Revised structure of *trans*-resveratrol: Implications for its proposed antioxidant mechanism

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ABSTRACT The crystal structure of *trans*-resveratrol has been redetermined by X-ray diffraction. The newly refined structure demonstrates that the previously reported, dynamically disordered hydrogen-bonding network is rather the superposition of two crystallographically independent molecules of *trans*-resveratrol. This latter arrangement possesses a well-defined hydrogen-bonding network in a unit cell of double the previously reported volume. While not meant as a criticism of the proposed antioxidant mechanism itself, the present studies clearly show that the X-ray diffraction data should no longer be used for its additional support.

GRAPHICAL ABSTRACT.

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is a phenolic natural product that has garnered considerable interest from the scientific and medical communities owing to its life-extending potential through the prevention of metabolic, neurodegenerative, and cardiovascular diseases, as well as cancer.^{1,2,3,4,5}

Scheme 1: Structure of *trans*-resveratrol

The X-ray crystal structure of resveratrol was first reported in 2004 by Caruso et al., where it was shown that each of the three hydroxyl groups participates in two intermolecular hydrogen bonding interactions.⁶ However, the locations of the hydroxyl hydrogen atoms were determined to be disordered over two positions and appeared to simultaneously participate in H-bond donor and acceptor interactions. This observation was rationalized in terms of a dynamically disordered hydrogen bonding network in which the hydrogen atoms “flip-flop” over two positions. The authors further suggest that the observed dynamic disorder may relate to the biological function of resveratrol, in particular providing “support for a hydrogen atom transfer antioxidant mechanism”. We became interested in the extensive hydrogen-bonding network observed in resveratrol as an opportunity to study weak interactions in the crystalline state by X-ray diffraction methods. In addition, resveratrol is an excellent candidate to expand our studies on ligand-receptor binding based on high resolution crystallographic measurements.^{7,8,9,10,11} In this context, we have redetermined the crystal structure of resveratrol and demonstrate herein that the dynamic disorder proposed by Caruso et al. is incorrect. The disordered structure derives from a previously unrecognized superstructure, resulting in the superposition of two crystallographically independent resveratrol molecules. Our revised model reveals that the hydrogen-bonding network in crystalline resveratrol is both well-defined and static.

General Procedures - *Trans*-resveratrol was purchased from Bulksupplements.com and used as received. Crystals of *trans*-resveratrol were grown by evaporation from a solution initially consisting of 1:1 acetonitrile/ethanol.

X-ray Crystallography - Diffraction data were collected at 120 K using a Bruker Duo diffractometer with an APEX II CCD detector using Mo-K α radiation ($\lambda=0.71073$ Å). Cell constants were obtained from the complete data set and frame data were integrated using SAINT V8.34A.¹² An absorption correction was made using the program SADABS.¹³ The structure was solved by direct methods and refined by least-squares methods against F^2 using the SHELXTL program suite.¹⁴ All non-H atoms were refined with anisotropic displacement parameters. Hydroxyl hydrogen atoms were located in the difference Fourier map and refined with isotropic atomic displacement parameters. Other hydrogen atoms were calculated on idealized positions and refined with a riding model. (CCDC 1440764)

The crystal structure of *trans*-resveratrol has been determined previously at 243 K in the monoclinic space group $P2_1/c$.⁶ The reported unit-cell volume was 1075.5(2) Å³ with lattice constants $a = 4.3791(5)$ Å, $b = 9.216(1)$, $c = 26.681(3)$ and $\beta = 92.748(2)^\circ$. The refined structural model was suggestive of dynamic disorder of the hydroxyl hydrogen atoms. Prior to beginning a high resolution experiment, we have crystallized *trans*-resveratrol and redetermined the structure at 120 K.

Crystallization - Initial crystallization experiments from ethanol-water solution confirmed the previously reported result, i.e., thin plates with the smallest dimension of ca. 10 μm were obtained, however, the unit cell volume was observed to be double that previously reported. Subsequently it was found that prismatic crystals can be obtained by slow evaporation from a mixture of 1:1 acetonitrile/ethanol.

Data collection - The crystal data and structure refinement details are listed in supplementary Table S1. The X-ray diffraction experiment at room temperature, at 243 K (as previously reported)⁶ and at 120 K did not agree with the published unit-cell parameters, the length of the *a* axis being doubled (*a* = 4.3791(5) Å reported vs. 8.796(8) at rt; 8.748(6) at 243 K and 8.667(2) Å at 120 K). Examination of reciprocal space showed the presence of superstructure reflections in the diffraction pattern (supplementary figure S1), which were clearly missed in the previous experiment.

Structure - The asymmetric part of the unit-cell consists of two *trans*-resveratrol molecules. The molecular structure and numbering scheme is presented in Fig. 1.

Figure 1. Molecular structure and numbering scheme of two symmetrically independent molecules of *trans*-resveratrol. Thermal ellipsoids are drawn at 50% probability.

The molecules are rather planar with dihedral angles between the planes of the aromatic rings of 8.66(6)° and 2.13(8)° respectively for molecules A and B. The values of valence bond distances and angles (supplementary material) do not differ significantly between the two molecules; no bond distances between non-hydrogen atoms differ by more than 4 σ . Small differences in bond angles are observed for the hydroxyl groups, $\angle\text{C}-\text{C}-\text{O}$, and are related to the difference in hydrogen bonding interactions around the two independent molecules. The most obvious structural difference between molecule A and B lies in the positions of the hydroxyl hydrogen atoms, and is clearly visible in an overlay of the two molecules (Fig. 2). This figure also confirms that the previously reported molecular structure⁶ of *trans*-resveratrol is actually the superposition of molecules A and B. In other words, the positions of the hydrogen atoms correspond with what was previously described as dynamic disorder of the hydroxyl H-atoms (Fig. 2). The C–C and C–O bond distances are normal and in good agreement with the average literature values.¹⁵ Full crystallographic and geometric details have been deposited.

Figure 2. Overlay diagram of A and B molecules of *trans*-resveratrol showing the different orientation of the OH groups.

In the crystal structure, molecules are connected by strong O–H···O hydrogen bonds. Each molecule forms six hydrogen bonds (3 donors and 3 acceptors), each with a different molecule (Fig. 3), with quite short donor-acceptor distances (H···O, 1.80 – 1.85 Å) and with $\angle$ D–H–A angles larger than 160° (Table 1). The molecules form extended chains with head-to-tail arrangement in the [101] direction through O1A–H21A···O3B^V and O3A–H23A···O1B^{II} hydrogen bonds (for symmetry codes, see Fig. 3; a packing diagram has been deposited). The chains are connected to each other by the four remaining hydrogen bonds which, together, form a complex, three dimensional network (Table 1). As most phenolic hydrogen atoms typically lie close to the aromatic plane,¹⁶ the strength of the hydrogen bonds in the present case is also manifested in the deviation of three of the hydrogen atoms from this plane (torsion angles 32.0(1), 26.7(1), and 18.2(1)° for O1A – C1A, O3B – C13B and O2A – C11A respectively).

Figure 3. Hydrogen bonding scheme of *trans*-resveratrol. Thermal ellipsoids are drawn at 50% probability. Symmetry codes: (I) $x, -y+1/2, z+1/2$ (II) $-x+1, -y+1, -z+1$ (III) $x+1, -y+1/2, z+1/2$ (IV) $x, y-1, z$ (V) $-x, -y+1, -z$

Table 1. Hydrogen bonds for *trans*-resveratrol [Å, °]

D–H...A	d(D–H)	d(H...A)	d(D...A)	$\angle$ DHA
O1A–H21A...O2B	0.88(2)	1.81(2)	2.6718(15)	165.2(19)
O2A–H22A...O1B ^{III}	0.90(2)	1.85(2)	2.7104(15)	160.2(18)
O3A–H23A...O3B ^I	0.92(2)	1.80(2)	2.7116(15)	170.6(17)
O1B–H21B...O3A ^V	0.90(2)	1.83(2)	2.7183(15)	166.7(18)
O2B–H22B...O2A ^{IV}	0.88(2)	1.81(2)	2.6799(15)	170.6(19)
O3B–H23B...O1A ^{II}	0.89(2)	1.84(2)	2.7057(15)	164.1(18)

Symmetry codes: (I) $x, -y+3/2, z+1/2$; (II) $-x, -y+1, -z$; (III) $x, y-1, z$; (IV) $x-1, -y+1/2, z-1/2$; (V) $-x+1, -y+1, -z+1$

High-quality crystals of *trans*-resveratrol were grown by evaporation from a mixture of acetonitrile/ethanol (1:1) and its crystal structure was redetermined at 120 K. In contrast to the previous report of the crystal structure of *trans*-resveratrol, our model includes superstructure reflections and reveals a static, well-defined hydrogen-bonding network. While not meant as a criticism of the proposed antioxidant mechanism itself, the present studies clearly show that the X-ray diffraction data should no longer be used for its additional support.

SUPPORTING INFORMATION

Crystallographic information file (CIF), reciprocal space maps, residual Fourier maps, summary of crystallographic data, tables of bond lengths and angles.

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