J Mol Model (2014) 20:2522
DOI 10.1007/s00894-014-2522-z

ORIGINAL PAPER

Exploratory conformational study of (+)-catechin. Modeling of the polarizability and electric dipole moment

Erika N. Bentz · Alicia B. Pomilio · Rosana M. Lobayan

Received: 28 February 2014 / Accepted: 3 November 2014 / Published online: 29 November 2014

Abstract The extension of the study of the conformational space of the structure of (+)-catechin at the B3LYP/6-31G(d,p) level of theory is presented in this paper. (+)-Catechin belongs to the family of the flavan-3-ols, which is one of the five largest phenolic groups widely distributed in nature, and whose biological activity and pharmaceutical utility are related to the antioxidant activity due to their ability to scavenge free radicals. The effects of free rotation around all C-O bonds of the OH substituents at different rings are taken into account, obtaining as the most stable conformer, one that had not been previously reported. One hundred seven structures, and a study of the effects of charge delocalization and stereoelectronic effects at the B3LYP/6-311++G(d,p) level are reported by natural bond orbital analysis, streamlining the order of these structures. For further analysis of the structural and molecular properties of this compound in a biological environment, the calculation of polarizabilities, and the study of the electric dipole moment are performed considering the whole conformational space described. The results are analyzed in terms of accumulated knowledge for ($4\alpha \rightarrow 6''$, $2\alpha \rightarrow O \rightarrow 1''$)-phenylflavans and (+)-catechin in previous works, enriching the study of both types of structures, and taking into account the importance of considering the whole conformational space in modeling both the polarizability and the electric dipole moment, also proposing to define a descriptive subspace of only 16 conformers.

Keywords (+)-catechin · Antioxidants · Conformational search · Density functional theory · Electric dipole moment · Molecular polarizability · Natural bond orbital analysis

This paper belongs to Topical Collection QUITEL 2013

Electronic supplementary material The online version of this article (doi:10.1007/s00894-014-2522-z) contains supplementary material, which is available to authorized users.

E. N. Bentz
Instituto de Investigaciones Científicas, Facultad de Ingeniería, Universidad de la Cuenca del Plata, Lavalle 50, 3400 Corrientes, Argentina

A. B. Pomilio
Instituto de Bioquímica y Medicina Molecular [IBIMOL (ex PRALIB), UBA-CONICET], Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, C1113AAD Buenos Aires, Argentina

R. M. Lobayan (✉)
Departamento de Física, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Avda. Libertad 5300, 3400 Corrientes, Argentina
e-mail: rlobayan@arnet.com.ar

R. M. Lobayan
e-mail: rmlb@exa.unne.edu.ar

Introduction

The flavonoids comprise a chroman moiety, and are classified by the degree of saturation of the pyran ring, the presence of a carbonyl group at C-4, and the substitution at C-3 for the monomers. These compounds consist of a benzene ring (A) fused to a six-membered ring (C), which in the case of the flavonols and flavones is a γ-pyran with a carbonyl group at C-4, and a phenyl group (B) at C-2 of ring C. Isoflavonoids have instead the latter substituent at C-3. Catechins lack both the carbonyl at C-4, and 2,3-double bond (Fig. 1) .

The structural diversity of flavonoids makes them exhibit antineoplastic, antiherpetic, antibacterial, anti-inflammatory, antimutagenic, antiallergic, antithrombotic, antiviral, and vasodilatory activities [1–3]. The potent antioxidant activity of flavonoids, their ability to scavenge hydroxyl radicals, superoxide anion and lipid peroxy radicals, could become the most important function of flavonoids, and underlies many of the processes listed above [4]. Since oxidative damage is involved

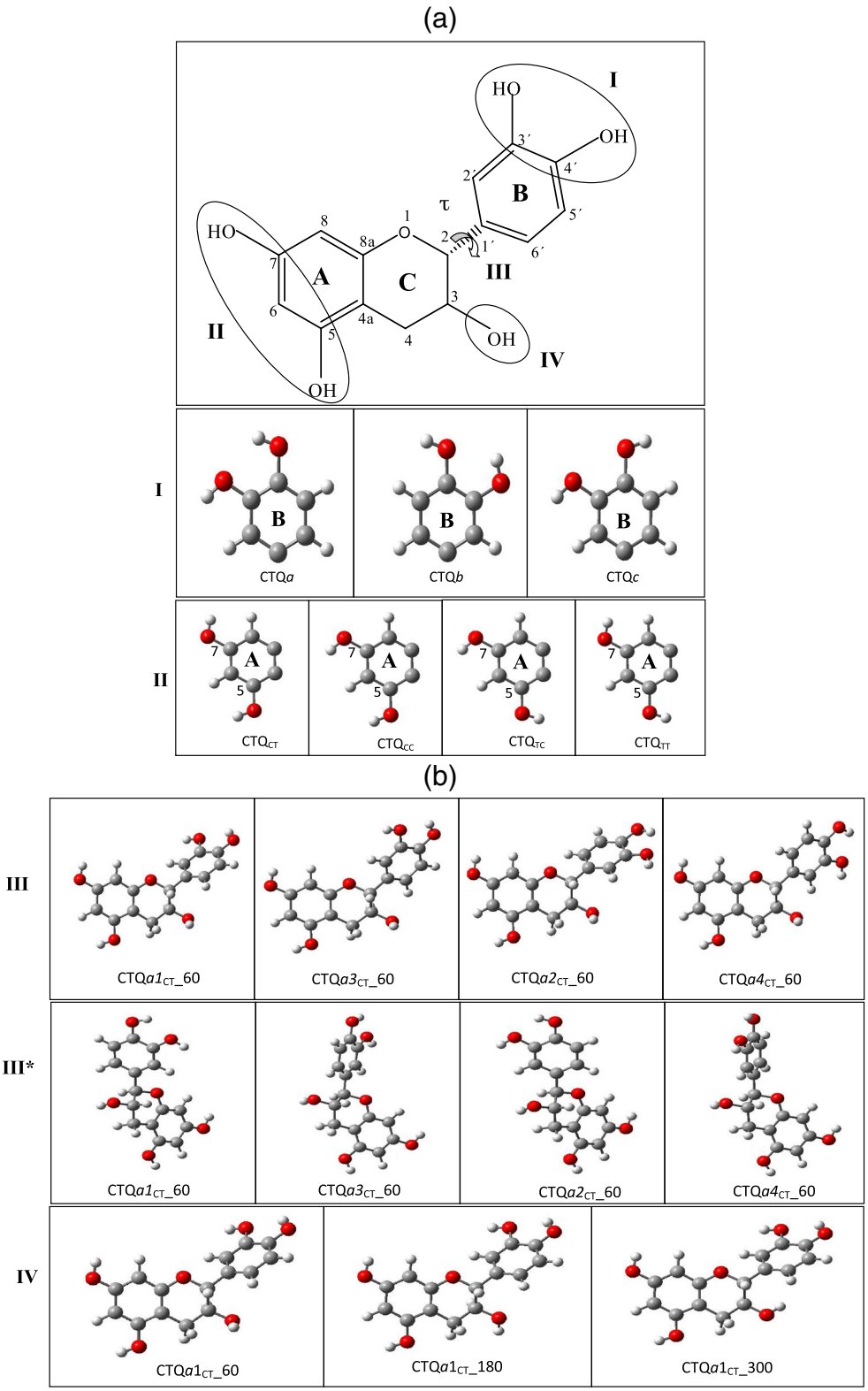

Fig. 1 Scheme of the molecule of (+)-catechin. Numbering and nomenclature used for the analysis are shown in this figure

in most disease processes supported by clinical and epidemiological research, flavonoids have been suggested for application in the prevention and treatment of a number of diseases [5, 6]. Furthermore, by scavenging free radicals, flavonoids are able to inhibit lipid peroxidation (LPO) induced by several factors [6].

In recent years the role of flavonoids as antioxidants is the subject of both theoretical and experimental intense research [7–10].

This paper refers to the study of the structure of (+)-catechin, which belongs to the flavan-3-ols, one of the five largest

phenolic groups, which are widely distributed in nature (mainly in green and black tea, and red wine). Hydroxylation occurs at the C-3′ and C-4′ of the catechol ring (ring B), and at C-5 and C-7 of the resorcinol ring (ring A), as shown in Fig. 1.

Theoretical studies, and progress in the detailed knowledge of the conformational space allow to correlate theoretical and experimental data. Since there is a mixture of conformers in the samples (even when very pure), consideration of the relative weight of each conformer is needed. Furthermore, variations in the structural, thermochemical and magnetic properties in commercial samples of different origin, have shown the existence of different conformational mixtures [11]. Reported research on this flavonoid [12–14] refers to the study of some properties in gas phase and calculations of descriptive reactivity parameters, also considering the effect of different solvents. Usually, studies of a single conformer that is chosen for its greater energy stability are reported.

However, except for a breakthrough in this regard recently reported [15], in the literature there is neither an exploratory study of the conformational space of this compound nor advances in the analysis of how this account can affect the theoretical prediction of the value of different properties of the electronic distribution of the compound, which is a central aim of the present work.

It is also of interest to analyze the order of stability of the described conformers by studying in depth the effects of electron delocalization that operate in the structures. All this knowledge is relevant to further study the molecular basis of the different mechanisms proposed in the literature to describe the antioxidant ability of these structures.

In addition, since another of our aims is to advance in research on structural and molecular properties of these kinds of compounds in a biological medium, the polarizability and dipole moment are properties of primary interest. Isotropic molecular polarizability is an important marker of the solubility and chemical reactivity of the molecules under study [16]. The dipole moment is also an index of reactivity, which is relevant to define biological properties, especially those related to interaction with an enzyme active sites [17]. It can be determined by a variety of techniques, and because of the fact that the magnitudes and directions of the dipole moments are sensitive to molecular size and shape, can also serve as a useful tool for conformational analysis [18]. There are QSAR studies, where it has been shown that the value of the dipole moment defines the orientation of the flavonoid and interaction rate, and therefore, it plays an important role in driving the interaction. Data analysis showed that, for example, an increase in the value of the dipole moment resulted in a decrease in the lipid peroxidation inhibition constant, so that it was suggested that a good inhibitor should have small values for such property [19].

In the present report the extension of the study of the conformational space of (+)-catechin is shown, taking into account the effects of free rotation around all C─O bonds of the OH substituents at different rings and studying the effects of charge delocalization and stereoelectronic effects, by a natural bond orbital analysis, streamlining the order of stability of these structures. In addition, we present the calculation of polarizabilities and electric dipole moments taking into account the whole conformational space described, analyzing the importance of considering it in the modeling of these properties, and also proposing to define a descriptive subspace of only 16 conformers. Results are interpreted in terms of the accumulated knowledge on ($4\alpha \rightarrow 6''$, $2\alpha \rightarrow O \rightarrow 1''$)-phenylflavans [20–23] and (+)-catechin in previous work [15], enriching the analysis of both types of structures.

Methods

The computational study is carried out using the density functional theory (DFT), as implemented in the Gaussian 03 program package [24]. The analysis of the conformational space is performed using the 3-parameter hybrid functional of Becke and correlation functional of Lee-Yang-Parr. This combination results in the method known as B3LYP [25, 26]. The 6-31G(d,p) basis set is used for all atoms. The reliability of the selected level for calculations has been previously demonstrated [10, 27, 28]. For the analysis, the effects of free rotation around the C─O bonds of the OH substituents at C-5 and C-7 (ring A), C-3′ and C-4′ (ring B), and C-3 (ring C) are taken into account (Fig. 1).

The minimum energies are confirmed as true minimum energy structures, through a harmonic vibrational frequencies calculation, and thermodynamic corrections are included in all electronic energies reported.

The characterization and description of charge delocalizations, interactions between different moieties of a molecule, and hyperconjugative effects are carried out by a natural bond orbital (NBO) analysis [29] using wave functions calculated at B3LYP level and with the improved base 6-311++G(d,p) implemented in the G03 program.

Results and discussion

Conformational and structural analysis

The progress in the study of the conformational space of (+)-catechin (CTQ) in gas phase is carried out considering the effects of free rotation around the C─O bonds of the OH substituents at C-5 and C-7 (ring A), C-3′ and C-4′ (ring B), and C-3 (ring C) (Fig. 1).

This study of the conformational space leads to one hundred and seven conformers, which are confirmed as energy minima, expanding the description given in our previous work [16], in which only 12 structures were described, as only rotation around the C─O bonds of the OH groups at C-3′ and C-4′ (ring B) were considered, fixing the substituents on C-5 and C-7 (ring A) in the most stable arrangement according to previous studies on (4α→6″, 2α→*O*→1″)-phenylflavans [21, 22].

In the present report the same notation is used as that defined in our previous work [15]. The possible arrangements of the OH groups of the catechol ring lead to the classification of the conformers into three groups, which are indicated with the *a, b, c* subscripts. Therefore, we have the CTQ*a* group, in which the H of the OH group that forms an intramolecular hydrogen bond (HB) is bonded to C-4′; the CTQ*b* group, in which the H of the OH group forms an intramolecular HB is bonded to C-3′; and finally, the group called CTQ*c*, whose arrangement of the OH groups does not allow the formation of an intramolecular HB in ring B (row I in Fig. 1a). The so defined groups are characterized according to which hydrogen atom of the OH groups is involved in the HB. According to other authors [8], the CTQ*a* and CTQ*b*-type structures have been named monohydrate-*syn* type; and CTQ*c*, dihydrate-*anti* type.

Conformers arising from the arrangement of O—H bonds in ring A are designated by C and T subscripts. This nomenclature refers to *syn* (*cis*) and *anti* (*trans*) configurations of the H—O5 and H— O7 bonds in resorcinol-type ring relative to the C5—C6 and C6—C7 bonds, respectively. The average values of the H—O5—C5—C6 and H—O7—C7—C6 dihedral angles are close to 0° (*cis*) or near 180° (*trans*) (row II in Fig. 1a).

As described for (4α→6″, 2α→*O*→1″)-phenylflavans substituted with R′=H and R=H, OH and OCH_3 [20–22], our results indicate that the free rotation around C2—C1′ leads to two types of minima, named Z1 and Z2 for each group (row III in Fig. 1b). These studies showed that for (4α→6″, 2α→*O*→1″)-phenylflavans Z1-type conformers have the minor energy, and are characterized by a τ C3—C2—C1′—C6′ dihedral angle of 88.2°. The same angle in Z2-type isomers accounts for about 0.0°. The angles between the bonds that define rings C and E, and their respective lengths were quite similar for both conformers; although the C3—C2—C1′ angle differed approximately 4.0° between conformers, Z2 was considered as rotamer of Z1 [20–22]. It is worth mentioning that in gas phase similar results were obtained in our previous report on CTQ, as Z1- and Z2-type rotamers were also defined [15], which is found again in this extended study of the conformational space. Taking into account in this work the 107 structures, the average value for τ: C3—C2—C1′—C-6′ dihedral angle is 87.45° or −89.26°, and 176.23° or −7.00° for Z1- and Z2-type rotamers, respectively. Given the asymmetric structure of the B ring, four types of minima arise, which are called CTQ1 and CTQ2 (for both Z1-type minima, τ: 87.45° and −89.26°, respectively), and CTQ3 and CTQ4 (for both Z2-type minima, τ: 176.23° or −7.00°, respectively) (rows III and III * in Fig. 1b).

Finally, the rotation of the OH group around the C3—O bond leads to three types of minimum energy structures, with a δ: C2—C3—O3—H dihedral angle, which takes values close to 60°, 180°, and 300° (row IV in Fig. 1b).

The minimum energy structures of each group are those in which the dihedral angle δ takes values close to 300° for Z1-type conformers, e.g., CTQ$a1_{CT_}$300, CTQ$b2_{CT_}$300, and CTQ$c1_{CT_}$300, as shown in Table 1 (Fig. 2).

Table 1 also shows that the most stable conformers for each group and those of greater relative population are on the Z1-type with an angle τ close to 90° for the minima of CTQ*a* and CTQ*c*, and an angle τ close to −90° for the minimum of CTQ*b*. The most stable structure is that called CTQ$a1_{CT_}$300, with an energy value of −647028.3892 kcal mol^{-1}.

When the angle δ is close to 300° and in some cases when it approaches 180° because of steric hindrance between the OH group of the catechol ring and the OH group of ring C, several Z2-type conformers disappear.

This study of the conformational space, therefore, leads to 107 lowest energy conformers of which only 35 are of Z2-type.

The coordinates of the minimum energy structures confirmed by vibrational calculations are shown in the Supplementary material.

The energy difference between Z1- and Z2-type conformers leads to sums of relative populations, calculated according to the Maxwell-Boltzmann distribution at 298.15 K, of 60.83, 38.90, and 0.06 % for Z1-type conformers of CTQ*a*, CTQ*b*, and CTQ*c* groups, respectively; while the sums of the relative populations for Z2-type conformers account for a total value of 0.16, 0.05, and 0.00 % for CTQ*a*, CTQ*b*, and CTQ*c* groups, respectively (Table 1).

Since Z1-type conformers of CTQ*a* and CTQ*b* groups with a value of δ close to 300° are the most stable and those with the greatest relative population as shown in Table 1, it is proposed in this work the analysis of the possibility of advancing in the study of some molecular properties by considering a subspace only constituted by such structures. With a bigger basis set (6-311++G(d,p)) similar behavior is found, see Table S2 and Table S3 in the Supplementary material. Relative populations calculated according to Maxwell-Boltzmann at room temperature considering the proposed subspace (16 conformers) are shown in Table 1. Relative weights so calculated do not differ appreciably from those obtained by considering the entire conformational space (107 conformers), being the reason of proposing this subspace as descriptive enough. On the other hand, the energy difference between the structures belonging to the *a* and *b* groups with

Table 1 Calculated energy at B3LYP/6-31G(d,p) level of theory, the energy difference with respect to the most stable conformer and relative population calculated according to Maxwell-Boltzmann distribution at 298.15 K, for different minimum energy conformers obtained for CTQ*a*, CTQ*b* and CTQ*c* in gas phase. Values of energy are expressed in kcal mol^{-1}, and corrected for zero point energy (ZPE)

CTQ*a* group				CTQ*b* group					CTQ*c* group			
Conformer	ΔE^a	Population[c]	Population[d]	Conformer	ΔE^a	ΔE^b	Population[c]	Population[d]	Conformer	ΔE^a	ΔE^b	Population[c]
CTQa1$_{CT}$_60*	2.42	0.37		*CTQb1*$_{CT}$_60*	2.73	2.32	0.22		*CTQc1*$_{CT}$_60*	6.60	2.46	0.00
CTQa1$_{CT}$_180	2.27	0.48		*CTQb1*$_{CT}$_180	2.65	2.24	0.25		*CTQc1*$_{CT}$_180	6.37	2.23	0.00
CTQa1$_{CT}$_300	0.00	22.03	22.92	*CTQb1*$_{CT}$_300	0.49	0.08	9.61	10.00	*CTQc1*$_{CT}$_300	4.14	0.00	0.02
CTQa1$_{CC}$_60	3.17	0.10		*CTQb1*$_{CC}$_60	3.51	3.11	0.06		*CTQcb1*$_{CC}$_60	7.37	3.24	0.00
CTQa1$_{CC}$_180	2.99	0.14		*CTQb1*$_{CC}$_180	3.46	3.05	0.06		*CTQc1*$_{CC}$_180	7.13	3.00	0.00
CTQa1$_{CC}$_300	0.64	7.51	7.82	*CTQb1*$_{CC}$_300	1.19	0.78	2.95	2.71	*CTQc1*$_{CC}$_300	4.82	0.68	0.01
CTQa1$_{TC}$_60	3.63	0.05		*CTQb1*$_{TC}$_60	3.99	3.58	0.03		*CTQc1*$_{TC}$_60	7.88	3.74	0.00
CTQa1$_{TC}$_180	3.46	0.06		*CTQb1*$_{TC}$_180	3.93	3.52	0.03		*CTQc1*$_{TC}$_180	7.64	3.50	0.00
CTQa1$_{TC}$_300	0.65	7.30	7.60	*CTQb1*$_{TC}$_300	1.18	0.77	2.99	2.23	*CTQc1*$_{TC}$_300	4.86	0.72	0.01
CTQa1$_{TT}$_60	3.70	0.04		*CTQb1*$_{TT}$_60	3.99	3.58	0.03		*CTQc1*$_{TT}$_60	7.90	3.76	0.00
CTQa1$_{TT}$_180	3.56	0.05		*CTQb1*$_{TT}$_180	3.93	3.52	0.03		*CTQc1*$_{TT}$_180	7.68	3.54	0.00
CTQa1$_{TT}$_300	0.80	5.68	5.91	*CTQb1*$_{TT}$_300	1.27	0.86	2.57	2.68	*CTQc1*$_{TT}$_300	4.96	0.83	0.01
CTQa3$_{CT}$_60*	4.13	0.02		*CTQb3*$_{CT}$_60*	4.43	4.02	0.01		*CTQc3*$_{CT}$_60*	8.09	3.96	0.00
CTQa3$_{CT}$_180	4.32	0.01		*CTQb3*$_{CC}$_60	5.25	4.84	0.00		*CTQc3*$_{CT}$_180	8.39	4.25	0.00
CTQa3$_{CC}$_60	4.13	0.02		*CTQb3*$_{TC}$_60	5.64	5.23	0.00		*CTQc3*$_{CC}$_60	8.84	4.70	0.00
CTQa3$_{CC}$_180	4.32	0.01							*CTQc3*$_{CC}$_180	9.16	5.03	0.00
CTQa3$_{TC}$_60	5.31	0.00							*CTQc3*$_{TC}$_60	9.33	5.2	0.00
CTQa3$_{TC}$_180	5.79	0.00							*CTQc3*$_{TC}$_180	9.94	5.8	0.00
CTQa3$_{TT}$_60	5.39	0.00							*CTQc3*$_{TT}$_60	9.39	5.25	0.00
CTQa2$_{CT}$_60*	2.90	0.16		*CTQb2*$_{CT}$_60*	2.76	2.35	0.21		*CTQc2*$_{CT}$_60*	6.99	2.85	0.00
CTQa2$_{CT}$_180	2.30	0.45		*CTQb2*$_{CT}$_180	2.50	2.10	0.32		*CTQc2*$_{CT}$_180	6.44	2.30	0.00
CTQa2$_{CT}$_300	0.51	9.36	9.74	*CTQb2*$_{CT}$_300	0.41	0.00	11.05	11.50	*CTQc2*$_{CT}$_300	4.53	0.39	0.01
CTQa2$_{CC}$_60	3.75	0.04		*CTQb2*$_{CC}$_60	3.64	3.23	0.05		*CTQc2*$_{CC}$_60	7.80	3.66	0.00
CTQa2$_{CC}$_180	3.16	0.11		*CTQb2*$_{CC}$_180	3.40	2.99	0.07		*CTQc2*$_{CC}$_180	7.25	3.11	0.00
CTQa2$_{CC}$_300	1.26	2.60	2.71	*CTQb2*$_{CC}$_300	1.20	0.79	2.89	3.01	*CTQc2*$_{CC}$_300	5.25	1.11	0.00
CTQa2$_{TC}$_60	4.39	0.01		*CTQb2*$_{TC}$_60	4.17	3.77	0.02		*CTQc2*$_{TC}$_60	8.38	4.24	0.00
CTQa2$_{TC}$_180	3.72	0.04		*CTQb2*$_{TC}$_180	3.92	3.51	0.03		*CTQc2*$_{TC}$_180	7.77	3.63	0.00
CTQa2$_{TC}$_300	1.38	2.14	2.23	*CTQb2*$_{TC}$_300	1.25	0.84	2.66	2.77	*CTQc2*$_{TC}$_300	5.33	1.19	0.00
CTQa2$_{TT}$_60	4.34	0.01		*CTQb2*$_{TT}$_60	4.10	3.69	0.02		*CTQc2*$_{TT}$_60	8.36	4.22	0.00
CTQa2$_{TT}$_180	3.68	0.04		*CTQb2*$_{TT}$_180	3.82	3.41	0.03		*CTQc2*$_{TT}$_180	7.78	3.64	0.00
CTQa2$_{TT}$_300	1.41	2.04	2.12	*CTQb2*$_{TT}$_300	1.24	0.83	2.72	2.83	*CTQc2*$_{TT}$_300	5.40	1.26	0.00
CTQa4$_{CT}$_60*	3.74	0.04		*CTQb4*$_{CT}$_60*	4.16	3.75	0.02		*CTQc4*$_{CT}$_60*	8.04	3.90	0.00
CTQa4$_{CT}$_180	4.26	0.02		*CTQb4*$_{CT}$_180	4.49	4.08	0.01		*CTQc4*$_{CT}$_180	8.45	4.31	0.00
CTQa4$_{CC}$_60	4.60	0.01		*CTQb4*$_{CC}$_60	5.05	4.64	0.00		*CTQc4*$_{CC}$_60	8.84	4.70	0.00
CTQa4$_{CC}$_180	5.17	0.00		*CTQb4*$_{CC}$_180	5.41	5.00	0.00		*CTQc4*$_{CC}$_180	9.31	5.18	0.00
CTQa4$_{TC}$_60	5.13	0.00							*CTQc4*$_{TC}$_60	9.40	5.27	0.00
CTQa4$_{TC}$_180	6.04	0.00							*CTQc4*$_{TC}$_180	10.16	6.02	0.00
CTQa4$_{TT}$_60	5.08	0.00							*CTQc4*$_{TT}$_60	9.32	5.18	0.00

[a] Energy difference compared to the most stable conformer

[b] Energy difference compared to the most stable conformer of each group (CTQ*a*, CTQ*b* and CTQ*c*)

[c] Relative percentage population calculated by Boltzmann, expressed in %

[d] Relative percentage population calculated by Boltzmann, expressed in % for a subspace containing only the most stable conformers (Z1-type of CTQ*a* and CTQ*b* groups, and with δ close to 300°)

*Conformers studied in a previous work [15]

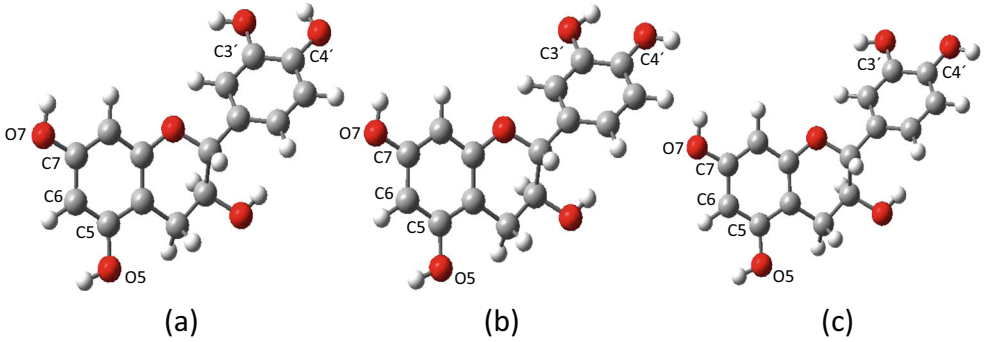

Fig. 2 Optimized structures at B3LYP/6-31G(d,p) level of the most stable conformers of CTQ*a* **a**, CTQ*b* **b**, and CTQ*c* **c** groups

respect to those of *c*-type, and the tiny population of the latter, indicate the stabilizing influence of the intramolecular hydrogen bonding (HB) in the catechol ring, as previously shown [15].

NBO analysis

The natural bond orbital (NBO) analysis [29] is a technique for obtaining localized orbitals from ab initio wave functions. These orbitals can be identified by the bonds, lone pairs, and antibonds. In simple terms, the delocalized molecular orbitals comprising an ab initio wave function are converted into natural bond orbitals through a series of matrix transformations. The transformation of the ab initio wave functions in localized natural orbitals defines representations that are in good agreement with the Lewis chemical structure concepts. Furthermore, the overall transformation to natural bond orbitals also leads to unoccupied orbitals in the formal structure of Lewis that can be used in the description of non-covalent effects.

From this methodology can also be derived information on the interactions between different moieties of a molecule by analyzing the interactions between occupied NBO of a region with additional orbitals of the valence shell of another one. The interaction energy can be obtained from the determination of second-order perturbation energy.

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

$$E^{(2)} = -n_i \frac{F_{ij}^2}{\varepsilon_j - \varepsilon_i},$$

where n_i is the occupation or population of the donor orbital *i*; ε_i and ε_j are the energies of the orbitals involved in the interaction, and $F_{(i,j)}$ is the *i,j* element of the Fock matrix.

In this case, the NBO analysis allows investigating which are the main hyperconjugative interactions involved in the stability of conformers differing in the δ: C2—C3—O3—H dihedral angle. Second order stabilization energies $E^{(2)}$ are shown in Table 2, which describe the effects of charge delocalization and explain the stability order of the conformers, whose dihedral angle δ is 60°, 180° or 300° for the CTQ$a1_{CT}$ group. With the same procedure, all groups of conformers are analyzed, in which the only structural variation corresponds to the different values of the angle δ. Our results indicate that the greater electronic delocalization of the bonding orbital for the O3—H bond to the rest of the compound, the greater the stability of the conformer.

The second order energies associated with charge transfers explaining the stability order of conformers that differ in the arrangement of the OH substituents of the resorcinol ring, in particular for CTQ*a1*-type conformers are shown in Table 3. Following the same procedure, the other conformers grouped so that the only structural change comes from the C-5 and C-7 substitution are discussed. Our results show that the greater stabilization in each group is associated with the more effective delocalization of the bonding orbitals corresponding to O5,7—H, O5,7—C5,7 toward the antibonding orbitals corresponding to the bonds of ring A (*sigma* electronic system).

The greater stability of the Z1-type versus Z2-type conformers is explained by electron charge delocalization effects from and to the bonds of ring C, as shown in a recent report on CTQ$_{CT}$_60 [15]. As observed for (4α→6″,2α→O→1″)-phenylflavan substituted with R=OH [21], and for a group of CTQ conformers, this shows that although CTQ is a non-planar structure, ring B is not independent of ring A; there are specific charge delocalization mechanisms that define the interaction between rings A, C, and B.

Table 2 Second order energies associated with the charge transfer explaining the stability order of conformers that differ in the values of the δ (C2—C3—O3—H) dihedral angle for CTQ$a1_{CT}$; calculated at the B3LYP/6-311++G(d,p) level of theory. Values are expressed in kcal mol^{-1}

Donor	Acceptor	CTQ$a1_{CT}$_300	CTQ$a1_{CT}$_180	CTQ$a1_{CT}$_60
Energy difference		0.00	2.27	2.42
$\sigma_{O3\text{-}H}$	$\sigma^*_{C3\text{-}C4}$	2.74		
$\sigma_{O3\text{-}H}$	$\sigma^*_{C2\text{-}C3}$		2.65	
$\sigma_{O3\text{-}H}$	$\sigma^*_{C3\text{-}H}$			2.43

Table 3 Second order energies associated with the charge transfer order explaining the stability order of the conformers that differ in the arrangement of the OH substituents of the resorcinol ring for CTQ*a1*; calculated at the B3LYP/6-311++G(d,p) level of theory. Values are expressed in kcal mol^{-1}

Donor	Acceptor	CTQ$a1_{CT}$_300	CTQ$a1_{CC}$_300	CTQ$a1_{TC}$_300	CTQ$a1_{TT}$_300
Energy difference		0.00	0.64	0.65	0.80
σ_{O5-H}	σ^*_{C4-C5}	4.45			
	σ^*_{C4a-C5}		4.40		
	σ^*_{C5-C6}			4.82	4.73
σ_{O5-C5}	σ^*_{C6-C7}	1.19	1.21	1.44	1.41
	σ^*_{C5-C6}	0.67	0.65		
	$\sigma^*_{C4a-C8a}$	2.05	2.06	1.72	1.72
	σ^*_{C4a-C5}	0.67	0.71	0.72	0.68
	$\sum$	4.58	4.62	3.88	3.81
σ_{O7-H}	σ^*_{C6-C7}	5.14			5.06
	σ^*_{C7-C8}		5.02	5.08	
σ_{O7-C7}	σ^*_{C5-C6}	1.37	1.20	1.17	1.34
	σ^*_{C7-C8}	0.61			0.57
	σ^*_{C8-C8a}	1.30	1.49	1.49	1.31
	σ^*_{C6-C7}		0.54	0.56	
	$\sum$	3.28	3.23	3.22	3.22
	$\sum_{TOTAL}$	17.45	17.28	17.00	16.82

Finally, the higher stability of *a*-type conformers compared to the *b*-type ones, as well as *b*-type conformers versus *c*, as also shown in a recent report on the CTQ_{CT}_60 group [15], is explained by the greater effectiveness of charge delocalization in the C-3′ and C-4′ regions, e.g., charge delocalizations of the bonding orbitals corresponding to O3′,4′—H bonds toward the antibonding orbitals of the ring B bonds, of the 1*n* lone pairs of the O-3′ and O-4′ oxygen atoms to ring B bonds, and $1n_{O3',4'} \rightarrow \sigma^*_{O4',3'—H}$ delocalizations. We characterized two lone pairs for each oxygen atom. One of them, *1n*, is sp-type and the other one, *2n*, is p-type. This is directly related to the stabilizing effects of the hydrogen bonding-type interactions (absent in *c*-type conformers), which are set out in the CTQ catechol ring in *a*- and *b*-type structures.

Molecular polarizability and dipole moment

The values of the dipole moments and molecular isotropic polarizability are important markers of the solubility and chemical reactivity of the molecules under study. Isotropic polarizability is a measure of the electronic distortion in a molecule caused by an external electric field, and is a good index of how the electric charge distribution of a molecule is affected [16]. The dipole moment is a general measure of charge density in a molecule and also is an important predictor of its behavior in physical, chemical, and biological processes [13].

Both properties are herein studied considering the extended conformational space and the subspace consisting of the most stable conformers of Z1-type CTQ*a* and CTQ*b* groups with a δ value close to 300°.

Calculations of the polarizability ⟨α⟩ for CTQ are performed according to the following expression:

$$\langle \alpha \rangle = \frac{1}{3}\left(\alpha_{xx} + \alpha_{yy} + \alpha_{zz}\right),$$

where the tensor components are obtained from the second derivative of the energy with respect to the Cartesian components of the applied electric field ε, $\alpha = [\partial^2 E/\partial \varepsilon^2]$. To take into account the weight of the different conformers the statistical average value according to the Maxwell-Boltzmann distribution at 298.15 K of the polarizability is calculated by the following expression:

$$< \alpha >= \sum_i < \alpha_i > \exp\left(\frac{-E_i}{RT}\right) \Big/ \sum_i \exp\left(\frac{-E_i}{RT}\right).$$

The polarizability value of the most stable conformer is 175.97 a.u. When considering the conformational space comprising 107 conformers, this value is 176.16 a.u., and a low fluctuation of the polarizability values is observed throughout the conformational space (those values of the subspace of δ close to 300° are shown in Table 4). Our results show the CTQ soluble nature in polar solvents, and its ability to polarize other atoms or molecules, also demonstrating that the polarizability is a property that can be modeled regardless of conformational variation with an error of 0.11 %. In addition, the value found for the subspace consisting of the 16 conformers selected above is 176.17 a.u., confirming this subspace as sufficiently representative. Polarizability values decrease in the following order CTQ*c*> CTQ*b*> CTQ*a*. The<α>value found for (4α→6″,2α→O→1″)-phenylflavan substituted with R=OH was

Table 4 Polarizability values of the conformers considered in the subspace δ (C2—C3—O3—H) next to the 300° calculated at B3LYP/6-31G(d,p) level of theory in gas phase[a]

Conformer	α_{xx}	α_{yy}	α_{zz}	<α>	Conformer	α_{xx}	α_{yy}	α_{zz}	<α>
CTQa1$_{CT}$_300	253.12	175.11	99.68	175.97	*CTQb1*$_{CT}$_300	259.70	169.95	99.12	176.26
CTQa1$_{CC}$_300	257.47	173.23	98.30	176.33	*CTQb1*$_{CC}$_300	264.34	167.13	98.40	176.63
CTQa1$_{TC}$_300	254.62	175.38	98.42	176.14	*CTQb1*$_{TC}$_300	261.56	169.03	98.64	176.41
CTQa1$_{TT}$_300	250.33	177.36	99.77	175.82	*CTQb1*$_{TT}$_300	256.97	171.94	99.33	176.08
CTQa2$_{CT}$_300	258.93	168.60	101.15	176.23	*CTQb2*$_{CT}$_300	255.81	173.15	99.45	176.14
CTQa2$_{CC}$_300	263.58	165.35	100.87	176.60	*CTQb2*$_{CC}$_300	260.25	170.08	99.26	176.53
CTQa2$_{TC}$_300	260.83	166.71	101.72	176.42	*CTQb2*$_{TC}$_300	257.36	171.84	99.79	176.33
CTQa2$_{TT}$_300	256.23	170.06	101.97	176.09	*CTQb2*$_{TT}$_300	252.95	174.98	99.99	175.97

[a] All values are expressed in a.u

222.24 a.u. [22], thus inferring that the expected CTQ stabilization in aqueous solution will be less than that of (4α→6″,2α→O→1″)-phenylflavan substituted with R=OH as we have previously reported.

The moduli values of the permanent electric dipole moments (μ) in the entire conformational space analyzed are shown in Table S4 in the Supplementary material; the values in the subspace δ (C2—C3—O3—H) next to the 300° are shown in Table 5.

Values ranging from 0.84 D to 5.27 D are found, showing that these compounds can be fitted to their surroundings based on dipole-dipole interactions.

Taking into account the entire conformational space, a statistical average is proposed at 298.15 K, based on the Maxwell-Boltzmann distribution, for each Cartesian component of μ according to the following expression:

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

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

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

The calculated value of μ for the most stable conformer is 3.077 D (CTQ*a1*$_{CT}$_300), while when all conformers at room temperature are considered according to the Maxwell-Boltzmann distribution, this is only 0.88 D.

Since the magnitudes and directions of the dipole moments are sensitive to molecular size and shape, they could also serve as a useful tool in conformational analysis [18]. Our results indicate that the μ modulus value varies over a wide range, which is also similar for the three groups (0.841 D to 5.792 D for CTQ*a* group; 1.146 D to 5.725 D for CTQ*b* group; and 0.836 D to 4.903 D for CTQ*c* group), indicating that the CTQ conformers cannot each be easily distinguished by this magnitude.

By comparison of CTQ*a1*$_{CT}$_300, CTQa*1*$_{TC}$_300, and CTQ*a2*$_{CT}$_300 structures, the μ modulus values are 3.077 D, 1.604 D, and 1.501 D, respectively (Table 5 and Fig. 3).

Table 5 Values of the modulus of the permanent dipole moment for different minimum energy conformers considered in the subspace δ (C2—C3—O3—H) next to the 300° in gas phase calculated at B3LYP/6-31G(d,p) level of theory in gas phase[a]

Conformer	μ_x	μ_y	μ_z	μ	Conformer	μ_x	μ_y	μ_z	μ
CTQa1$_{CT}$_300	0.544	2.743	1.284	3.077	*CTQb1*$_{CT}$_300	−1.611	−3.126	−1.763	3.934
CTQa1$_{CC}$_300	−2.808	−1.494	1.678	3.596	*CTQb1*$_{CC}$_300	0.539	−1.634	−1.948	2.599
CTQa1$_{TC}$_300	−0.12	−0.45	1.535	1.604	*CTQb1*$_{TC}$_300	−2.243	−0.811	−1.903	3.052
CTQa1$_{TT}$_300	−2.178	1.706	1.158	2.999	*CTQb1*$_{TT}$_300	−4.423	−2.308	−1.74	5.283
CTQa2$_{CT}$_300	−0.445	0.358	1.388	1.501	*CTQb2*$_{CT}$_300	−2.802	0.092	−1.085	3.006
CTQa2$_{CC}$_300	1.692	1.888	1.193	2.802	*CTQb2*$_{CC}$_300	0.564	1.215	−0.651	1.489
CTQa2$_{TC}$_300	−1.074	2.661	1.253	3.131	*CTQb2*$_{TC}$_300	3.237	2.244	−0.839	4.028
CTQa2$_{TT}$_300	−3.243	1.13	1.423	3.717	*CTQb2*$_{TT}$_300	5.507	0.932	−1.256	5.725

[a] All values are expressed in Debye

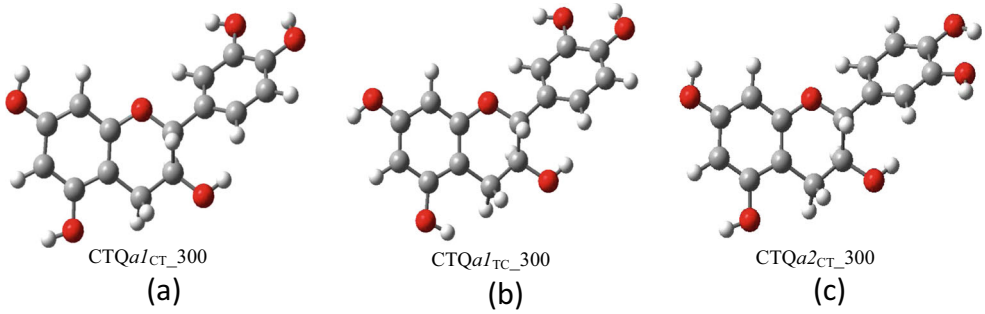

Fig. 3 Clockwise and anticlockwise arrangements of the OH substituents of resorcinol and catechol rings in structures optimized at B3LYP/6-31G(d,p) level

If we analyze these variations, when the OH groups of ring A have a clockwise orientation (CT-type conformation) the μ modulus value is greater than when the OH groups have a counterclockwise orientation (TC-type conformation) (Fig. 3a and b). In the case of two structures in which the variation occurs in the OH substituent arrangement of the catechol ring, if the ring B substituents have a counterclockwise orientation the μ value is greater than in structures where ring B substituents have a clockwise orientation (Fig. 3a and c).

Dipole moment changes against small structural modifications have been observed in other flavonoids [13, 30]. Moreover, these results show that μ has separable contributions, one associated with changes in the catechol ring and another related to the different conformations of the resorcinol ring, such as has been reported for other compounds [18, 22, 30].

The high flexibility of this type of structure results in a large conformational space and, as suggested previously [13], the large variation of the dipole moment modulus values and its directions indicate that it is very difficult to obtain a relationship between this descriptor and its biological activity without the knowledge of the orientation of the flavonoid in the internal portion of the enzyme. Which in turn shows that in order to understand the biological activity of CTQ it is important to know the conformational space and the values of the dipole moment for each conformer, also considering that the interaction with biomolecules can occur in complex ways, e.g., by metal chelation, hydrogen bonds of the OH groups, or van der Waals interactions.

Interestingly, for CTQ the μ_Z component can be either positive or negative, while this component was positive for all conformers of the (4α→6″,2α→O→1″)-phenylflavan substituted with R=OH [22]; this is precisely the reason why in CTQ the averaged modulus value over the whole conformational space is close to zero.

If we consider for calculating the representative value of μ in the subspace formed by conformers with δ (C2—C3—O3—H) next to 300°, the value obtained (μ =0.89 D) differs by only 0.01 D of the value obtained when considering the whole conformational space, which further supports that this subspace is sufficiently representative also in the case of modeling a property that appears to be highly sensitive to conformational change.

Conclusions

In this paper we present a deep analysis of the conformational space, considering the effects of free rotation around the C—O bonds of all OH substituents at different rings. It is concluded that at the level of calculation used the conformational space in vacuum comprises 107 lowest energy conformers, which are classified according to their structural characteristics. From the analysis of the stability order and percentage contribution of each of the described conformers at room temperature it is concluded that it is possible to propose a representative subspace of only 16 conformers.

It can also be concluded that there are specific charge delocalizations that explain the energy system, and that an analysis of natural bond orbitals (NBO) allows describing them, characterizing the hyperconjugative effects of charge delocalization, which are relevant for stabilizing structures. For further analysis of the structural and molecular properties of this compound in a biological environment, progress is made in modeling the polarizability and electric dipole moment, considering all the conformational space described; concluding that the former varies only slightly throughout the conformational space, while the second shows appreciable changes even under small conformational modifications. A Maxwell-Boltzmann statistical average is proposed for modeling these properties at room temperature, and considering the entire conformational space described. We conclude that for the electric dipole moment property, there is a major difference between this value and that which accounts for the most stable conformer. Furthermore, it is concluded that the subspace proposed from the energetic analysis, of only 16 structures, also shows to be representative for the analysis of both polarizability and electric dipole moment.

Since the magnitudes and directions of the dipole moments are sensitive to molecular size and shape, they can also serve in the conformational analysis to distinguish groups of the structures. However, the found fluctuations allow us to conclude that the dipole moment for CTQ is not useful to differentiate between different conformers.

The accumulated knowledge in this study in vacuum allowed to describe relevant intrinsic esteroelectronic standpoints, and is an essential input to proceed with the measure of

the effects of different solvents, and modeling of the antioxidant ability of CTQ.

Acknowledgments Thanks are due to the National Council of Scientific and Technical Researches of Argentina (CONICET) and Universidad de Buenos Aires (Argentina) for financial support. A.B..P. is a Senior Research Member of the National Research Council of Argentina (CONICET). E.N.B. acknowledges a fellowship from the National Council of Scientific and Technical Researches of Argentina (CONICET) and Universidad de la Cuenca del Plata (Corrientes, Argentina). R.M.L. acknowledges Centro de Cómputos de Alto Desempeño de la Universidad Nacional del Nordeste (CADUNNE) and Universidad Nacional del Nordeste for facilities provided during the course of this work, and financial support of the Secretaria General de Ciencia y Técnica de la Universidad Nacional del Nordeste (Corrientes, Argentina).

References

1. Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 247: 60–64
2. Visioli F, Galli C (1998) Olive oil phenols and their potential effects on human health. J Agric Food Chem 46:4292–4296
3. Mercader AG, Pomilio AB (2012) (Iso)Flav(an)ones, chalcones, catechins, and theaflavins as anticarcinogens: mechanisms, anti-multidrug resistance and QSAR studies. Curr Med Chem 19:4324–4347
4. Mercader AG, Pomilio AB (2011) Biflavonoids: occurrence, structural features and bioactivity. Nova Science Publishers, Inc., New York. ISBN 978-1-62100-354-0
5. Mercader AG, Pomilio AB (2013) Naturally-occurring dimers of flavonoids as anticarcinogens. Anticancer Agents Med Chem 13(8): 1217-35
6. Cook NC, Samman S (1996) J Nutr Biochem 7:2
7. Pérez-González A, Rebollar-Zepeda AM, León-Carmona JR, Galano A (2012) Reactivity indexes and O-H Bond dissociation energies of a large series of polyphenols: implications for their free radical scavenging activity. J Mex Chem Soc 56(3):241–249
8. Mendoza-Wilson AM, Lardizabal-Gutiérrez D, Torres-Moye E, Fuentes-Cobas L, Balandrán-Quintana RR, Camacho-Dávila A, Quintero-Ramos A, Glossman-Mitnik D (2007) Optimized structure and thermochemical properties of flavonoids determined by the CHIH(medium) DFT model chemistry versus experimental techniques. J Mol Struct 871:114–130
9. Mendoza-Wilson AM, Glossman-Mitnik D (2006) Theoretical study of the molecular properties and chemical reactivity of (+)-catechin and (−)-epicatechin related to their antioxidant ability. J Mol Struct THEOCHEM 761:97–106
10. Zhang J, Du F, Peng B, Lu R, Gao H, Zhou Z (2010) Structure, electronic properties, and radical scavenging mechanisms of daidzein, genistein, formononetin, and biochanin A: a density functional study. J Mol Struct THEOCHEM 955:1–6
11. Olejniczak S, Potrzebowski MJ (2004) Solid state NMR studies and density functional theory (DFT) calculations of conformers of quercetin. Org Biomol Chem 2:2315–2322
12. Zhang HY, Wang LF, Sun YM (2003) Why B-ring is the active center for genistein to scavenge peroxyl radical: a DFT study. Bioorg Med Chem Lett 13:909–911
13. Antonczak S (2008) Electronic description of four flavonoids revisited by DFT method. J Mol Struct THEOCHEM 856:38–45
14. Leopoldini M, Russo N, Toscano M (2007) A comparative study of the antioxidant power of flavonoid catechin and its planar analogue. J Agric Food Chem 55:7944–7949
15. Bentz EN, Pomilio AB, Lobayan RM (2014) Structure and electronic properties of (+)-catechin: aqueous solvent effects. J Mol Model 20: 2105. doi:10.1007/s00894-014-2105-z
16. Weber KC, Honório KM, Bruni AT, da Silva ABF (2006) The use of classification methods for modeling the antioxidant activity of flavonoid compounds. J Mol Model 12:915–920
17. Olivero-Verbel J, Pacheco-Londoño L (2002) Structure–activity relationships for the anti-HIV activity of flavonoids. J Chem Inf Comput Sci 42:1241–1246
18. Nguyen TV, Pratt DW (2006) Permanent electric dipole moments of four tryptamine conformers in the gas phase: a new diagnostic of structure and dynamics. J Chem Phys 124:1216–1219
19. Rasulev BF, Abdullaev ND, Syrov VN, Leszczynski J (2005) A quantitative structure-activity relationship (QSAR) study of the antioxidant activity of flavonoids. QSAR Comb Sci 24:1056–1065
20. Lobayan RM, Jubert AH, Vitale MG, Pomilio AB (2009) Conformational and electronic (AIM/NBO) study of unsubstituted A-type dimeric proanthocyanidin. J Mol Model 15:537–550
21. Bentz EN, Jubert AH, Pomilio AB, Lobayan RM (2010) Theoretical study of Z isomers of Atype dimeric proanthocyanidins substituted with R=H, OH and OCH3: stability and reactivity properties. J Mol Model 16:1895–1909
22. Lobayan RM, Bentz EN, Jubert AH, Pomilio AB (2012) Structural and electronic properties of Z isomers of (4α→6″,2α→O→1″)-phenylflavans substituted with R=H, OH and OCH3 calculated in aqueous solution with PCM solvation model. J Mol Model 18:1667–1676
23. Lobayan RM, Bentz EN, Jubert AH, Pomilio AB (2013) Charge delocalization in Z- isomers of (4α→6″,2α→O→1″)-phenylflavans with R = H, OH and OCH3. Effects on bond dissociation enthalpies and ionization potentials. J Comput Theor Chem 1006:37–46
24. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA, Vreven TJr, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2003) Gaussian 03, revision B.02. Gaussian Inc, Pittsburgh
25. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B 37:785–789
26. Becke AD (1993) Density-functional thermochemistry. III. The role of exact exchange. J Chem Phys 98:5648–5642
27. Leopoldini M, Marino T, Russo N, Toscano M (2004) Antioxidant properties of phenolic compounds: H-atom *versus* electron transfer mechanism. J Phys Chem A 108:4916–4922
28. Zhang HY, Sun YM, Wang XL (2003) Substituent effects on O—H bond dissociation enthalpies and ionization potentials of catechols: a DFT study and its implications in the rational design of phenolic antioxidants and elucidation of structure–activity relationships for flavonoid antioxidants. Chem A Eur J 9:502–508
29. Glendening ED, Reed AE, Carpenter JE, Weinhold F (2009) NBO 3.1. Program as implemented in the Gaussian 98 package. Madison, WI
30. Aparicio S (2010) A systematic computacional study on flavonoids. Int J Mol Sci 11:2017–2038