Journal of Hazardous Materials 163 (2009) 285–294

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

A theoretical study on vomitoxin and its tautomers

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ARTICLE INFO

Article history:
Received 16 April 2008
Received in revised form 30 May 2008
Accepted 21 June 2008
Available online 1 July 2008

Keywords:
Vomitoxin
Deoxynivalenol
Tautomers
Mycotoxin
Trichothecenes

ABSTRACT

In the present work, the structural and electronic properties of vomitoxin (deoxynivalenol, a mycotoxin) and all of its possible tautomers have been investigated by the application of B3LYP/6-31G(d,p) type quantum chemical calculations. According to the results of the calculations, tautomer V_4 has been found to be the most stable one among all the structures both in the gas and aqueous phases. The calculations also indicated that, vomitoxin and V_2 possess the deepest and the highest lying HOMO levels, respectively. Hence, V_2 is to be more susceptible to oxidations than the others. On the other hand, V_5(S) and vomitoxin have the lowest and the next lowest LUMO energies, respectively. Whereas, V_1 and V_2 possess quite highly lying (within the group) LUMO energy levels which result in comparatively unfavorable reduction potentials. Some important geometrical and physicochemical properties and the calculated IR spectra of the systems have also been reported in the study.

1. Introduction

Mycotoxins are secondary metabolites of molds, contaminating a wide range of crops of plants and fruits before or after harvest. The most important mycotoxins are: aflatoxins, vomitoxin, ochratoxin A, fumonisins, zearalenone, patulin and T-2 toxin.

The acute and chronic impact of mycotoxins on human and animal health is a scientifically proven fact. Mycotoxin contamination is recognized as an unavoidable risk because the formation of fungal toxins is weather dependent and effective prevention is impossible. According to the FAO reports, more than 25% of the world's agricultural production is contaminated with mycotoxins [1].

Trichothecenes are mycotoxins (a group of sesquiterpenes) produced by various *Fusarium* species like *Fusarium graminearum*, *Fusarium sporotrichioides*, *Fusarium poae* and *Fusarium equiseti*. Trichothecenes can be divided into four types (A–D) according to characteristic functional groups (see Fig. 1) [1]. T-2 and HT-2 toxins are A-type trichothecenes in which an oxygen functionality is different from a carbonyl function at the C-8 position (see Fig. 1). Whereas, B-type trichothecenes have a carbonyl function at this position [2]. The most frequently detected mycotoxin of this category is deoxynivalenol. C-type trichothecenes are characterized by a second epoxide function at C-7, -8 or C-9, -10, whereas D-type includes trichothecenes containing a macrocyclic ring between C-4 and C-15 having two ester linkages (see Fig. 1).

The most important structural features of trichothecenes responsible for their biological activities are the 12,13-epoxy ring (present in all the structures), the presence of hydroxyl or acetyl groups at the appropriate positions of the trichothecene nucleus and the structure and position of the side-chain. They are produced on many different grains like wheat, oats or maize.

Vomitoxin (12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-ene-8-one) also known as deoxynivalenol (DON) is a B-type trichothecene, which has been fully characterized as a tetracyclic, epoxy-sesquiterpene with seven stereo centers [3]. Vomitoxin possesses two secondary and one primary alcoholic OH groups, in addition to the presence of two chemically reactive functional groups, namely a conjugated ketone and an epoxide ring. At least one, but maybe both of these functionalities can be associated with the toxic activity of deoxynivalenol [4]. As a B-type trichothecene, deoxynivalenol is soluble even in water and in polar solvents such as aqueous methanol, aqueous acetonitrile, and ethyl acetate. The 12,13-epoxy group is extremely stable to nucleophilic attack, and deoxynivalenol is stable at 120 °C and is not decomposed under mildly acidic conditions [3,4].

Vomitoxin contamination occurs predominantly in grains such as wheat, barley, oats, rye, and maize, and less often in rice, sorghum, and triticale. The occurrence of vomitoxin is associated primarily with *F. graminearum* (*Gibberella zeae*) and *Fusarium culmorum*, both of which are important plant pathogens which cause *Fusarium* head blight in wheat and *Gibberella* ear rot in maize [4,5]. A direct relationship between the incidence of *Fusarium* head blight and contamination of wheat with deoxynivalenol has been established. Vomitoxin (deoxynivalenol) has been implicated in incidents of mycotoxicoses in both humans and farm animals [5].

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doi:10.1016/j.jhazmat.2008.06.087

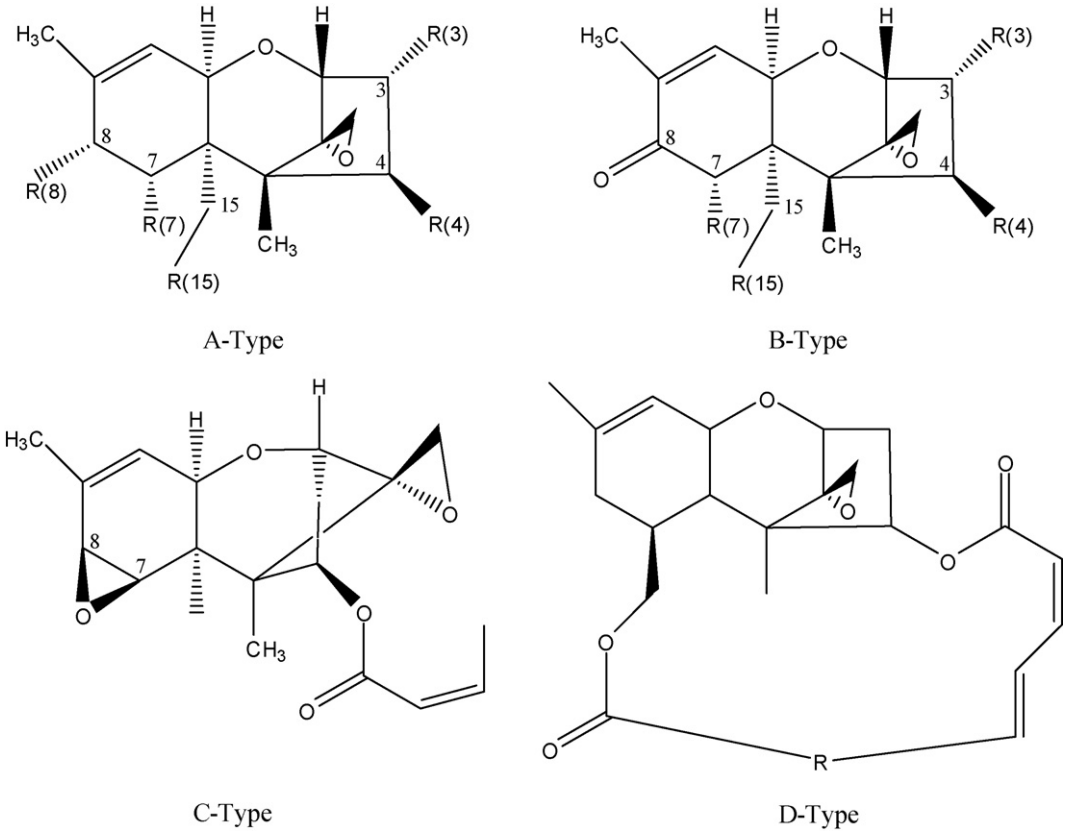

Fig. 1. Some types of trichothecenes.

It was reported that vomitoxin inhibits protein synthesis [6]. The immunosuppressive and carcinogenic character of *Fusarium* mycotoxins (They may possibly appear in some domestic food products.) led scientists to investigate the immunological effects of them on human peripheral blood mononuclear cells [7]. The synthesis and characterization of vomitoxin have been reported in Ref. [8] and the effective detection methods of deoxynivalenol and other trichothecenes have been described in Refs. [9–12].

Different toxic properties have been associated to trichothecenes [12–19]. As mentioned in the references given, there is considerable evidence that mycotoxins produced by microorganisms in our foodstuffs constitute a serious threat to human health through likely reactions of their enols and epoxides. Deoxynivalenol, being one of the trichothecenes has capability for keto–enol tautomerism. Moreover, the presence of epoxide group makes it capable of reacting directly with thiol forms of the vitaletheine modulators [7].

In the present study, vomitoxin and its possible keto–enol tautomers have been subjected to theoretical analysis within the level of density functional theory. Although there are few hundreds of publications on vomitoxin, none of its tautomers has been pronounced in the literature up to date.

2. Method

The initial geometry optimizations of all the structures leading to energy minima were achieved by using MM2 method followed by semi-empirical PM3 self-consistent fields molecular orbital (SCF MO) method [20,21] at the restricted level [22]. Then, further geometry optimizations were achieved using STO and RHF levels of theory and then within the framework of density functional theory (DFT, B3LYP) [23] at the level of 6-31G(d,p) (restricted closed-shell) [22]. The exchange term of B3LYP consists of hybrid Hartree–Fock and local spin density (LSD) exchange functions with Becke's gradient correlation to LSD exchange [24]. The correlation term of B3LYP consists of the Vosko, Wilk, Nusair (VWN3) local correlation functional [25] and Lee, Yang, Parr (LYP) correlation correction functional [26]. The normal mode analysis for each structure resulted in no imaginary frequencies for all the three methods of calculations.

In addition to calculations in the gas phase, the stabilities of all the structures in the aqueous phase have also been investigated. This is practically important since it is well known that these kinds of molecules should exist in body fluid of humans or animals, so that how to describe the interaction between the solute and the surrounding solvent molecules and the corresponding effect on the stability are essentially significant. Traditionally, self-consistent reaction field (SCRF) model was usually used to describe the effect of medium on chemical reactions. In this model, the microscopic information of molecular interaction between biomolecules and its surrounding molecules were neglected. Instead, the small water clusters were used to model solvent effects on some properties of the solute, such as tautomeric stabilities. Solvent effects were explored by using the SCRF method, and the polarizable continuum model (PCM) method was employed [27].

All these computations were performed by using Spartan 06 [28] and Gaussian 03 [29] package programs, and some geometrical and QSAR properties were calculated using Hyperchem (release 7.5) package program [30].

3. Results and discussion

In the present work, the structural and electronic properties of vomitoxin, V_0 (deoxynivalenol, a mycotoxin) and its tautomers,

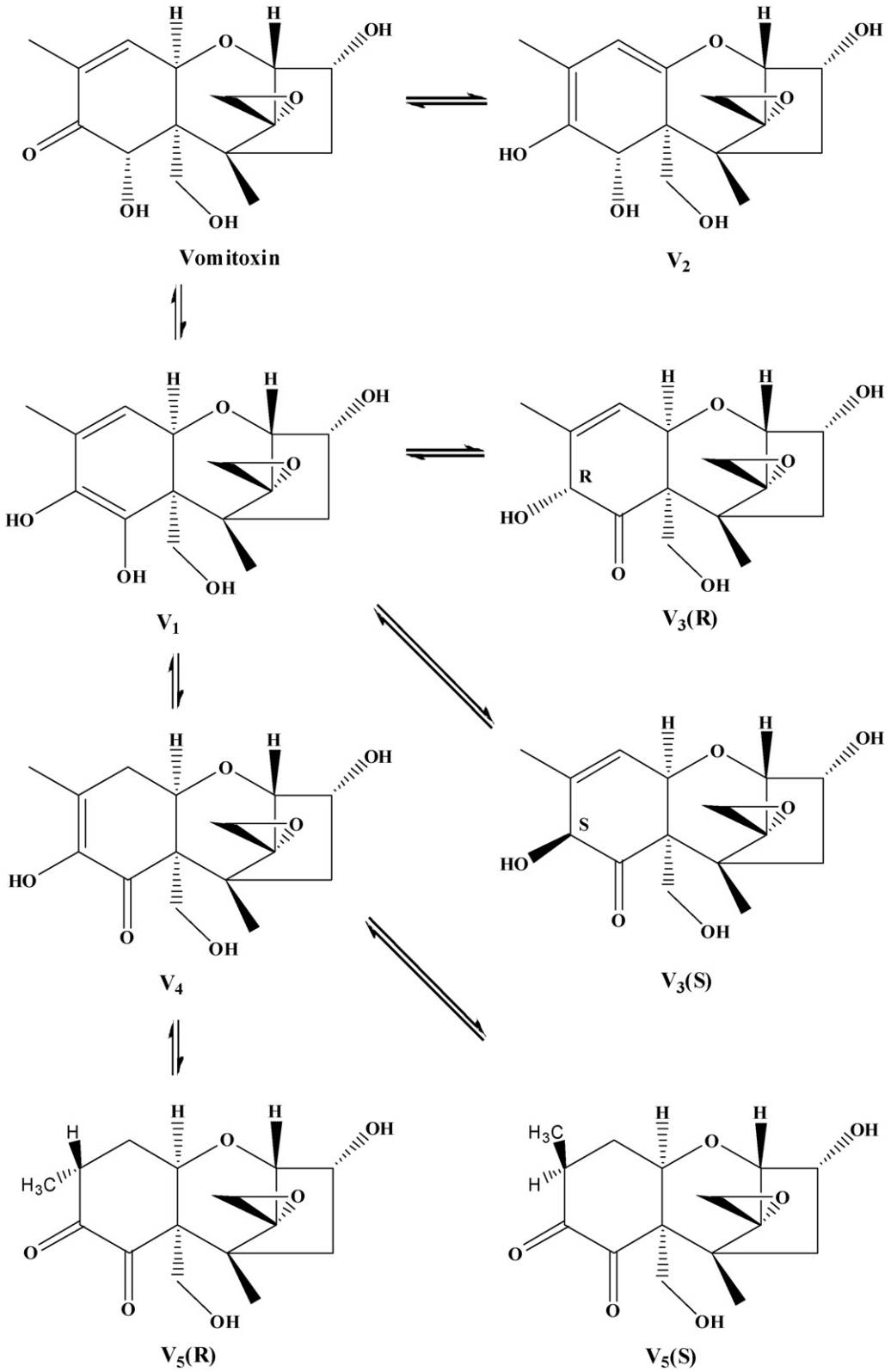

Fig. 2. Structures of vomitoxin and all of its possible tautomers.

V_1–V_5 (see Fig. 2 for the structures) have been investigated by performing B3LYP/6-31G(d,p) type quantum chemical calculations. The interesting features (see Section 1) of vomitoxin and the possibility of presence of various tautomeric forms make this study worthwhile.

Fig. 3 shows the geometry optimized (B3LYP/6-31G(d,p)) structures of the presently considered systems. The tautomer V_1 is obtained by 1,3-keto–enol tautomerism, while V_2 is obtained by 1,5-tautomerism of the parent compound vomitoxin. V_3(R) and V_3(S) are tautomeric forms of V_1. They have opposite absolute

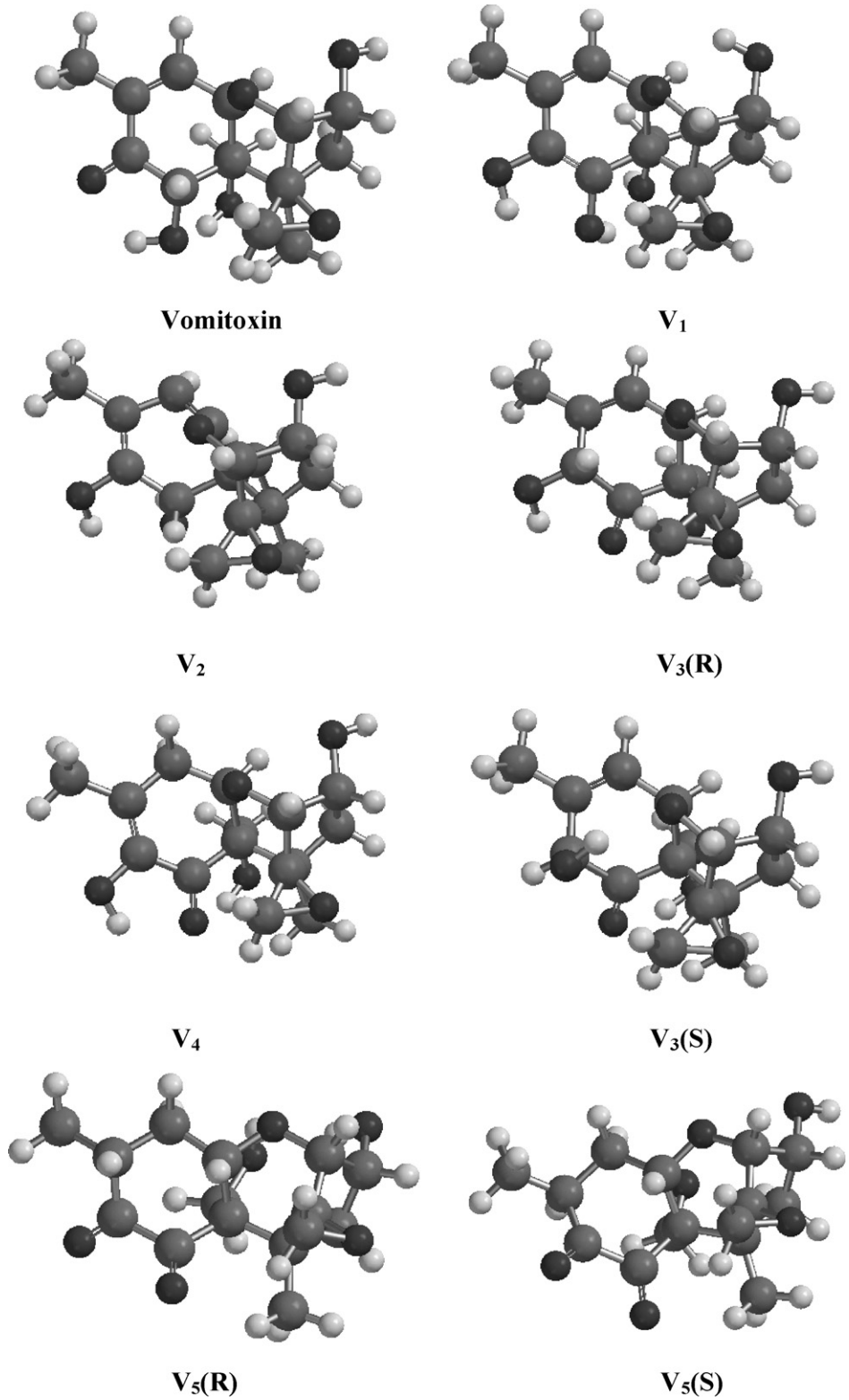

Fig. 3. Geometry optimized structures of vomitoxin and its possible tautomers.

configuration on the α-carbon next to the carbonyl group. V_3(R) and V_3(S) are enantiomeric pairs and possess R and S configurations on the carbon considered, respectively (see Fig. 2). Thus, the keto–enol tautomerism of V_1 should result in enantiomeric pairs, V_3(R) and V_3(S). On the other hand, V_4 derived from V_1, is a 1,5-tautomer. The 1,3-keto–enol tautomerism of V_4 results two enantiomeric pairs of tautomers V_5(R) and V_5(S). In the structure of vomitoxin, α-hydrogen atom, at least in theory, can easily undergo tautomerism because it is (due to the presence of adjacent C=O and –OH group) more acidic and better enolizable than any other α-methylenic or α-methine hydrogens. Therefore, at first glance V_1 seems to be a quite likely structure accompanying vomitoxin,

Table 1
Calculated (B3LYP/6-31G(d,p)) total energies (Hartree), energy of solvation (kcal/mol) and total energy in aqueous solution (Hartree) of vomitoxin and its tautomers

System	Total energy	Energy of solvation	Total energy (aquated)
Vomitoxin	−1034.8950029	12.40	−1034.875244
V_1	−1034.8658703	12.82	−1034.845443
V_2	−1034.7778816	13.26	−1034.756747
V_3(R)	−1034.8922613	12.72	−1034.871990
V_3(S)	−1034.8909691	12.11	−1034.871674
V_4	−1034.9026165	13.33	−1034.881371
V_5(R)	−1034.8852293	13.04	−1034.864456
V_5(S)	−1034.8850934	13.14	−1034.864151

Table 3
Gibbs free energy difference of tautomerization of the presently considered structures (kJ/mol)

Tautomerization	ΔG°
$V_0 \rightarrow V_1$	44.01
$V_0 \rightarrow V_2$	288.21
$V_1 \rightarrow V_3$ (R)	−25.39
$V_1 \rightarrow V_3$ (S)	−16.76
$V_1 \rightarrow V_4$	−79.40
$V_4 \rightarrow V_5$(R)	63.34
$V_4 \rightarrow V_5$(S)	90.64

V_0: vomitoxin.

especially in polar solvents. On the other hand, in vomitoxin, the hydrogen on the bridgehead position, flanked by etheric oxygen should undergo tautomerism to yield V_2. In this case, the geometry of the six-membered ring allows double bond formation at the bridgehead position, contrary to the Bredt's rule [31]. V_3 and V_4 are derived from V_1 (a dienol) by a shift of proton from alcoholic group to 2- and 4-positions of the diene π-system.

Table 1 tabulates the total energies (both in gas and aqueous phases) and the energy of solvation, obtained by means of B3LYP/6-31G(d,p) type quantum chemical calculations. According to the results obtained, V_4 has been found to be the most stable one among all. The predicted stability order is the same for both phases: V_4 > vomitoxin > V_3(R) > V_3(S) > V_5(R) > V_5(S) > V_1 > V_2. The greater stability of V_4 and vomitoxin over the other tautomers can be attributed to the conjugated enone system in their structures, in addition to the hydrogen bonding. According to the present level of calculations, V_4 appears to be more stable than vomitoxin. However, conversion of vomitoxin to V_4 necessitates the formation of a much less stable tautomer V_1, which should act as an energy barrier for the conversion of vomitoxin to V_4 or vice versa.

Vomitoxin and its various tautomers discussed in the present work possess many oxygens potentially capable of interacting with some Lewis acids including metal cations involved in structures of enzymes. Thus, overall nucleophilic character of these vomitoxin-related tautomers and vomitoxin itself should be interesting to investigate. Therefore, firstly we calculated the solvation energies (see Table 1). Although, the solvation energies for these species contribute little to overall energy, V_4 and V_3(S) have the greatest and the smallest solvation energy in the group, respectively. The solvent effects for neutral molecules acting as bases would be similar to ions but much less in magnitude [32].

Generally, –OH groups are solvated better in aqueous medium than the keto and ether groups because an –OH group can donate or accept hydrogen atom to form hydrogen bonding with water molecules. Whereas, keto and ether groups can only accept hydrogen for bonding of that sort. Therefore, solvation energies are expected to show some parallelism with the number of –OH groups among the isomeric (or tautomeric) structures. In the present case, the solvation energy order is partly explainable on this basis. However, configuration and conformation of the groups and neighboring group effect should also be operative. For instance, the solvation energy of V_4 is greater than V_1, although the former has less number of –OH groups.

Also it is noteworthy that the stereoisomers of the same tautomer exhibit some stability difference. The total energy difference between V_3(R) and V_3(S) is as small as 0.8 kcal/mol and in the case of V_5(R) and V_5(S), it is even smaller. Zero point energies, enthalpy, entropy and C_v values (standard thermodynamic quantities at 298.15 K and 1.00 atm) of the present species have been tabulated in Table 2.

Table 3 shows the ΔG° of tautomeric equilibria for vomitoxin-related species. The least probable tautomeric conversion seems to

Table 2
Some calculated thermodynamic properties of the present systems (B3LYP/6-31G(d,p)) (standard thermodynamic quantities at 298.15 K and 1.00 atm)

System	ZPE (kJ/mol)	Enthalpy[a] (kJ/mol)	Entropy (J/(mol K))	Gibbs free energy[b] (kJ/mol)	C_v (J/(mol K))
Vomitoxin	895.9031	948.2135	567.4109	779.0399	323.0283
V_1	894.4814	947.7286	571.9629	777.1979	329.4794
V_2	889.2196	943.2590	584.5859	768.9647	332.6701
V_3(R)	894.2055	946.8125	569.6010	776.9860	324.9301
V_3(S)	897.0435	949.1778	563.4169	781.1951	322.8843
V_4	894.5553	947.6375	576.3085	775.8111	324.8995
V_5(R)	895.9972	948.2360	569.4669	778.4494	320.8596
V_5(S)	887.7725	941.4308	585.0898	766.9863	321.2499

[a] Temperature correction.
[b] Entropy correction (Hv – TSv).

Table 4
Some geometrical and physicochemical properties of the systems considered presently (area, volume, polarizability, refractivity, hydration energy and dipole moment are in the order of 10^{-20} m^2, 10^{-30} m^3, 10^{-30} m^3, 10^{-30} m^3, kcal/mol, Debye, respectively)

	Vomitoxin	V_1	V_2	V_3(R)	V_3(S)	V_4	V_5(R)	V_5(S)
Area	278.85	280.30	284.19	280.03	275.54	281.72	276.92	286.38
Volume	279.76	280.26	281.53	280.23	279.84	279.96	278.80	281.20
Polarizability	28.28	28.64	28.64	28.28	28.28	28.28	27.92	27.92
Refractivity	71.62	73.92	73.93	71.62	71.62	72.09	70.36	70.36
log *P*	−0.17	−1.67	−1.92	−0.17	−0.17	−0.45	0.86	0.86
Hydration energy	−1.44	−2.67	−2.40	−1.77	−1.45	−2.26	−5.87	−5.99
Dipole moment	2.20	3.54	1.74	1.66	2.53	2.17	2.67	3.66

Table 5
B3LYP/6-31G(d,p) calculated LUMO, HOMO, $\Delta\varepsilon$, Mulliken's electronegativity and chemical hardness values for the structures presently considered (energies, electronegativities and hardness values are in eV) ($\Delta\varepsilon = \varepsilon_{LUMO} - \varepsilon_{HOMO}$)

Energy	Vomitoxin	V_1	V_2	V_3(R)	V_3(S)	V_4	V_5(R)	V_5(S)
LUMO	−2.02	−0.64	−0.99	−1.33	−1.34	−1.80	−1.93	−2.16
HOMO	−6.71	−5.24	−4.71	−6.70	−6.57	−6.19	−6.61	−6.43
$\Delta\varepsilon$	4.69	4.60	3.72	5.37	5.23	4.39	4.68	4.27
Mulliken's electronegativity	4.36	2.94	2.85	4.01	3.95	3.99	4.27	4.29
Chemical hardness	2.34	2.30	1.86	2.68	2.61	2.19	2.34	2.13

be $V_0 \rightarrow V_2$. The probable reason for it is not only low acidity of the hydrogen next to the etheric oxygen but also inductive effect of the methyl group in vomitoxin. The electron donating ability of the methyl group polarizes the double bond in such a way that negative partial charge development occurs, thus the acidity of the hydrogen theoretically involved in 1,5-tautomerism to yield V_2 is further decreased. Also, $V_4 \rightarrow V_5$(S) and $V_4 \rightarrow V_5$(R) conversions are not favorable according to ΔG° values. The conversion of vomitoxin

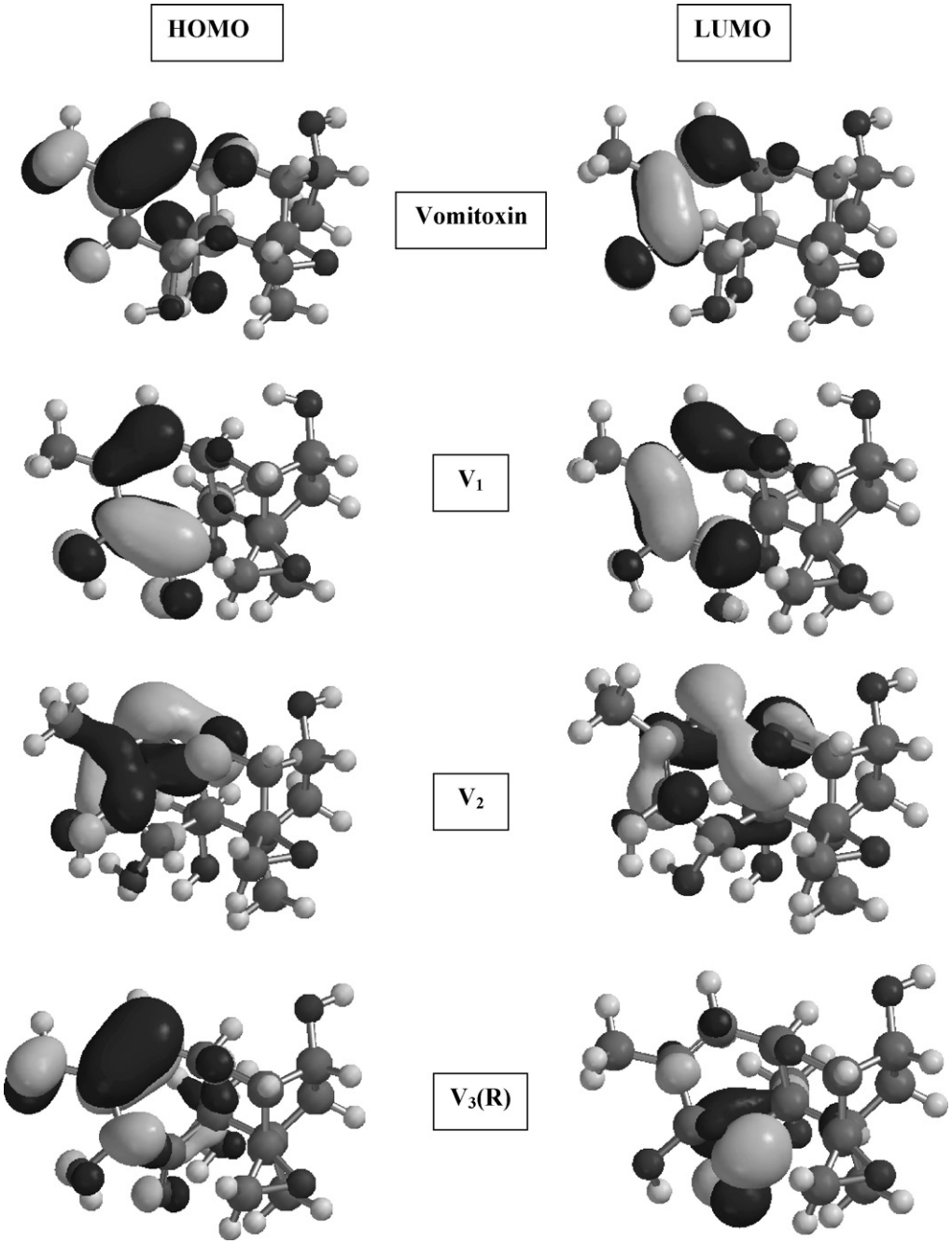

Fig. 4. Frontier molecular orbital schemes of the present systems.

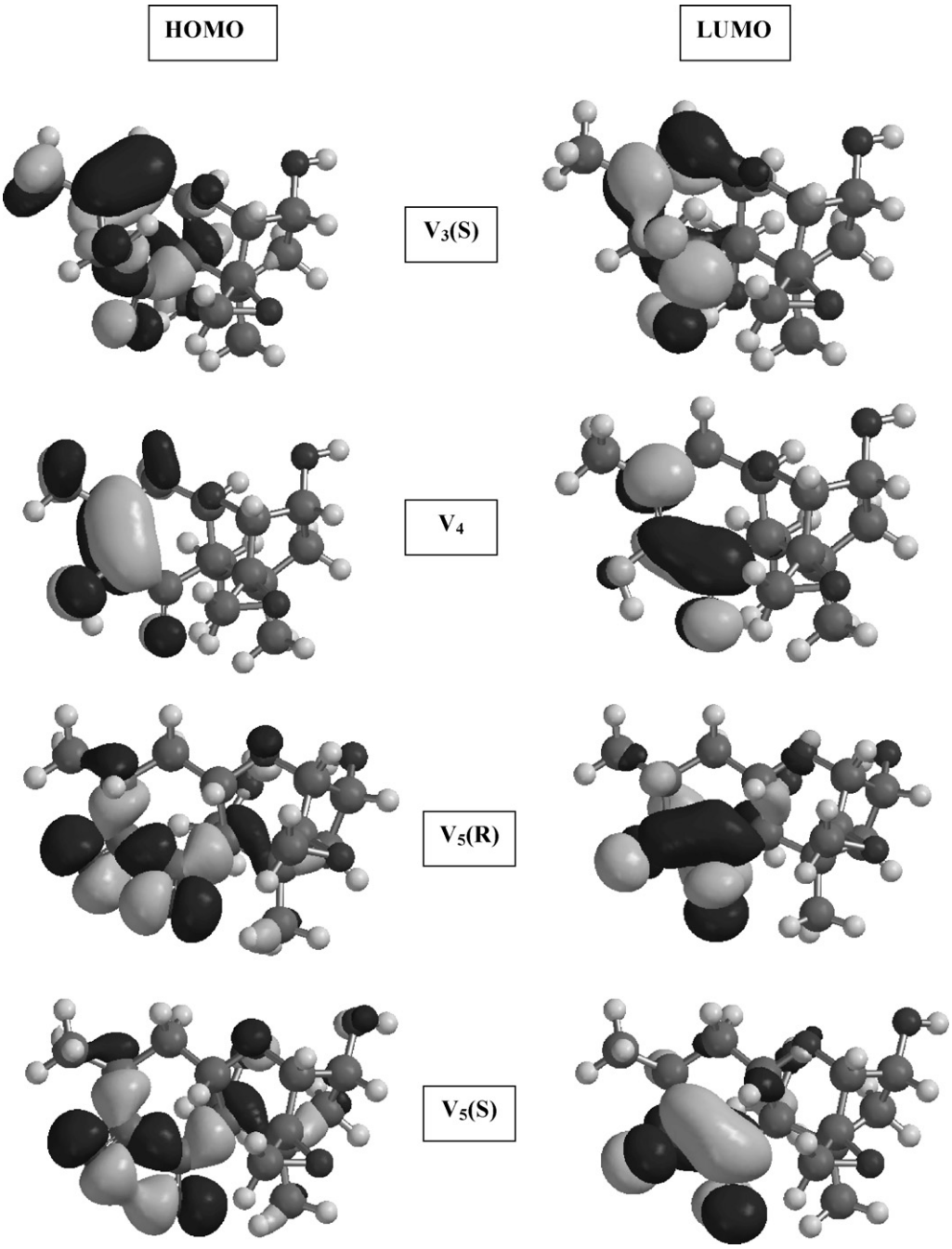

Fig. 4. (*Continued*).

to V_1 is also unlikely although the energy barrier for it is less. On the other hand, $V_1 \rightarrow V_4$, $V_1 \rightarrow V_3$ (R) and $V_1 \rightarrow V_3$ (S) conversions are characterized with negative free energy values.

In Table 4 various geometrical, physicochemical and QSAR properties of the vomitoxin-related systems are shown. These QSAR data can be used to correlate chemical structure quantitatively with a well-defined process, such as biological activity or chemical reactivity. Some data in the table are obtained based on group additivities which mean that they are independent of the geometry optimizations (such as refractivities and polarizabilities). On the other hand, log *P* (partition coefficient) values and hydration energies are to be considered on the relative basis, within the group but not to be taken as absolute values. The polarizabilities are due to atomic contributions whereas the refractivities are based on group contributions. Note that polarizability is the relative tendency of a charge distribution, like the electron cloud of an atom or molecule, to be distorted from its normal shape by an external electric field, which may be caused by the presence of a nearby ion or dipole. Hence, some structures considered have identical values in terms of these properties.

Table 5 tabulates the frontier molecular orbital energies (HOMO and LUMO), as well as the interfrontier energy gaps ($\Delta\varepsilon$). The data in Table 5 reveal that the enol form of vomitoxin, V_1, is characterized with narrowing of the interfrontier energy gap ($\Delta\varepsilon$ value) [33] as compared to the parent compound. This is due to the extended conjugation present in V_1, because the lone-pair electrons of –OH group may undergo conjugation with the diene π-system. However, V_2 has the smallest interfrontier energy gap within the group.

Whereas, $V_3(R)$ and $V_3(S)$ possess the highest and the next highest values, respectively. It is all because of no π-delocalization is possible in their structures in contrast to the other tautomers.

The stereochemistry of the groups in the enantiomers causes some varying degrees of torsions in the bond angles which consequently affect the conjugation, etc. For instance, in the case of V_5, the dione system of *S*-isomer is better conjugated than the *R*-isomer. Hence, the extended conjugation in the *S*-isomer lowers the LUMO but raises up the HOMO energy level resulting in the narrowing of interfrontier molecular orbital energy gap. Additionally, differences between the intramolecular hydrogen bonding capabilities and intramolecular quasi interactions between the lone pairs of –OH groups and the carbonyl carbon atom(s) could be responsible for the stability as well as spectral (see Fig. 5 for IR spectra) differences between the enantiomeric pairs.

Within the group, vomitoxin and V_2 possess the deepest and the highest lying HOMO levels, respectively. Hence, V_2 is to be more susceptible to oxidations than the others. Although, vomitoxin is practically quite stable, if its degradation occurs by atmospheric oxidation, it is probably via V_2 tautomer, although its mole fraction in the mixture is expected to be low as compared to vomitoxin. The calculations indicate that the behavior of enantiomers, $V_3(R)$ and $V_3(S)$ has to be more or less like vomitoxin in oxidation reactions. The species have many oxidizable groups, such as –OH and C=C. Chemical oxidations, including air oxidation, occur over these groups. The overall tendency of the system to undergo oxidations directed by the HOMO energy level and the electron density on the oxidizable groups.

On the other hand, $V_5(S)$ and vomitoxin have the lowest and the next lowest LUMO energies, respectively. Whereas, V_1 and V_2 possess quite highly lying (within the group) LUMO energy levels which result in comparatively unfavorable reduction potentials.

Fig. 4 shows the HOMO and LUMO of vomitoxin and its tautomers considered. As it is seen there, the frontier molecular

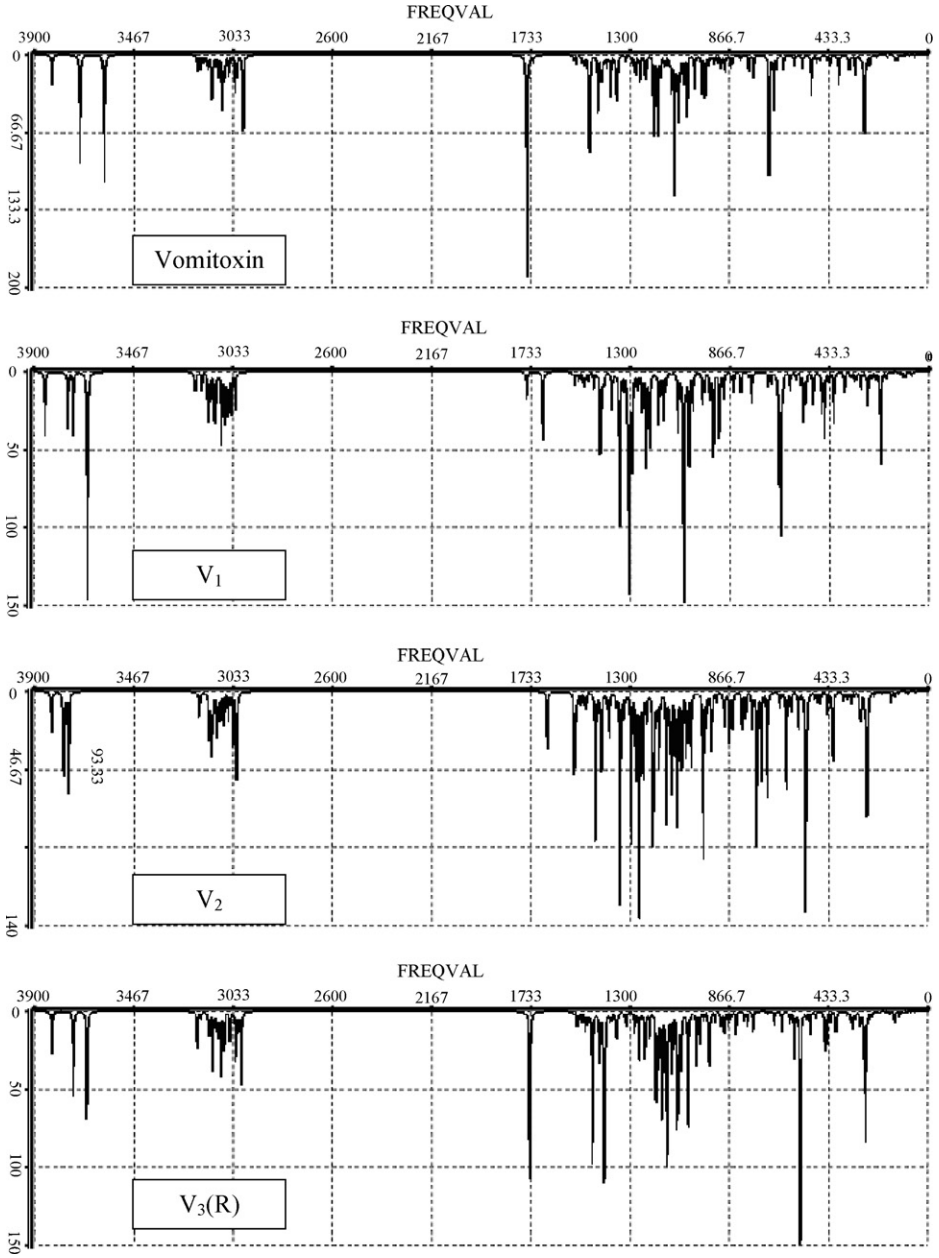

Fig. 5. Calculated IR spectra of vomitoxin and its tautomers (frequency values are in cm^{-1}).

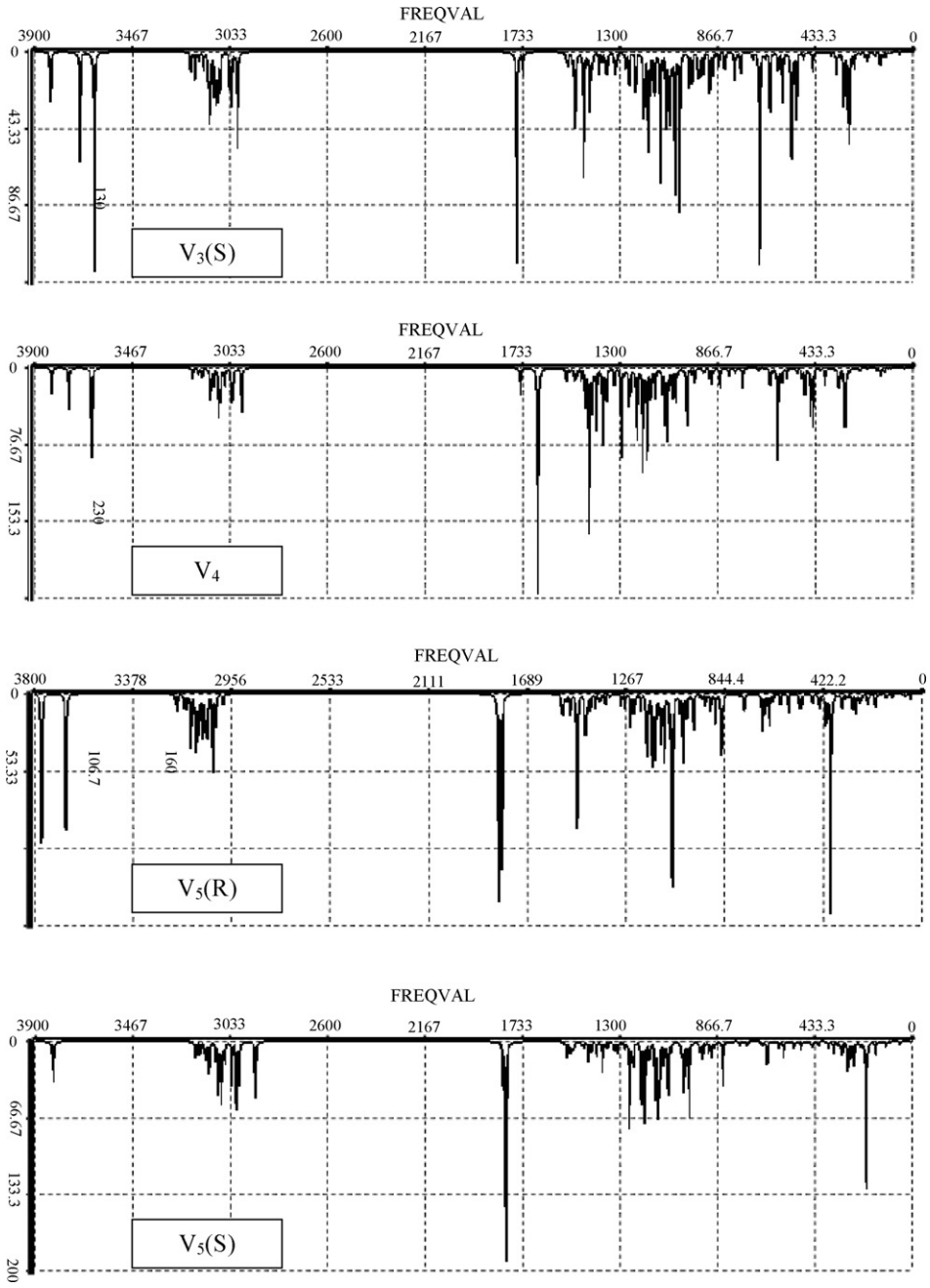

Fig. 5. (*Continued*).

orbitals (HOMO and LUMO) in almost every case are constituted by the contribution of atomic orbitals of the six-membered hydrocarbon ring system (cyclohexenone or cyclohexadienol moiety depending of the structure). Generally, the frontier molecular orbitals of the structures possess π-type (either π or π*) symmetry.

On the other hand, the chemical potential (μ) which is equal to χ_M (Mullikan's electro negativity) and η (absolute or chemical hardness) are important in reflecting chemical reactivity. The calculated χ_M and η values for vomitoxin and its tautomers are given in Table 5. χ_M and η are defined as [32],

$$\chi_M = \frac{I + A}{2}$$

$$\eta = \frac{I - A}{2}$$

where I and A are the ionization potential and electron affinity, respectively. Note that $I = -\varepsilon_{HOMO}$ and $A = -\varepsilon_{LUMO}$ within the validity of the Koopman's theorem [34].

When χ_M values are compared, vomitoxin and V_2 appear as the least and most susceptible species to oxidations, as directly predicted from the respective HOMO energies. On the other hand, the LUMO energies generally are very dependent on the size of the basis set used and its quality. This means that the results for both χ_M and η can contain some errors [32]. Fortunately, relative values for a series of related molecules or a series of possible structures for a given molecule are often quite reliable [32]. Therefore, χ_M and η values in Table 5 should be taken on relative basis rather than absolute values. Within this limitation, the η values predict that V_2 is the softest and V_3(R) is the hardest nucleophile among the group.

Fig. 5 shows the calculated (B3LYP/6-31G(d,p)) infrared spectra of the vomitoxin and its tautomers presently considered. All these structures possess many OH groups. Vomitoxin and V_4 additionally contain a conjugated enone group. $V_3(R)$ and $V_3(S)$ have a carbonyl group but unconjugated with an olefinic bond. Whereas, $V_5(R)$ and $V_5(S)$ possess two carbonyl groups in their structures. The calculated spectra give rather meaningful values for both O–H stretchings and C=O stretching ($\sim$1700 cm^{-1}) of the α,β-unsaturated keto group of vomitoxin (experimental value 1700 cm^{-1} [35]). The calculated carbonyl stretchings for $V_3(R)$, $V_3(S)$, $V_5(R)$ and $V_5(S)$ are all acceptable, too. The calculated IR spectra of vomitoxin shows strong peaks at 1733 (1698) cm^{-1} for C=O stretching, and 3644 (3480) cm^{-1} and 3701 (3644) cm^{-1} for the OH stretchings of different alcoholic groups. The values in parenthesis are obtained by Young and Games [36] from the FTIR spectra of vomitoxin. The % error in the calculation of the infrared spectra is only around 2% in the present case, which is quite reasonable. Unfortunately, in the literature there are no spectral data for the tautomers of vomitoxin to compare. In the spectra, the peaks at 3200–3000 cm^{-1} are to be due to various symmetric and asymmetric C–H stretchings and C=C stretchings mostly occur at 1500–1450 cm^{-1} (somewhat low). Actually, one should keep in mind that the positions of bands in calculated spectra are useful for relative comparison rather than absolute.

4. Conclusion

The present DFT calculations performed (both in gas and aqueous phases) at the level of B3LYP/6-31G(d,p) have revealed that tautomer V_4 should be more stable than vomitoxin itself. However, its co-presence with vomitoxin has not been mentioned in the literature, up to best of our knowledge. Assuming that the lack of mention about the existence of V_4 is indeed the result of some meticulous research, then the probable reason for it lies in the fact that the formation of V_4 is via another tautomer, V_1, which has relatively unfavorable energy content. Thus, the formation of V_4 should be blocked by V_1 considerably. The other tautomer V_2 is also found to be unfavorable energetically. Hence, the tautomers discussed presently should be present only in tiny amounts together with vomitoxin. However, by changing the conditions, such as solvent or temperature, they can be accessible for experimental studies.

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