) was performed from $\langle\alpha\rangle = \frac{1}{3}(\alpha_{xx} + \alpha_{yy} + \alpha_{zz})$, where the tensor components were obtained from the second derivatives of the energy with respect to the Cartesian components of the applied electric field ϵ , ($\alpha = [\partial^2 E / \partial \epsilon^2]$).

To the best of our knowledge, investigations concerning the molecular polarizabilities of proanthocyanidins have not yet been reported, and therefore, our results are the first prediction of them. The average polarizabilities of (4 α →6'',2 α →O→1'')-phenylflavans substituted with R=OH and R=OCH₃ were ≈ 223 a.u. and ≈ 249 a.u., respectively, indicating their nature of being soluble in polar solvents, and their ability to polarize other atoms or molecules.

Moreover, we found, as expected, that the Z2 structures, which are more stabilized in solution than the Z1 structures, showed the largest value of $\langle\alpha\rangle$ in vacuum, while Z1 had minor $\langle\alpha\rangle$, which correlated with the lower stabilization in solution.

The permanent electric dipole moment of an isolated molecule (μ) is also an important predictor of its behavior in physical, chemical, and biological processes [27]. The dipole moment represents a generalized measure of the charge density in a molecule and is a reactivity index, which is very important to define biological properties, especially those related to the interaction with the active sites of an enzyme [2]. It can be determined by a variety of techniques. Because the magnitudes and directions of dipole moments are sensitive to molecular size and shape they can also serve as a useful tool in conformational analysis [28].

The values of the modulus of the permanent dipole moments (μ values) of Z-isomers of (4 α →6'',2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃ are listed in Table 6. The observed variation allowed us to predict that the values of dipole moments can be used to distinguish between different conformers. In fact, in vacuum as in solution, μ decreased as CC>TC>TT>CT.

Besides the changes of the μ modulus, for R=OH conformers we found also an important variation of the μ

vector projection on the X-Y plane, as shown in Fig. 3. This behavior can be rationalized by the analysis of the contribution of dipole moments associated to the hydroxyls following a scheme similar to that used in the vector addition model to predict the dipole moments of a compound, based on the known dipole moments of the "component parts" [28]. In fact, our results mainly showed that in vacuum as in solution, μ had two separable contributions, one of them associated to the changes in O—C—O moiety, which were uniform for all conformers (also in unsubstituted structures), and another related to the conformational changes of the hydroxy groups.

The μ variation found when all conformers are considered warns about the use of theoretical μ values considering only the most stable conformer. To take into account properly the conformational landscape, we propose a Maxwell-Boltzmann statistical average at 298.15 K of each μ Cartesian component according to:

$$\langle\mu_j\rangle = \sum_i \mu_{j,i} \exp\left(-\frac{E_i}{RT}\right) / \sum_i \exp\left(\frac{E_i}{RT}\right)$$

with $j=x, y, z$, where E_i is the relative energy of the i th conformer, and $\mu_{j,i}$ is the component j th of the i th conformer. Then, obtaining the Maxwell-Boltzmann statistical average of the magnitude of total dipole moment:

$$\langle\mu\rangle = \sqrt{\langle\mu_x\rangle^2 + \langle\mu_y\rangle^2 + \langle\mu_z\rangle^2}$$

The average Maxwell-Boltzmann values for Z1 conformers are shown in Table 6. We found an increase of 25.8% with respect to the value of the most stable conformer for R=OH, and a decrease of 43.4% with respect to the value of the most stable conformer for R=OCH₃. The same trend, but enhanced, was found in solution (54.8% for R=OH, and 47.1% for R=OCH₃).

Moreover, our calculations of molecular dipole moment and polarizabilities implied that the Z-isomers of

Table 5 Polarizability data for the (4 α →6'',2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃ calculated at B3LYP/6-31 G** level of theory in gas phase

	R=OH								R=OCH ₃			R=H	
	Z1 _{CT}	Z1 _{CC}	Z1 _{TC}	Z1 _{TT}	Z2 _{CT}	Z2 _{CC}	Z2 _{TC}	Z2 _{TT}	Z1 _{CT}	Z1 _{CC}	Z2 _{CC}	Z1	Z2
α_{xx}	275.64	279.29	275.45	271.82	277.60	281.05	274.94	271.25	298.65	311.71	308.03	260.02	264.69
α_{yy}	252.45	251.62	255.23	256.09	215.49	214.42	226.69	228.21	284.00	278.28	248.08	232.97	191.57
α_{zz}	137.90	136.20	136.72	138.47	176.41	175.10	170.23	171.49	162.20	157.63	194.97	124.66	165.01
$\langle\alpha\rangle$	222.00	222.37	222.46	222.13	223.17	223.52	223.95	223.65	248.28	249.20	250.36	205.89	207.09
Average	222.24				223.57				248.74		250.36		

^a All values are expressed in a.u.

Table 6 Values of the modulus of the permanent dipole moment for the (4 α →6'',2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃ calculated at B3LYP/6-31 G** level of theory in aqueous solvent and gas phase^a

	R=OH	Vacuum		Solution		Δ^c
R=OH	Z1 _{CT}	1.45	1.83 ^b	2.32	3.59 ^b	0.86
	Z1 _{CC}	3.56		5.53		1.97
	Z1 _{TC}	2.57		5.08		2.51
	Z1 _{TT}	2.57		3.52		0.95
	Z2 _{CT}	1.31		2.18		0.87
	Z2 _{CC}	3.28		5.31		2.03
	Z2 _{TC}	2.34		4.36		2.01
	Z2 _{TT}	2.63		3.61		0.98
	R=OCH ₃	Z1 _{CT}	1.88	1.06 ^b	1.89	1.00 ^b
Z1 _{CC}		3.88		4.99		1.11
Z2 _{CC}		3.61		4.77		1.16
R=H	Z1	1.20		2.73		1.53
	Z2	1.89		2.59		0.70

^a All values are expressed in Debye

^b Values of the Maxwell-Boltzmann statistical averages for Z1 conformers

^c Δ represents the difference of the modulus of the permanent dipole moment for the structure in aqueous solvent and gas phase

(4 α →6'',2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃ are polarized systems, and consequently, soluble in polar solvents, and with ability to polarize other atoms.

As expected, increased dipole moment values were found in solution, however, the solvent effect was not uniform. In fact, we found higher changes in CC and TC structures, and lower changes when the configuration of the H—O-3'' bond was *anti* (*trans*) to the C-3''—C-4'' bond.

The highest value was found in CC structures in agreement with the higher stabilization in aqueous solution. This finding

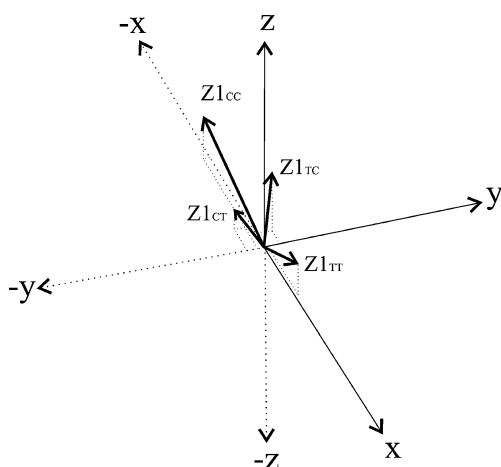


Fig. 3 Variation of the electric dipole moment vector (μ) projection on the X-Y plane

explained the marked increase in relative population (ca. 19% in vacuum and ca. 42% in solution for Z1_{CC}; ca. 1% in vacuum and ca. 4% in solution for Z2_{CC}; (Table 1).

Conclusions

The stereochemistry of (4 α →6'',2 α →O→1'')-phenylflavan was studied in aqueous solution, emphasizing the description of the factors that determine it, and the changes that occur with R=OCH₃, R=H, and R=OH, R'=H as substituents. As in gas phase, two lowest-energy conformers were characterized for R=H, eight for R=OH, and three for R=OCH₃.

In relation to the structural solvation effects we reached the following conclusions:

- As expected, all structures were stabilized in solution, however, the stabilization in solution was higher for R=OH than for R=OCH₃. The effects of charge delocalization that we described, allowed us to rationalize it (improved structure stabilization of the compounds with R=OH in solution).
- The Z2 were always more stabilized than the Z1 rotamers. We rationalize this through the higher Z2 polarizability.
- The solvent effect on structure stabilization was not uniform for all conformers. The relative population of CC conformers increased significantly in aqueous solution in accordance with the highest values of dipole moment in CC structures.

Intramolecular interactions were studied and characterized by the theory of atoms in molecules, characterizations being similar to those in vacuum. Changes on electron density at nuclear critical points (NCPs) in solution were related to charge delocalization effects and changes at the electrostatic potential maps. We described the intrinsic properties of (4 α →6'',2 α →O→1'')-phenylflavan and also showed some delocalization improved (decreased) in solution as well as the topological and structural consequences.

Our study was enriched by a deep analysis of electrostatic potential maps and their changes in solution; with the description of the underlying charge delocalization effects.

Moreover, our findings allowed us to propose an enhanced stabilization in polar solvents of the cation radical and the radical generated from the scavenging process for the substitution with R=OH due to the higher resonance and further charge delocalizations found in (4 α →6'',2 α →O→1'')-phenylflavans substituted with R=OH.

To the best of our knowledge, our results are the first predictions of molecular polarizabilities and dipole moments of proanthocyanidins and phenylflavans, which led to propose the Z-isomers of (4 α →6'',2 α →O→1'')-phenylflavans substituted with R=H, OH and OCH₃ as

molecules soluble in polar solvents with the ability to polarize other atoms and molecules.

We remark on the use of dipole moments to distinguish between different conformers. In this regard, we calculated the dipole moment variation taking into account all conformers, using Maxwell-Boltzmann statistical average of each Cartesian μ component. Results warn about using μ values only calculated considering the most stable conformer.

In this paper we studied in depth the stereoelectronic features of the Z isomers of ($4\alpha\rightarrow 6''$, $2\alpha\rightarrow O\rightarrow 1''$)-phenylflavans substituted with R=H, OH and OCH₃ in a simulated aqueous solution. The study of substitution with R'=R=H, OH and OCH₃, and the ability to scavenge free radicals are in progress.

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