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Resveratrol: a representative of a phytoalexin polymeric family

by

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Abbreviations

COX-2	Cyclooxygenase-2
EpRE	Electrophile response element
ER β	Oestrogen receptor β
$HO^{\bullet}$	Hydroxyl radical
H_2O_2	Hydrogen peroxide
LC	Liquid chromatography
LC – MS	Liquid chromatography – mass spectrometry
LPS	Lipopolysacharides
$O_2^{\bullet -}$	Superoxide
PMA	Phorbol esters
QR	Quinone reductase
ROI	Reactive oxygen intermediates
ROS	Reactive oxygen species
RSV	Resveratrol

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Abstract

Resveratrol is a representative of the plant antibiotics family called *phytoalexins*. It is found in red wine and a variety of plant species. Resveratrol exists in two isomeric forms, *trans* and *cis*, with the last one being less biologically active. The structure of resveratrol is hard to detect because of the "flip-flop" motion of its hydroxyl groups. Once fixed in place, the real structure of the molecule can be reflected using diffraction procedure. Among popular methods of detecting resveratrol are: UV light absorption, gas chromatography, electrochemical processes, etc. A selective multichannel liquid chromatography – electrochemistry method is described to detect resveratrol in rat blood. Resveratrol is considered to be an important activator of phase II enzymes (UDP-glucuronyl transferase, quinone reductase 1). Resveratrol has strong antioxidant qualities in the presence of reducing agents. It is necessary to perform more clinical studies to fully realize resveratrol potential.

Introduction

It has been known for centuries that natural products prevent many disease states. Consequently, exceptional interest in applying the properties of natural compounds for treating ailments has led to advances in biomedical research. Amid the compounds in the scope of investigation is a *viniferin* polymeric family. It is in the family of plant antibiotics called *phytoalexins*. This family consists of toxic compounds produced by higher plants in response to attack by pathogens and to other stresses, particularly fungal invasion[*].

One of the representatives of phytoalexins is resveratrol (RSV), a very active ingredient among stilbene phytoalexins [1]. Resveratrol is found in high concentrations in red wine [2]. This fact helps in explaining the so-called "French paradox": epidemiological studies with the French population that demonstrated an inverse correlation between consumption of red wine and incidents of deaths resulting from coronary heart diseases [2]. Resveratrol has been more frequently studied since it was linked to its influences on cardiovascular regulation [*e.g.* 3]. However, since the reports have been made on cancer chemopreventive activity of RSV [4], such effects as induction of apoptosis [5] and protection against oxidative DNA damage [6] have been vigorously investigated. This paper will review RSV isomeric forms, structure, detection, interaction with phase II enzymes, and antioxidant effects.

Isomeric Forms

Resveratrol (3,5,4'-trihydroxistilbene) exists in two isomeric forms: *trans* and *cis* (**Fig. 1**). Both forms were found in red wine [7]. However, the *trans* isomer was found in a greater variety of plant species, such as grapevines, peanuts, mulberries, and the dried roots of *Polygonum cuspidatum* [8].

[*] Dictionary: http://cancerweb.ncl.ac.uk/cgi-bin/omd?query=phytoalexin&action=Search+OMD

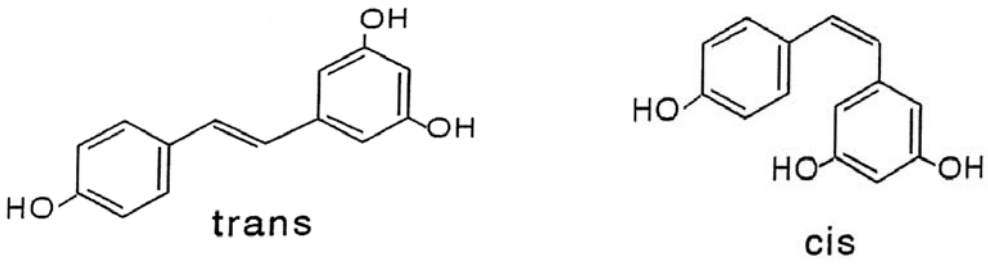

Fig. 1. Isomeric forms of resveratrol: *trans* and *cis*. [9].

Biomedical properties of *trans*-RSV include anticarcinogenic [5], anti-inflammatory [10], anti-oxidant [11], and other effects. The role of *cis*-RSV is less clear. Even though *cis* isomer is present in some foods, it is not as biologically active as its stereoisomer. A study on MCF-7 breast cancer cells [12] has shown that the agonistic/antagonistic interaction of the *trans*-RSV with estrogen receptor-α (ER- α) is much stronger than *cis* isomer interaction. Therefore, the interaction occurs in a stereoselective manner. Difference in behavior of RSV stereoisomers is dependent on the three-dimensional molecular structure.

Structure

In 1937, crystal X-ray diffraction was performed on a "parent" compound of RSV, stilbene [mentioned in 13]. The results showed surprisingly short ethylene bonds. The outcome was explained by orientational and dynamical disorder of the RSV molecule during measurements. The disorder issue was eliminated by keeping the molecule fixed in place during diffraction [13]. **Figure 2** shows a structure of ***trans*-**RSV. The structure of the molecules shows its relative coplanarity. The molecular packing of RSV (**Fig. 3**) shows a hydrogen bond network where H bonds are formed and broken by the "flip-flop" motion of the hydroxyl groups. This motion allows for the transfer of up to three hydroxyl hydrogen atoms along the chain of H bonds. The transfer of hydrogen atoms explains the mobility of a resveratrol molecule and suggests its participation in chemical reactions of biological systems. It is considered that the dynamics of hydrogen atoms in the crystal studying of ***trans*-**RSV allows for its participation in biological reactions as an antioxidant[13].

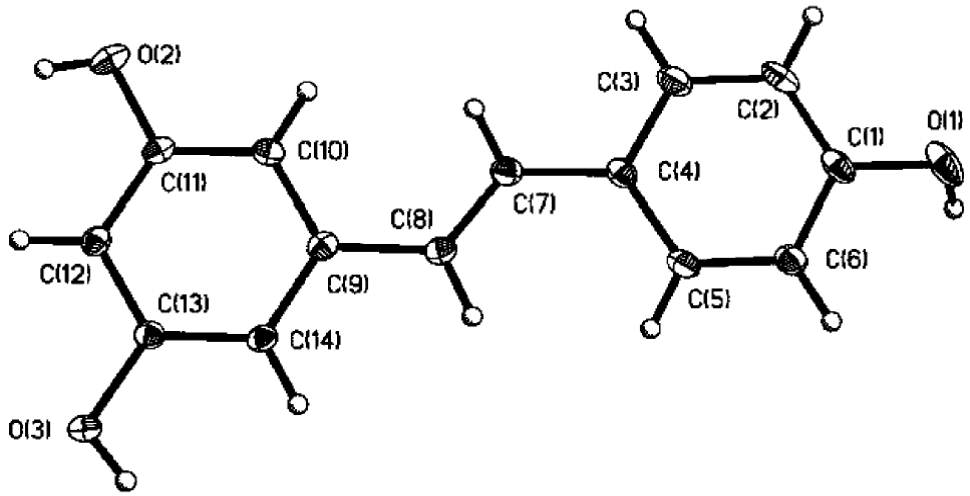

Fig. 2. Molecular structure of trans-resveratrol obtained from crystal X-ray diffraction. [13].

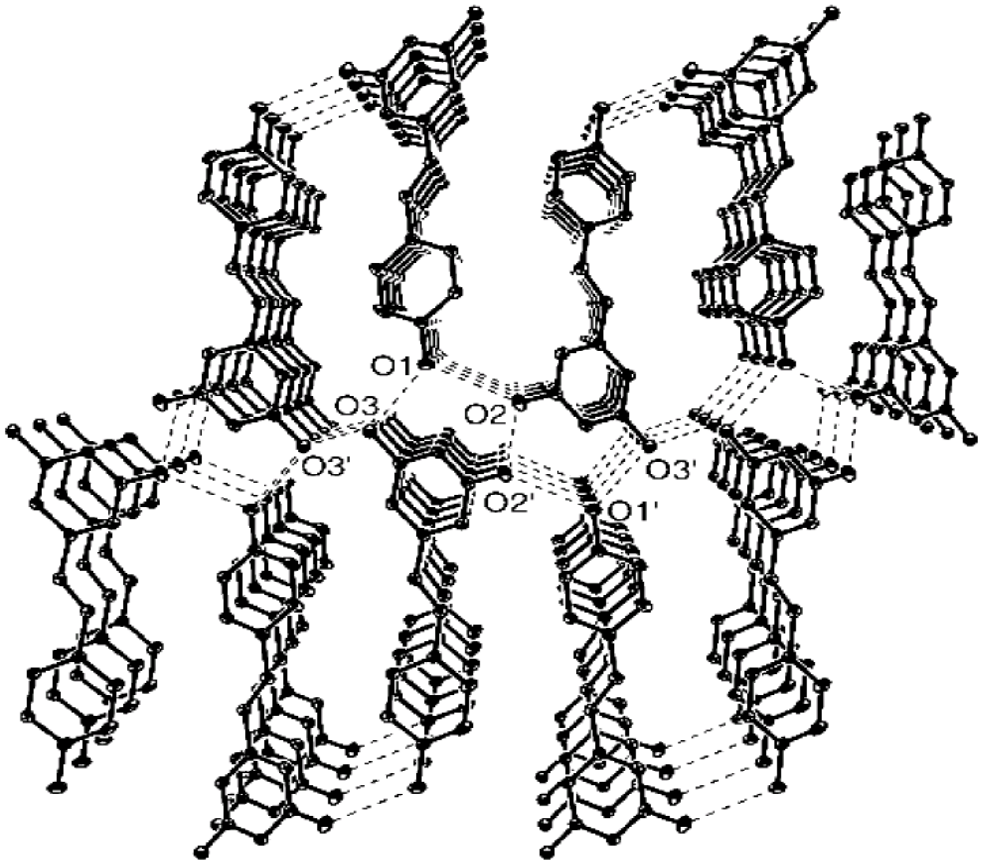

Fig. 3. *trans*-Resveratrol packing diagram down the a axis showing the extended network of H bonds (dashed lines) obtained from X-ray diffraction. Disordered hydrogen atoms are not shown for clarity [13].

Detection

Resveratrol content can be measured in wine [7], in plants, and in tissues by various techniques. Among popular methods are: detecting RSV by UV light absorption, by gas

chromatography, by electrochemical processes, etc. Recently such techniques as liquid chromatography – mass spectrometry (LC – MS) have been introduced as powerful tools for the determination of phenolic compounds in food [14].

In order to detect and determine RSV in rat blood, a selective multichannel liquid chromatography (LC)-electrochemistry method was developed [9]. In the study, four different electric potentials (+800 mV, +700 mV, +600 mV, +500 mV) were applied by a multichannel detector to receive a better voltammetric description of blood samples. The reason for choosing LC – electrochemistry method was due to its usefulness in identifying phenolic compounds. By comparing peaks of standards and samples at various energies, it was found that the optimum potential for determining pure RSV in blood was +700mV. **Figure 4** shows a chromatogram of blank rat blood (top) and rat blood that is spiked with RSV (bottom).

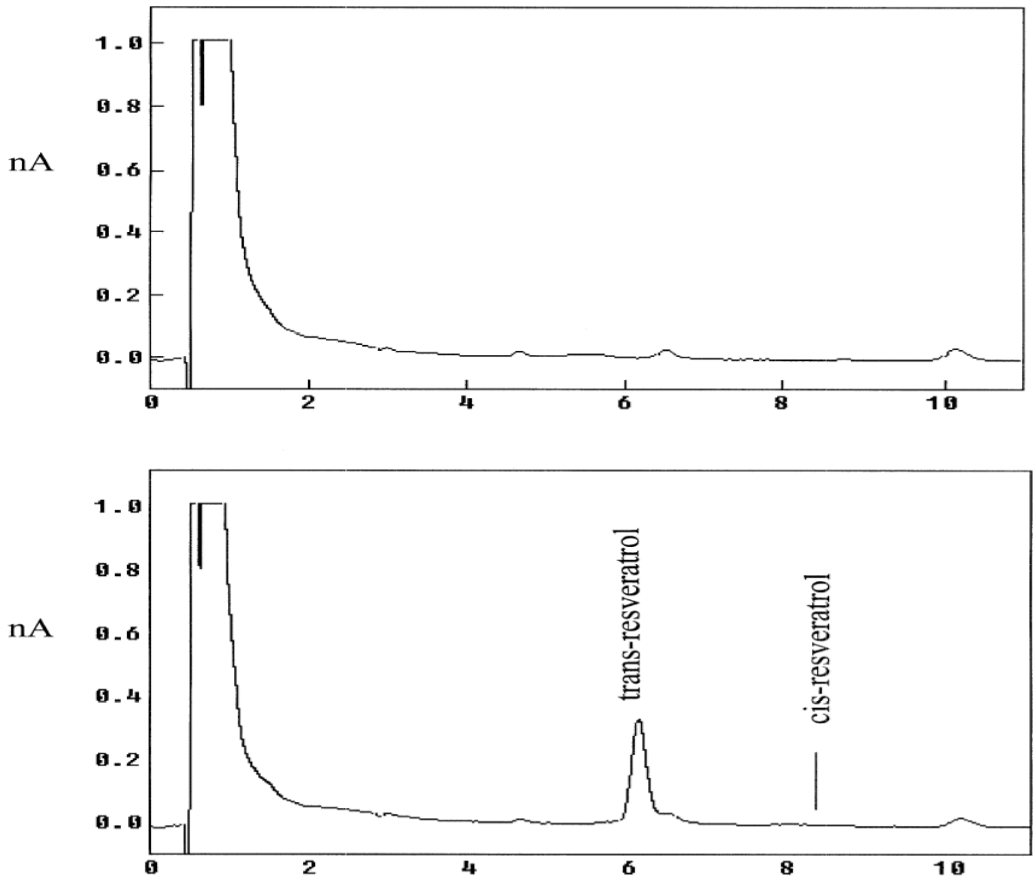

Fig. 4. Chromatogram of extract from blank rat blood (top) and blood spiked with 100ng/mL of resveratrol (bottom). Applied potential: +700 mV vs. Ag/AgCl [9].

Because of different retention times, *trans* and *cis* forms of RSV can be separated (in the experiment, time of separation was 6.1 and 8.4 min respectively). This method allows for accuracy and short time of chromatographic run [9].

Phase I &II Enzymes

Phase I and phase II enzymes are the first lines of defense against cancer in the organism. The enzymes are able to enter a wide variety of biochemical reactions and perform catalytic functions; the catalytic ability allows them to defend the organism from carcinogens. The efficiency of enzymatic work depends on environmental and nutritional factors of the organism. In most cases, enzymes of both phases work in tandem. The enzymes of phase I turn pre-carcinogenic compounds into more reactive forms, which allows for an easier disposal of harmful substances from the organism. Often the disposal occurs with the involvement of phase II enzymes whose main role is to detoxify the carcinogens, produced in phase I. The detoxification occurs by directly attacking cancer-causing agents or inactivating and escorting them from the body [15]. Resveratrol is considered to be an important activator of phase II enzymes (UDP-glucuronyl transferase [16], NADPH-dependent quinone reductase 1 (QR) [17], etc.). In studying how RSV affects QR enzyme, Bianco *et al*. used breast cancer cells. They showed that RSV induces QR at the protein level of the cells. It was determined in transcriptional studies that increases in QR occur at the electrophile response element (EpRE) through oestrogen receptors β (ER β) transactivation. Resveratrol up-regulates QR enzyme through enhanced binding of ER β to QR. Even more, the QR up-regulation protects the cell against oestrogen-induced oxidative DNA damage. Regulation of detoxification genes through their enzymes, such as QR, may partly explain the protective effect of RSV [17].

Antioxidant effects

Reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, are continuously generated in cells of aerobic environment. The accumulation of ROS that oxidize cell environment is associated with cell growth and tumor formation [18]. Resveratrol has been

shown to possess qualities of an antioxidant: it suppressed ROS formation [19] produced by lipopolysacharides (LPS) and macrophages that are stimulated by phorbol esters (PMA). In the same study, it was shown that RSV decreased amino acid release, originally induced by exposure to hydrogen peroxide (amino acid decrease by 51%) and superoxide (decrease by 67%). Along with lowering amino acid release, treatment by resveratrol caused an impairment of cyclooxygenase-2 (COX-2), originally stimulated by exposure to $O_2^{\bullet -}$ and H_2O_2 [19]. COX-2 is an enzyme that provides prostaglandin (PG) synthesis. Prostaglandin accumulation leads to inflammation, pain, and fever. Hence, regulating COX-2 by resveratrol allows for the regulation of inflammatory responses. Jang and Surh have shown that resveratrol can inhibit reactive oxygen intermediates (ROI) and by this attenuate apoptotic death of the cell [20].

Antioxidant capacity of RSV has been shown *in vivo*. DNA damage was caused in rat kidneys by kidney-specific carcinogen potassium bromate ($KBrO_3$). To estimate the damage, a reliable marker for oxidative DNA damage, deoxyguanosine, was measured by high performance liquid chromatography with electrochemical-coulometric and UV detection. The levels of deoxyguanosine in the renal genomic DNA doubled when using $KBrO_3$. After treatment with RSV, damage was completely abolished and kidney weight was prevented [6].

There has been evidence that RSV promotes DNA fragmentation when copper ions are around [reviewed in 11]. By acting as a reducing agent, RSV promoted formation of hydroxyl radicals ($HO^{\bullet}$) by DNA-bound copper (II) ions. However, one detail was not taken to consideration: in physiological conditions (when the ascorbic acid or glutathione are present), RSV behaves as an antioxidant and does not influence on $HO^{\bullet}$ formation; instead, RSV acts as a radical-scavenging antioxidant and protects DNA from damaging. In the glutathione system, RSV inhibits $HO^{\bullet}$ formation by inhibiting glutathione disulfide formation. Therefore, it was concluded that resveratrol is a powerful antioxidant in the presence of reducing; even more, it can suppress $HO^{\bullet}$ formation by reacting with glutathione as in the following reaction [11]:

$$GSH + HO^{\bullet} \rightarrow H_2O + GS^{\bullet}$$

Conclusion

Resveratrol is a relatively new class of chemopreventive agent. It has proven both *in vivo* and *in vitro* to be capable of impeding or even preventing carcinogenic steps. It is apparent today that resveratrol can arbitrate differential responses with various tissues, organs, and assay models. Some of the activities of RSV have been or remain controversial. One encouraging fact is that the component is found in a soluble form in red wine, although not in most other fruits and vegetables consumed daily. Other sources of RSV would be grapevines, peanuts, mulberries, certain kinds of brussel sprouts, or dietary supplements. The structure of the chemopreventive agent is simple and at the same time capable of regulating enzyme pathways. The unique part of the situation is that resveratrol is already consumed by humans, even though studying of the mechanism of the compound is still underway. In traditional medicines of India, Japan, and China, resveratrol has been used for ages as an antioxidant, antimutagen, and anti-inflammatory. Nevertheless, full potential of resveratrol can be realized during clinical studies that are yet to be performed.

References

1. Rodrigue CM, Arous N, Bachir D, Smith-Ravin J, Romeo PH, Galacteros F, Garel MC. (2001) Resveratrol, a natural dietary phytoalexin, possesses similar properties to hydroxyurea towards erythroid differentiation. *Br J Haematol*. **113(2)**:500-7.

2. Kopp P. (1998) Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'? *Eur J Endocrinol*. **138(6)**:619-20.

3. Pendurthi UR, Williams JT, Rao LV. (1999) Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells : A possible mechanism for the cardiovascular benefits associated with moderate consumption of wine. *Arterioscler Thromb Vasc Biol*. **19(2)**:419-26.

4. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*. **275(5297)**:218-20.

5. Fulda S, Debatin KM. (2004) Sensitization for anticancer drug-induced apoptosis by the chemopreventive agent resveratrol. *Oncogene*. **23(40)**:6702-11.

6. Cadenas S, Barja G. (1999) Resveratrol, melatonin, vitamin E, and PBN protect against renal oxidative DNA damage induced by the kidney carcinogen $KbrO_3$. *Free Radic Biol Med*. **26(11-12)**:1531-37.

7. Goldberg DM, Tsang E, Karumanchiri A, Diamandis E, Soleas G, Ng E. (1996) Method to assay the concentrations of phenolic constituents of biological interest in wines. *Anal Chem*. **68(10)**:1688-94.

8. Fremont L. (2000) Biological effects of resveratrol. *Life Sci*.**66(8)**:663-73.

9. Zhu Y, Huang T, Cregor M, Long H, Kissinger CB, Kissinger PT. (2000) Liquid chromatography with multichannel electrochemical detection for the determination of trans-resveratrol in rat blood utilizing an automated blood sampling device. *J Chromatogr B Biomed Sci Appl*. **740(1)**:129-33.

10. Shigematsu S, Ishida S, Hara M, Takahashi N, Yoshimatsu H, Sakata T, Korthuis RJ. (2003) Resveratrol, a red wine constituent polyphenol, prevents superoxide-dependent inflammatory responses induced by ischemia/reperfusion, platelet-activating factor, or oxidants. *Free Radic Biol Med*. **34(7)**:810-7.

11. Burkitt MJ, Duncan J. (2000) Effects of *trans*-resveratrol on copper-dependent hydroxyl-radical formation and DNA damage: evidence for hydroxyl-radical scavenging and a novel, glutathione-sparing mechanism of action. *Arch Biochem Biophys*. **381(2)**:253-63.

12. Basly JP, Marre-Fournier F, Le Bail JC, Habrioux G, Chulia AJ. (2000) Estrogenic/antiestrogenic and scavenging properties of (E)- and (Z)-resveratrol. *Life Sci*. **66(9)**:769-77.

13. Caruso F, Tanski J, Villegas-Estrada A, Rossi M. (2000) Structural basis for antioxidant activity of trans-resveratrol: ab initio calculations and crystal and molecular structure. *J Agric Food Chem*. **52(24)**:7279-85.

14. Dominguez C, Guillen DA, Barroso CG. (2001) Automated solid-phase extraction for sample preparation followed by high-performance liquid chromatography with diode array and mass spectrometric detection for the analysis of resveratrol derivatives in wine. *J Chromatogr A*. **918(2)**:303-10.

15. Backgrounder: The Phase I – Phase II enzyme system: a delicate balance. *Brassica*. http://www.brassica.com/sci/ph12backgnd.htm 18 Feb 2005.

16. Szaefer H, Cichocki M, Brauze D, Baer-Dubowska W. (2004) Alteration in phase I and II enzume activities and polycyclic aromatic hydrocarbons-DNA adduct formation by plant phenolics in mouse epidermis. *Nutr Cancer*. **48(1)**: 70-77.

17. Bianco NR, Chaplin LJ, Montano MM. (2005) Differential induction of quinone reductase by phtyoestrogens and protection against oestrogen-induced DNA damage. *Biochem J*. **385**: 279-87.

18. Asensi M, Medina I, Ortega A, Carretero J, Bano MC, Obrador E, Estrela JM. (2002) Inhibition of cancer growth by resveratrol is related to its low bioavailability. *Free Radic Biol Med*. **33(3)**:387-98.

19. Martinez J, Moreno JJ. (2000) Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. *Biochem Pharmacol*. **59(7)**:865-70.

20. Jang JH, Surh YJ. (2003) Protective effect of resveratrol on beta-amyloid-induced oxidative PC12 cell death.*Free Radic Biol Med*. **34(8)**:1100-10.