JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Structural Basis for Antioxidant Activity of *trans*-Resveratrol: Ab Initio Calculations and Crystal and Molecular Structure

FRANCESCO CARUSO,[†] JOSEPH TANSKI,[‡] ADRIEL VILLEGAS-ESTRADA,[‡] AND MIRIAM ROSSI*,[‡]

Istituto di Chimica Biomolecolare, ICB-CNR, Piazzale Aldo Moro 5, I-00185, Roma, Italy, and Department of Chemistry, Vassar College, Poughkeepsie, New York 12604-0484

From the experimental crystal structure and ab initio calculations on resveratrol and its derivatives, structural features of mechanistic importance are described. The molecular structure reveals the relative coplanarity of the *trans*-stilbene skeleton, and the molecular packing in the solid state shows an extensive hydrogen bond network that elucidates the flip-flop motion of the three hydroxyl groups that alternately form and break H bonds with each of the neighboring phenolic oxygens. The dynamic behavior provoked by the alternation of hydrogen bond formation and breaking can result in the ready mobility of up to three hydrogen atoms per resveratrol molecule that can be transferred to reactive oxidants that are rich in electron density. In addition, theoretical studies confirm the planarity of resveratrol as well as for half of the molecule of a condensation dimeric derivative of resveratrol, *trans*-$\sigma$-viniferin. Furthermore, these studies show the *p*-4′-OH group to be more acidic compared to the other two *m*-OH groups. These features correlate with the biological activity of resveratrol as an antioxidant and support earlier studies showing H-atom transfer to be the dominant mechanism by which phenolic antioxidants intercept free radicals.

**KEYWORDS: Resveratrol; crystal structure; ab initio calculations; flip-flop hydrogen bonding; structural features; antioxidant activity**

## INTRODUCTION

Increasingly, phenolic compounds usually found in fruits and vegetables are of interest because many studies have shown that diet plays an important role in the prevention of many disease states (*1*−*4*). In particular, chronic degenerative conditions such as Alzheimer's and Parkinson's diseases, cancer, cardiovascular disease, and multiple sclerosis can be the result of cell damage or death due to severe oxidative stress (*5, 6*). Oxidative stress is caused by reactive oxygen species (ROS) that include free radicals such as the hydroxyl radical and nonradical compounds such as ozone. When the original chemical bond in the parent compound is cleaved homolytically because enough energy is provided to break the covalent bonds, a free radical is formed. This can occur either spontaneously or through heat or light. The body combats oxidative stress with antioxidants, compounds that prevent oxidation of important cellular targets, and with free radical scavengers that act on free radicals to give stable products. These defenses include endogenous compounds such as enzymes, for example, superoxide dismustase (SOD), and small molecules, for example, uric acid, as well as exogenous compounds obtained from the diet. Because naturally occurring antioxidants found in fruits and vegetables are generally less toxic and less likely to promote drug resistance, the mechanisms by which they act are of biomedical importance (*1*−*4*).

In this study, we focus on *trans*-resveratrol (*trans*-3,5,4′-trihydroxystilbene), a triphenolic phytoalexin found in a variety of plant species (*4, 7*), such as grapevines, mulberries, peanuts, and the dried roots and stems of *Polygonium cuspidatum* (Japanese knotweed). Its synthesis is triggered by plant stress conditions such as fungal infection, UV irradiation, and exposure to ozone or heavy metal ions (*4*). For human consumption, the greatest amounts of this compound are present in the grape skin and in red wine, although when isolated from *P. cuspidatum* it is used as an ingredient in traditional Asian medicine ("Kojo-kon") as a circulatory tonic (*4*). Related compounds such as the stereoisomer *cis*-resveratrol and the family of polymeric viniferins (condensed resveratrol entities) are also present in some foods, but their role is less clear.

*trans*-Resveratrol has extensive biomedical properties (*4*), as it is a strong antioxidant and anti-inflammatory agent, as well as antiestrogenic and anticarcinogenic activities. The absence of negative effects in rats when high doses of *trans*-resveratrol were administered is highly encouraging (*8*). As an anti-inflammatory agent, resveratrol appears to be unique because it discriminates between the two different cyclooxygenase (COX) isoforms and it is a potent inhibitor of both the peroxidase and cyclooxygenase active sites of COX-1 (*9*). It helps to prevent osteoporosis in menopausal women (*10*).

* Author to whom correspondence should be addressed (telephone 001 845 437 5746; fax 001 845 437 5732; e-mail rossi@Vassar.edu).
[†] Istituto di Chimica Biomolecolare.
[‡] Vassar College.

10.1021/jf048794e CCC: $27.50 
Published on Web 11/02/2004

Resveratrol appears to reduce the incidence of cardiovascular disease by curtailing the oxidation of low-density lipoproteins (LDLs) by free radicals or $Cu^{2+}$ (*11*−*14*). Data show that its activity differs from that of other LDL protective plant polyphenols, such as quercetin and catechin, because it is a much better $Cu^{2+}$ ligand (*11*−*13*).

Resveratrol has shown activity as an effective anticarcinogenic agent against several tumor cells in vitro and in vivo (*15*−*20*). Data suggest that resveratrol is a polycyclic aromatic hydrocarbon (PAH) antagonist, which can inhibit the proliferation of carcinogens through its interaction with the cytochrome P450 enzyme system (*20*). Recently, Cao and Li (*14*) have shown that resveratrol induces the production of cellular antioxidants and phase 2 enzymes in cardiovascular tissues/cells, followed by increased resistance to oxidative and electrophilic injury to these cells.

*cis*-Resveratrol is not as biologically active and demonstrates that the difference in molecular behavior of the two isomers is dependent on the three-dimensional molecular structure. For example, resveratrol interacts with the estrogen receptor in a stereoselective manner. Studies of the *cis*- and *trans*-resveratrol isomers on MCF-7 breast cancer cells show that the *trans* isomer is a much stronger mixed estrogen receptor-α (ER-α) agonist/antagonist than *cis*-resveratrol (*21*). Recently, docking experiments from molecular dynamics studies revealed greater hydrogen bond interactions between the *trans*-resveratrol binding to ER-α, providing an explanation for the stereoselective ligand binding with the protein (*22*). Structure−activity studies by Farines et al. (*23*) have shown that *trans*-resveratrol inhibits SOD in vitro and that it also binds to the estrogen receptor-β (ER-β) in vitro. The authors point out that its anticarcinogenic potential could result from these two effects in tandem: its binding to the ER-β in tumor cells and the resulting accumulation of the dioxygen radical, $O_2^{[\bullet-]}$ due to SOD inhibition could cause free radical damage and apoptosis of these cancer cells.

Important earlier work by Wright et al. demonstrated hydrogen atom transfer to be the dominant mechanism for phenolic antioxidants (*24*). Currently, the mechanisms of action of resveratrol are not completely understood despite its wide range of biochemical activities. To shed light on this, we examined the molecular structure of resveratrol and performed theoretical energy calculations on several excited states of *trans*-resveratrol and a dimeric derivative, *trans*-σ-viniferin. In particular, our results highlight the structural and mechanistic importance of the 4′-OH group of resveratrol to its biological activities. The acidity of this group and subsequent transfer of protons or hydrogen atoms to reactive species appear to be crucial to its antioxidant mechanism. The essential nature of the *p*-4′-OH is confirmed by several structure−activity studies showing it to be (a) the most genotoxic (*26*), (b) required for inhibition of cell proliferation (*27*), and (c) more effective at scavenging free radicals than the *m*-OH groups (*28*).

## MATERIALS AND METHODS

*trans*-Resveratrol was purchased from Sigma-Aldrich. Long, thin crystals were obtained from an ethanol/water 1:1 solution after ∼2 days. Several crystals had to be studied before one suitable crystal for data collection could be found. An initial assignment of a triclinic unit cell that was subsequently changed to the correct monoclinic one gave the unit cell parameters. X-ray diffraction data for resveratrol were collected on a Bruker P4 diffractometer equipped with a Smart CCD detector and crystal data; data collection and refinement parameters are summarized in **Table 1**. The structure was solved using direct methods and standard Fourier difference map techniques and was refined by full-matrix least-squares procedures on $F^2$ with SHELXTL (version 5.03) (*29*). From the difference map, two positions for each of the three disordered hydroxyl hydrogen atoms were seen, and the calculated positions agreed with the stronger of the two peaks. The quality of our data prevented us from refining these partially occupied sites, however, and all hydrogen atoms on carbon and oxygen were included in calculated positions and were refined as riding on the heavy atoms to which they were attached.

**Table 1.** Crystal Data of *trans*-Resveratrol

| | |
|---|---|
| elemental formula | $C_{14}H_{12}O_3$ |
| $M_f$ | 228.25 |
| *a* | 4.3791(5) Å |
| *b* | 9.2158(11) Å |
| *c* | 26.681(3) Å |
| σ | 92.748(2) |
| *V* | 1075.5(2) Å$^3$ |
| *z* | 4 |
| crystal system, space group | monoclinic, $P2_1/c$ (no. 14) |
| radiation, λ | Mo Kα, 0.71069 Å |
| temp | 243 K |
| crystal growth method | evaporation (2 days) |
| solvent system | 1:1 ethanol/water |
| crystal size, color | 0.20 × 0.10 × 0.10 mm, colorless |
| $D_c$ | 1.410 g cm$^{-3}$ |
| *F*(000) | 480 |
| μ (Mo Kα) | 0.099 cm$^{-1}$ |
| absorption (σ − scan), $T_{min} - T_{max}$ | 0.9805–0.9902 (SADABS) |
| data collection apparatus, method | Bruker Smart CCD area detector, phi omega scans |
| X-ray source | fine focus sealed X-ray tube |
| monochromator | graphite |
| *h* min,max/*k* min,max/*l* min,max | –2,5/–12,11/–35,34 |
| $2\sigma_{min} - 2\sigma_{max}$ | 1.53–28.30 |
| total no. of reflections | 2491 |
| reflections with $F_o^2 > 2\sigma(F_o^2)$ | 1254 |
| *R* (*R* all reflections) | 0.0602 (0.1327) |
| $R_w$ | 0.1509 |
| decay correction | Bruker (SAINT) |
| no. of parameters refined | 155 (0 restraints) |
| highest/lowest difference Fourier peak | 0.231/–0.329 |

The structural features of the molecules in the gaseous state were analyzed with theoretical methods [density functional theory (DFT)] and were performed using the Accelrys program Cerius 2.4.6, subroutine DMol3 (*30*), on an SGI Octane computer. The X-ray molecular structure of *trans*-resveratrol provided starting coordinates, and the optimized geometry was obtained through energy minimization. Standard local density was the Perdew and Wang (PWC) functional (*31*) using a double numeric basis set with polarization functions (DNP) (*32*) on all atoms. The same procedure was applied to three resveratrol anions obtained after the elimination of $H^+$ from the corresponding hydroxyls. Joining two *trans*-resveratrol units and proceeding to geometrical minimization as above gave the σ-viniferin coordinates. The phenyl ring rotational barrier in *trans*-resveratrol was analyzed by rotating the phenyl group containing the *p*-OH group and performing a single-point energy calculation every 10°.

## RESULTS AND DISCUSSION

The X-ray molecular structure, depicted in **Figure 1**, with geometrical bond distances and angles listed in **Table 2**, shows three sets of C−C bonds: the shortest is the olefin double bond, C(7)−C(8) = 1.333(3) Å, in agreement with the standard ethylene bond length of 1.337(6) Å (*33*); the intermediate lengths are those within the phenyl rings, ranging from 1.37 to 1.40 Å, and the longest bonds are at the olefin−phenyl junctions, C(4)−C(7) = 1.460(3) Å and C(8)−C(9) = 1.468(3) Å. The latter bonds are relatively short, however, compared with the normal C−C single bond of 1.54 Å, and indicate some resonance and subsequent double-bond character. As a consequence, the compound is quite planar, with torsion angles of C(7)−C(8)−C(9)−C(10) = −3.0° (demonstrating coplanarity between the

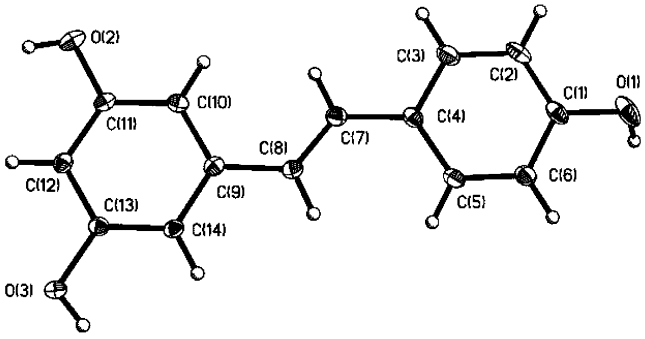


**Figure 1.** Molecular structure of *trans*-resveratrol obtained from crystal X-ray diffraction.

**Table 2.** (A) Interatomic Bond Distances [with esds in Parentheses (Angstroms)] and (B) Bond Angles [esds in Parentheses (Degrees)] for X-ray Structure of Resveratrol

(A) Bond Distances

| | | | |
|---|---|---|---|
| O(1)–C(1) | 1.385(3) | C(5)–C(6) | 1.373(3) |
| O(2)–C(11) | 1.379(3) | C(7)–C(8) | 1.333(3) |
| O(3)–C(13) | 1.392(3) | C(8)–C(9) | 1.468(3) |
| C(1)–C(2) | 1.371(4) | C(9)–C(10) | 1.398(3) |
| C(1)–C(6) | 1.377(4) | C(9)–C(14) | 1.399(3) |
| C(2)–C(3) | 1.372(4) | C(10)–C(11) | 1.366(3) |
| C(3)–C(4) | 1.396(3) | C(11)–C(12) | 1.391(3) |
| C(4)–C(5) | 1.396(4) | C(12)–C(13) | 1.383(3) |
| C(4)–C(7) | 1.460(3) | C(13)–C(14) | 1.374(3) |

(B) Bond Angles

| | | | |
|---|---|---|---|
| C(2)–C(1)–C(6) | 120.2(3) | C(10)–C(9)–C(14) | 118.7(2) |
| C(2)–C(1)–O(1) | 120.2(2) | C(10)–C(9)–C(8) | 122.0(2) |
| C(6)–C(1)–O(1) | 119.6(3) | C(14)–C(9)–C(8) | 119.3(2) |
| C(1)–C(2)–C(3) | 119.3(3) | C(11)–C(10)–C(9) | 120.3(2) |
| C(2)–C(3)–C(4) | 122.3(3) | C(10)–C(11)–O(2) | 118.8(2) |
| C(3)–C(4)–C(5) | 116.7(2) | C(10)–C(11)–C(12) | 121.5(2) |
| C(3)–C(4)–C(7) | 119.3(2) | O(2)–C(11)–C(12) | 119.8(2) |
| C(5)–C(4)–C(7) | 123.9(2) | C(13)–C(12)–C(11) | 117.9(2) |
| C(6)–C(5)–C(4) | 121.2(2) | C(14)–C(13)–C(12) | 121.8(2) |
| C(5)–C(6)–C(1) | 120.2(3) | C(14)–C(13)–O(3) | 120.4(2) |
| C(8)–C(7)–C(4) | 128.5(3) | C(12)–C(13)–O(3) | 117.8(2) |
| C(7)–C(8)–C(9) | 126.5(2) | C(13)–C(14)–C(9) | 119.8(2) |

olefin and the di-*m*-OH ring) and C(5)−C(4)−C(7)−C(8) = 8.0° (coplanarity between the olefin and the *p*-OH ring).

The X-ray structure of the parent compound, stilbene, was first studied by Robertson and Woodward (*34*) and has been the subject of numerous studies because in the solid-state crystal structure it, and molecules having the *trans*-stilbene skeleton, seemed to have an anomalously short ethylene bond. This was attributed to two factors: orientational disorder (flipping motion of the resveratrol molecule) and dynamical disorder due to torsional vibration about the C-phenyl bonds in a direction perpendicular to the molecular plane (*35*). Recently, Harada and Ogawa have performed accurate single-crystal diffraction studies that demonstrate that some of these dynamic disorders in stilbenes are mediated through a molecular pedal motion in the crystal and suggest that this is quite common for these structures (*36*). Resveratrol, unlike stilbene, has three hydroxyl groups that participate in an extensive three-dimensional hydrogen-bonding network, however. These additional interactions, despite the dynamic behavior shown by the hydroxyl hydrogen atoms, should curtail significant molecular orientational disorder by keeping the molecules more fixed in place. An examination of the thermal ellipsoids for the atoms in **Figure 1** shows that they appear to be elongated for some atoms [O(1), O(2), C(1), C(2), C(3), C(6)], and this is indicative of possible disorder; the disordered hydrogen bonding no doubt contributes to some of this anisotropic motion as well as some possible torsional vibration about the C(7)−phenyl bond.

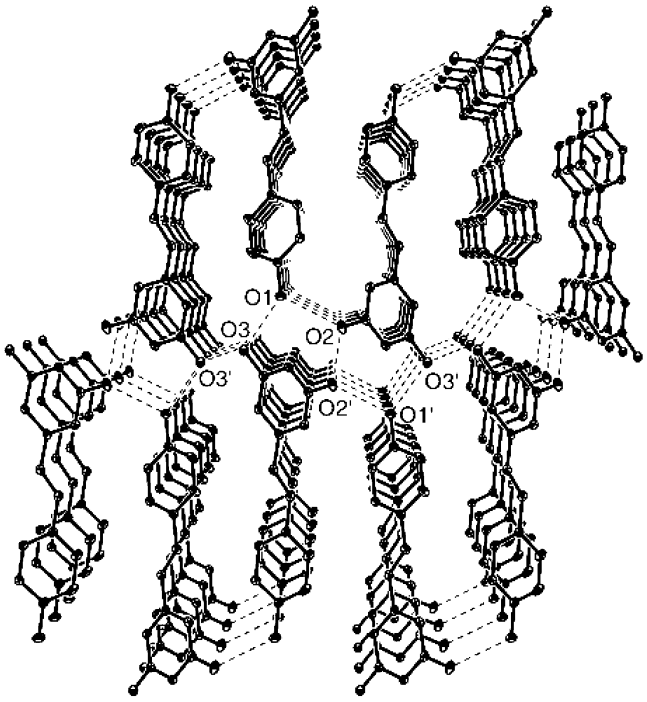


**Figure 2.** *trans*-Resveratrol packing diagram down the *a* axis showing the extended network of H bonds (dashed lines) obtained from X-ray diffraction. Disordered hydrogen atoms are not shown for clarity.

The X-ray structure determination of a related substituted stilbene having methoxy groups in place of the hydroxyl groups in resveratrol has been reported (*37*). Because the methoxy groups limit the hydrogen-bonding capability and also are significantly larger than the hydroxyl group, the structure is different, as expected, from that of resveratrol. There are two molecules in the asymmetric unit; one of the molecules is less planar than resveratrol, and the ethylene double bond is shorter than in resveratrol.

The crystal structure of the title compound shows the packing to be extremely interesting. The major intermolecular interactions are through hydrogen bonds. Each of the three oxygen atoms in the hydroxyl groups participates in two hydrogen bond intermolecular interactions. This three-dimensional solid-state structure rich in dynamic hydrogen bonds provides support for a hydrogen-atom transfer antioxidant mechanism for resveratrol.

The stacking distance between the molecules is close to 4.1 Å, similar to the *a* axis length (4.379 Å). The packing of the resveratrol molecules down the *a* axis is shown in **Figure 2**; this ensures maximal hydrogen bonding between the molecules. The hydrogen-bonding scheme is listed in **Table 3**. It consists of an infinite chain of hydrogen bonds down the *a* and *b* axes, and because of the centrosymmetrical orientation of the molecules, this chain is present at about $z = 0$ and the inverse chain at about $z = 0.5$. The geometrical details of the hydrogen bond oxygen chain are described in **Table 3** and shown in **Figure 2** with only oxygen atoms labeled. All three of the hydroxyl hydrogens must be disordered and simultaneously act as H-bond donors and H-bond acceptors to accommodate this hydrogen-bonding scheme. The three hydroxyl hydrogens, H(1), H(2), and H(3) [respectively bound to O(1), O(2), and O(3)] exist half the time in one position and half the time in another well-defined orientation, giving rise to a "flip-flop" H-bonding scheme as first described for cyclodextrins by Saenger (*38*). It is assumed that the disorder is dynamic, being associated with

**Table 3.** Intermolecular Hydrogen Bonds (D = Donor, A = Acceptor)

| D–H...A | D–H (Å) | H...A (Å) | D...A (Å) | D–H...A (deg) |
|---|---|---|---|---|
| O1–H1...O2 (–*x*, –1/2 + *y*, 1/2 – *z*) | 0.829 | 1.875 | 2.685 | 164.9 |
| O2–H2..O2 (–1 – *x*, 1 – *y*, 1 – *z*) | 0.830 | 1.928 | 2.727 | 161.4 |
| O3–H3...O3 (1 – *x*, –*y*, 1 – *z*) | 0.830 | 1.935 | 2.754 | 169.1 |

| hydrogen bond oxygen chain | distance (Å) | distance (Å) | angle (deg) |
|---|---|---|---|
| O2 (–*x*, –1/2 + *y*, 1/2 – *z*)...O1...O3 (*x*, –1/2 – *y*, *z* – 3/2) | O2...O1, 2.685 | O1...O3, 2.687 | O2...O1...O3, 96.0 |
| O2 (–1 – *x*, 1 – *y*, 1 – *z*)...O2...O1 (–*x*, 1/2 + *y*, 1/2 – *z*) | O2...O2, 2.727 | O2...O1, 2.685 | O2...O2...O1, 104.8 |
| O1 (*x*, –1/2 – *y*, –1/2 + *z*)...O3...O3 (1 – *x*, – *y*, 1 – *z*) | O1...O3, 2.687 | O3...O3, 2.754 | O1...O3...O3, 112.7 |

jumps of the hydrogens over the partially occupied sites, and that it is dictated by optimization of the hydrogen-bonding scheme. The flip-flop motion of the hydroxyl groups alternately forms and breaks H bonds with each of the neighboring phenolic oxygens and can support the transfer of up to three hydroxyl hydrogen atoms. This transfer along the chain of H bonds is involved in many chemical reactions in aqueous solutions and could be a fundamental process in many biological systems. An excess of the ROS rich in electron density such as the superoxide anion, the hydroxyl radical, and hydrogen peroxide can cause disease states. The dynamic mobility of electrophilic hydrogen atoms due to the flip-flop hydrogen-bonding scheme evident in the crystal structure of *trans*-resveratrol suggests its participation in the biological activity of resveratrol as an antioxidant. This antioxidant mechanism is used by one of the most potent phenolic antioxidants found in nature: a form of vitamin E, α-tocopherol, that transfers its phenolic proton to a propagating lipid peroxyl radical to terminate the chain reaction (*39*).

Because the extended network of H bonds in the crystal may be different or even absent in the isolated molecule, we performed theoretical ab initio calculations using Dmol3. These confirm that the most stable optimized structure for the *trans*-resveratrol molecule is planar. Thus, the torsion angles concerning both phenyl groups and the bridging CH═CH moiety are C(7)—C(8)—C(9)—C(10) = 2.9° (for the di-*m*-OH ring) and C(5)—C(4)—C(7)—C(8) = 7.2° (for the *p*-OH ring). Compared to the solid-state crystal structure, the *p*-OH ring keeps the same level of coplanarity with the attached phenyl ring. The olefin double bond appears to be elongated in the isolated molecule, C(7)—C(8) = 1.346 Å, compared with 1.333(3) Å in the crystal; however, the standard deviation in the latter accounts for such difference, and both bonds are equal.

The relative stability of the different phenolato species is calculated with additional ab initio calculations as there are three protons associated with OH that are involved in H bonds. The calculated bond distances of these structures are shown in **Figure 3**. The difference in energy between both *m*-phenolato anions is only 1 kcal/mol; that is, they are equally stable. The most stable phenolato is obtained after extraction of the $H^+$ in the para position, which is ∼7 kcal/mol lower than those having extracted meta protons. Therefore, removal of the hydrogen from the *p*-4′-OH group is highly preferred. It is known that the antioxidant activity of phenolic compounds depends on the nature and position of the substituents. For example, electron-withdrawing substituents, such as phenyl groups and −CH═CH—Ph groups in para positions, produce an increase in the antioxidant activity of the phenols by increasing the acidity of the hydroxyl hydrogen atom (*40*). These substituted phenols have lower bond dissociation energies for the phenolic O—H bond and are better antioxidants and effective radical scavengers. Therefore, the 4′-OH in resveratrol provides the most acidic hydrogen and contributes most to the compound's antioxidant activity, in agreement with its biological activity (*23*, *26*, *27*).

The importance of the *p*-hydroxyl group to the reactivity of resveratrol was recently suggested by analyzing excited states of *trans*-resveratrol (*41*, *42*) with theoretical methods, where charge rearrangement of the *p*-hydroxyl was more marked than for *m*-OH ones. Additional DFT analysis showed the same conclusions regarding higher tendency of the *p*-OH group, in comparison with the *m*-hydroxyls, to be engaged in radical

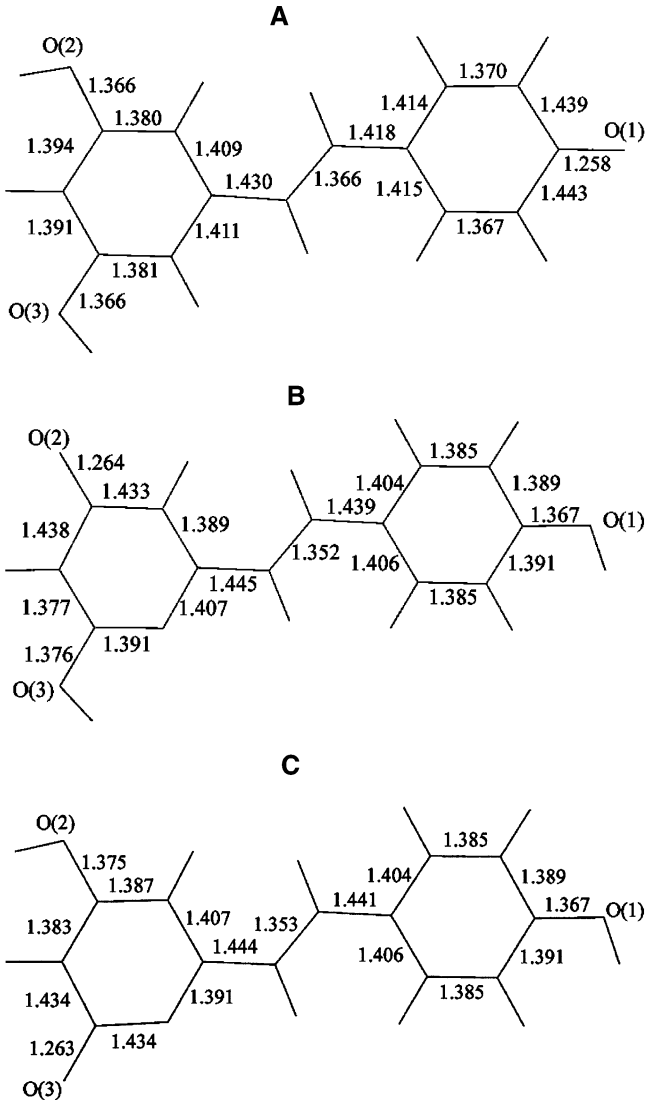


**Figure 3.** Bond distances (in Å) for the ab initio structure of (**A**) the most stable phenolato species of *trans*-resveratrol (obtained after extracting the proton at the *p*-4′-OH position), (**B**) the phenolato obtained after extracting the proton on one *m*-O(2)–H position, and (**C**) the phenolato obtained after extracting the proton bound to the *m*-O(3)–H position.

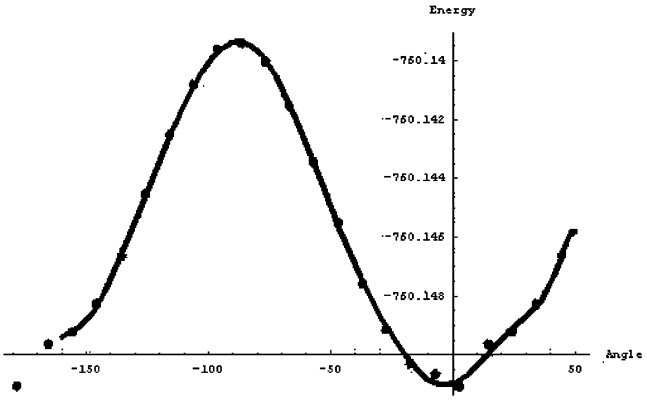


**Figure 4.** DFT single-point energy (hartrees) versus torsional angle C(5)–C(4)–C(7)–C(8) (rotation of the Ph group containing the *p*-4′-OH substituent).

activity (*43*). Therefore, any chemical or biological features that modify the acidity of resveratrol will probably increase its activity. These include the presence of reactive oxygen species that can interact with the 4′-OH.

Additional calculations have been performed to determine the rotational energy for the phenyl group containing the *p*-OH group, as such information can be important for resveratrol accommodation in enzyme pockets or interaction with DNA. **Figure 4** shows that the maximum energy corresponds to the phenyl arranged perpendicular to the CH═CH moiety and is 7.3 kcal/mol higher than that of the planar conformation.

Two examples of resveratrol cocrystallized with an enzyme exist: one with chalcone synthase, an enzyme used in the biosynthesis of plant flavonoid compounds (*44*), and one with transthyretin (TTR), involved in the formation of amyloid diseases (*45*). In both cases, the protein−ligand interactions include (1) resveratrol stacking between aromatic side chains due to the maintenance of its planar structure and (2) utilization of its hydroxyl groups to form extensive hydrogen bonds with the protein amino acids. In particular, for the latter case, the high potency of resveratrol as an inhibitor of TTR fibril formation is ascribed to the multiple hydrogen bonds it can form, with the *p*-4′-OH group being particularly strong.

*trans*-*σ*-Viniferin is a dimeric derivative of *trans*-resveratrol, formed by the addition of a *m*-hydroxyl and a neighboring aryl CH of one resveratrol unit across the double bond of the second resveratrol. It is found in grapes and extracted from *Vitis vinifera* (*46*) and also obtained after oxidation of *trans*-resveratrol by horseradish peroxidase (*47*). By taking advantage of the X-ray structure of *trans*-resveratrol, we also calculated the *σ*-viniferin DFT molecular structure; see **Figure 5**. The resveratrol unit structurally less affected is the one that reacted one *m*-OH (depicted as capped stick in **Figure 5**) and conserves some coplanarity; thus, torsion angles equivalent to C(5)−C(4)−C(7)−C(8) and C(7)−C(8)−C(9)−C(10) in *trans*-resveratrol (X-ray structure: 8.0° and −3.0°) become 15.4° (para) and −12.8° (di-meta). On the other hand, the resveratrol group that has lost its ethylene double bond, depicted as ball-and-stick in **Figure 5**, shows a marked loss of coplanarity with C(5)−C(4)−C(7)−C(8) and C(7)−C(8)−C(9)−C(10) of 30.2° and −10.8°, respectively. It must be recalled that coplanarity of *trans*-resveratrol is retained in the two enzyme−resveratrol complexes described earlier (*44*, *45*). Therefore, because half a molecule of *σ*-viniferin posseses marked coplanarity, a potential interaction of *σ*-viniferin with similar or related biological macromolecules requiring guest coplanarity suggests possible similar

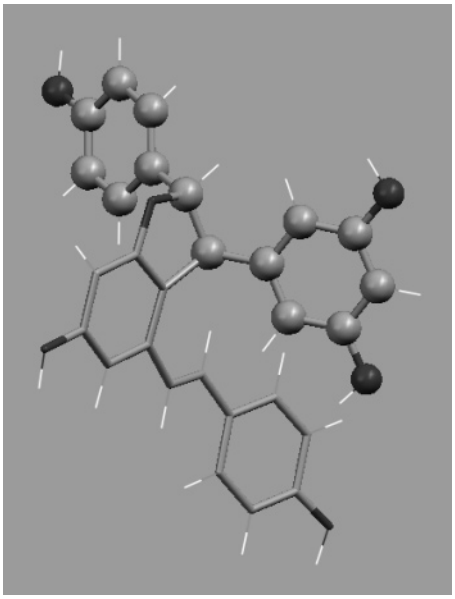

**Figure 5.** DFT structure of a dimeric derivative of *trans*-resveratrol, *σ*-viniferin. The capped-stick style atoms represent the more planar resveratrol moiety, whereas the atoms in the ball style represent the second resveratrol moiety that has lost its planarity and its central double bond. (One hydrogen of the former ethylene double bond is pointing toward the back of the picture and is hidden from view.)

structural roles for resveratrol and *σ*-viniferin, provided that the pocket size is large enough to accept the latter.

Theoretical analyses on the five viniferin phenolato derivatives are also performed. The most stable phenolato is that obtained after extraction of $H^+$ from the *p*-4′-OH on the planar moiety (capped stick, see **Figure 5)**: it is 4.6 kcal/mol more stable than the other possible *p*-phenolato species (from a less planar moiety). All *m*-phenolato species are even less stable. In particular (the comparison is to the most stable planar *p*-phenolato species), these values are 8.6 kcal/mol for the *m*-phenolato (planar) and 9.8 and 12.0 kcal/mol for the *m*-phenolato (nonplanar). These results support those for *trans*-resveratrol; that is, the *p*-4′-OH proton is the most labile, and the more planar the resveratrol unit, the more stable its phenolato derivative.

In conclusion, the experimental and theoretical results help to explain the biological activity of this simple substituted stilbene. The hydrogen bonding due to the molecular packing in the crystal structure demonstrates the ready mobility of up to three hydrogen atoms per resveratrol molecule. These hydrogen atoms may be transferred to reactive cell species to neutralize their harmful effects. The importance of the *p*-4′-OH group to the compound's activity is explained on the basis of its increased acidity compared to the other two *m*-OH groups.

## ACKNOWLEDGMENT

We thank Danya Machnes for growing the crystals at Vassar College, Prof. Ged Parkin at Columbia University for use of the diffractometer, the Camille and Henry Dreyfus Foundation (M.R.), the Vassar College URSI program (M.R., A.V.-E.), and Drs. Paolo Bovicelli and Roberto Antonioletti (ICB-CNR) for helpful conversations and use of facilities while on sabbatical leave.

**Supporting Information Available:** Crystallographic CIF file. This material is available free of charge via the Internet at http://pubs.acs.org

## LITERATURE CITED


(1) Havsteen, B. H. The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther.* **2002**, *96*, 67−202.

(2) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: History, production, and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313

(3) Birt, D. F.; Hendrich, S.; Wang, W. Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol. Ther.* **2001**, *90*, 157−177.

(4) Frémont, L. Minireview: Biological effects of resveratrol. *Life Sci.* **2000**, *66*, 663−673.

(5) Arouma, O. I.; Bahorun, T.; Jen, L.-S. Neuroprotection by bioactive components in medicinal and food plant extracts. *Mutat. Res.* **2003**, *544*, 203−215.

(6) Conte, A.; Pellegrini, S.; Tagliazucchi, D. Synergistic protection of PC12 cells from $\beta$-amyloid toxicity by resveratrol and catechin. *Brain Res. Bull.* **2003**, *62*, 29−38.

(7) Sanders, T. H.; McMichael, R. W.; Hendrix, K. W. Occurrence of resveratrol in edible peanuts. *J. Agric. Food Chem.* **2000**, *48*, 1243−1246.

(8) Juan, M. E.; Vinardell, M. P.; Planas, J. M. The daily oral administration of high doses of *trans*-resveratrol to rats for 28 days is not harmful. *J. Nutr.* **2002**, *132*, 257−260.

(9) Szewczuk, L. M.; Forti, L.; Stivala, L. A.; Penning, T. M. Resveratrol is a peroxidase-mediated inactivator of COX-1 but not COX-2. *J. Biol. Chem.* **2004**, *279*, 22727−22737.

(10) Calabrese, G. Nonalcoholic compounds of wine: the phytoestrogen resveratrol and moderate red wine consumption during menopause. *Drugs Exp. Clin. Res.* **1999**, *25*, 111−114.

(11) Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. The red wine phenolics *trans*-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin. Chim. Acta* **1995**, *235*, 207−219.

(12) Frémont, L.; Belguendouz, L.; Delpal, S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Sci.* **1999**, *64*, 2511−2521.

(13) Belguendouz, L.; Frémont, L.; Gozzelino, M.-T. Interaction of *trans*-resveratrol with plasma lipoproteins. *Biochem. Pharmacol.* **1998**, *55*, 811−816.

(14) Cao, Z.; Li, Y. Potent induction of cellular antioxidants and phase 2 enzymes by resveratrol in cardiomyocytes: protection against oxidative and electrophilic injury. *Eur. J. Pharm.* **2004**, *489*, 39−48.

(15) Surh, Y.-J.; Hurh, Y.-J.; Kang, J.-Y.; Lee, E.; Kong, G.; Lee, S. J. Resveratrol, an antioxidant present in red wine, induces apoptosis in human promyeolocytic leukemia (HL-60) cells. *Cancer Lett.* **1999**, *140*, 1−10.

(16) Asensi, M.; Medina, I.; Ortega, A.; Carretero, J.; Baño, M. C.; Obrador, E.; Estrela, J. M. Inhibition of cancer growth by resveratrol is related to its low bioavailabilty. *Free Radical Biol. Med.* **2002**, *33*, 387−398.

(17) Hsieh, T.-C.; Wu, J. M. Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines. *Exp. Cell Res.* **1999**, *249*, 109−115.

(18) Soleas, G. J.; Grass, L.; Josephy, P. D.; Goldberg, D. M.; Diamandis, E. P. A comparison of the anticarcinogenic properties of four red wine polyphenols. *Clin. Biochem.* **2002**, *35*, 119−124.

(19) Hsieh, T.-C.; Burfeind, P.; Laud, K.; Backer, J. M.; Traganos, F.; Darzynkiewicz, Z.; Wu, J. M. Cell cycle effects of gene expression by resveratrol in human breast carcinoma cell lines with different metastatic potentials. *Int. J. Oncol.* **1999**, *15*, 245−252.

(20) Casper, R. F.; Quesne, M.; Rogers, I. M.; Shirota, T.; Jolivet, A.; Milgrom, E.; Savouret, J.-F. Resveratrol has antagonist activity on the aryl hydrocarbon receptor: implications for prevention of dioxin toxicity. *Mol. Pharm.* **1999**, *56*, 784−790.

(21) Basly, J. P.; Marre-Fournier F.; LeBail, J. C.; Habrioux, G.; Chulia, A. J. Estogenic/antiestrogenic and scavenging properties of (*E*)- and (*Z*)-resveratrol. *Life Sci.* **2000**, *66*, 769−777.

(22) Abou-Zeid, L. A.; El-Mowafy, A. M. Differential recognition of resveratrol isomers by the human estrogen receptor-$\alpha$: molecular dynamics evidence for stereoselective ligand binding. *Chirality* **2004**, *16*, 190−195.

(23) Farines, V.; Monje, M.-C.; Telo, J. P.; Hnawia, E.; Sauvain, M.; Nepveu, F. Polyphenols as superoxide dismustase modulators and ligands for estrogen receptors. *Anal. Chim. Acta* **2004**, *513*, 103−111.

(24) Wright, J. S.; Johnson, E. R.; DiLabio, G. A. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. *J. Am. Chem. Soc.* **2001**, *123*, 1173−1183.

(25) Kirlin, W. G.; Cai, J.; DeLong, M. J.; Patten, E. J.; Jones, D. P. Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. *J. Nutr.* **1999**, *129*, 1827−1835.

(26) Matsuoka, A.; Takeshita, K.; Furuta, A.; Ozaki, M.; Fukuhara, K.; Miyata, N. The 4′-hydroxy group is responsible for the in vitro cytogenetic activity of resveratrol. *Mutat. Res. G* **2002**, *521*, 29−35.

(27) Stivala, L. A.; Savio, M.; Carafoli, F.; Perucca, P.; Bianchi, L.; Maga, G.; Forti, L.; Pagnoni, U. M.; Albini, A.; Prosperi, E.; Vannini, V. Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol. *J. Biol. Chem.* **2001**, *276*, 22586−22594.

(28) Brede, O.; Stojanovic, S.; Sprinz, H. Antioxidant action of *trans*-resveratrol and its analogues in the radiation-induced liposome oxidation. *Free Radical Res.* **2002**, *36* (Suppl. 1), 76−77.

(29) Sheldrick, G. M. *SHELXTL, An Integrated System for Solving, Refining and Displaying Crystal Structures from Diffraction Data*; University of Göttingen: Göttingen, Germany, 1981.

(30) Delley, B. An all-electron numerical method for solving the local density functional for polyatomic molecules. *J. Chem. Phys.* **1990**, *92*, 508−517.

(31) Perdew, J. P.; Wang, Y. Accurate and simple analytic representation of the electron gas correlation energy. *Phys. Rev. B* **1992**, *45*, 13244−13249.

(32) Delley, B. Fast calculation of electrostatics in crystals and large molecules. *J. Phys. Chem.* **1996**, *100*, 6107−6110.

(33) Allen, F. H.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. Chapter 9, Sec 5. Typical Interatomic Distances: Organic Compounds. In *International Tables for Crystallography, Vol. C: Mathematical, Physical and Chemical Tables*, 3rd ed.; Prince, E., Ed.; Kluwer Academic Publishers: Boston, MA, 2004; pp 790−812.

(34) Robertson, J. Monteath; Woodward, I. X-ray analysis of the bibenzyl series. IV. Detailed structure of stilbene. *Proc. R. Soc. London* **1937**, *A162*, 568−583.

(35) Ogawa, K.; Sano, T.; Yoshimura, S.; Takeuchi, Y.; Toriumi, K. Molecular structure and intramolecular motion of (*E*)-stilbenes in crystals. An interpretation of the unusually short ethylene bond. *J. Am. Chem. Soc.* **1992**, *114*, 1041−1051.

(36) Harada, J.; Ogawa, K. Invisible but common motion in organic crystals: a pedal motion in stilbenes and azobenzenes. *J. Am. Chem. Soc.* **2001**, *123*, 10884−10888.

(37) Yin, Q.; Shi, Y.-M.; Liu, H.-M.; Li, C.-B.; Zhang, W.-Q. (*E*)-3,5,4′-Trimethoxystilbene. *Acta Crystallogr.* **2002**, *E58*, o1180−o1181.

(38) Saenger, W.; Betzel, C.; Hingerty, B.; Brown, G. M. Flip-flop hydrogen bonding in a partially disordered system. *Nature (London)* **1982**, *296*, 5857. Saenger, W.; Betzel, C.; Hingerty, B.; Brown, G. M. Flip-flop hydrogen bridging bonds in $\beta$-cyclodextrin—a general principle in polysaccharides? *Angew. Chem.* **1983**, *95*, 908−909.

(39) Bowry, V. R.; Ingold, K. U. The unexpected role of vitamin E ($\alpha$-tocopherol) in the peroxidation of human low-denisty lipoprotein. *Acc. Chem. Res.* **1999**, *32*, 27−34.

(40) Brigati, G.; Lucarini, M.; Mugnaini, V.; Pedulli, G. F. Determination of the substituent effect on the O−H bond dissociation enthalpies of phenolic antioxidants by the EPR radical equilibration technique. *J. Org. Chem.* **2002**, *67*, 4828−4832.
(41) Del Nero, J.; de Melo, C. P. Quantum chemistry calculations of resveratrol and related stilbenes. *Opt. Methods* **2001**, *21*, 455−460.
(42) Del Nero, J.; de Melo, C. Investigation of the excited states of resveratrol and related molecules. *Int. J. Quantum Chem.* **2003**, *95*, 213−218.
(43) Cao, H.; Pan, X.; Li, C.; Zhou, C.; Deng, F.; Li, T. Density functional theory calculations for resveratrol. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1869−1871.
(44) Ferrer, J.-L.; Jez, J. M.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis. *Nat. Struct. Biol.* **1999**, *6*, 775−784.
(45) Klabunde, T.; Petrassi, H. M.; Oza, V. B.; Raman, P.; Kelly, J. W.; Sacchettini, J. C. Rational design of potent human transthyretin amyloid disease inhibitors. *Nat. Struct. Biol.* **2000**, *7*, 312−321.
(46) Privat, C.; Telo, J. P.; Bernardes-Genisson, V.; Vieira, A.; Souchard, J.-P.; Nepveu, F. Antioxidant properties of *trans*-σ-viniferin as compared to stilbene derivatives in aqueous and nonaqueous media. *J. Agric. Food Chem.* **2002**, *50*, 1213−1217.
(47) Langcake, P.; Pryce, R. J. Oxidative dimerization of 4-hydroxystilbenes in vitro: prodution of a grapevine phytoalexin mimic. *J. Chem. Soc., Chem. Commun.* **1977**, *7*, 208−210.

**Received for review July 20, 2004. Revised manuscript received September 9, 2004. Accepted September 13, 2004.**

JF048794E