

Specific Structural Determinants Are Responsible for the Antioxidant Activity and the Cell Cycle Effects of Resveratrol*

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Lucia A. Stivala‡, Monica Savio, Federico Carafoli, Paola Perucca, Livia Bianchi, Giovanni Maga§, Luca Forti¶, Ugo M. Pagnoni¶, Angelo Albini||, Ennio Prosperi**, and Vanio Vannini

From the Dipartimento di Medicina Sperimentale, sez. Patologia Generale, the ||Dipartimento di Chimica Organica, Università di Pavia, the **Centro di Studio per l'Istochimica del CNR, §Istituto di Genetica Biochimica ed Evoluzionistica IGBE-CNR, 27100 Pavia, and the ¶Dipartimento di Chimica, Università di Modena e Reggio Emilia, 41100 Modena, Italy

Resveratrol (3,4',5-trihydroxy-*trans*-stilbene) is a natural phytoalexin found in grapes and wine, which shows antioxidant and antiproliferative activities. In this study we have investigated whether these properties are dependent on similar or different structural determinants of the molecule. To this purpose, resveratrol derivatives, in which all or each single hydroxylic function were selectively substituted with methyl groups, were synthesized. Analogues with the stilbenic double bond reduced or with the stereoisomery modified were also investigated. The antioxidant activity of these compounds was evaluated by measuring the inhibition of citronellal thermo-oxidation, or the reduction of 2,2-diphenyl-1-picrylhydrazyl radical. In addition, the protection against lipid peroxidation was determined in rat liver microsomes, and in human primary cell cultures. The antiproliferative activity was evaluated by a clonogenic assay, and by analysis of cell cycle progression and DNA synthesis. The results showed that the hydroxyl group in 4' position is not the sole determinant for antioxidant activity. In contrast, the presence of 4'-OH together with stereoisomery in the *trans*-conformation (4'-hydroxystyryl moiety) was absolutely required for inhibition of cell proliferation. Enzymatic assays *in vitro* demonstrated that inhibition of DNA synthesis was induced by a direct interaction of resveratrol with DNA polymerases α and δ .

Resveratrol (3,4',5-trihydroxystilbene) is synthesized by several plants in response to adverse conditions such as environmental stress or pathogenic attack. For this reason, it is classified as a phytoalexin, a class of antibiotics of plant origin (1–3). Resveratrol has been found in a multitude of dietary plants, such as peanuts, mulberries, and in grape skin (4). Thus, relatively high concentrations of this compound are present in grape juice and, especially, in red wine (5–8). Growing evidence suggest that resveratrol plays a role in the prevention of human pathological processes, such as inflammation (9–11), atherosclerosis (12–14), and carcinogenesis (4, 15, 16). The protective effect has been attributed to its antioxidant properties (11, 17, 18), to an anticyclooxygenase activity (4, 20), and to

a modulating activity of lipid and lipoprotein metabolism (9, 21, 22). Resveratrol also inhibits platelet aggregation (13, 23) and exhibits antiestrogenic activity (24, 25). However, these effects do not exhaustively explain the antiproliferative and anticarcinogenic properties of resveratrol. The proliferation of various human malignant cell lines is slowed down by resveratrol (15, 16, 26). The inhibition of cell growth, which has also been described in normal cells (27–29), is accompanied by the accumulation of cells in S and G₂ phases (16, 26, 30). Conflicting results have been reported on the induction of apoptosis by resveratrol (26, 31, 32).

A number of antioxidants, such as vitamin E, *N*-acetylcysteine, flavonoids, and carotenoids have been reported to interfere with cell cycle progression by inducing the expression of cdk inhibitors, like p21^{waf1-cip1}, p16^{ink4a}, and p27^{kip1} (33–36). In the case of resveratrol, such a mechanism is still controversial (26, 27). The effects on cell cycle progression may be also explained by the direct inhibition of ribonucleotide reductase (37) and DNA polymerase (38).

The structural determinants of these diverse properties of the resveratrol molecule are obscure, but the number and position of the hydroxylic groups have been suggested to play an important role in the antioxidant activity (14, 19, 39). The aim of this study was to extend these studies on the structural determinants of the activity of resveratrol, and in particular to establish whether the antioxidant and antiproliferative activities are dependent on (i) the stereoisomery, (ii) the position of the different phenolic hydroxyl groups, and (iii) the stilbenic double bond of the molecule. For this purpose, the *cis* form (II) was obtained by UV irradiation of *trans*-resveratrol; three different derivatives were synthesized in which the hydroxylic functions were selectively protected by methyl groups: 3,5-dihydroxy-4'-methoxystilbene (III), 3,5-dimethoxy-4'-hydroxystilbene (IV), and 3,4',5-trimethoxystilbene (V). Finally, the α,β -dihydro-3,4',5-trihydroxystilbene (VI) was obtained by reduction of the stilbenic double bond.

The biological properties of *trans*-resveratrol were compared with those of the above derivatives. In particular, the antioxidant activity was investigated *in vitro* by measuring the inhibition of citronellal thermo-oxidation or the radical scavenging ability using the free radical DPPH.¹ The protection against

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‡ To whom correspondence should be addressed: Dipartimento di Medicina Sperimentale Sez. Patologia generale "C. Golgi," Università di Pavia Piazza Botta, 10-27100 Pavia, Italy. Tel.: 39-0382-506333; Fax: 39-0382-303673; E-mail: l.stivala@botta.unipv.it.

¹ The abbreviations used are: DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; TBHP, *tert*-butylhydroperoxide; BrdUrd, bromodeoxyuridine; Me₂SO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; PMSF, phenylmethylsulfonyl fluoride; pol α , DNA polymerase α ; pol δ , DNA polymerase δ ; RPA, replication protein A; TBARS, thiobarbituric acid reactive substances; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

lipid peroxidation induced by Fe/ascorbate and *tert*-butylhydroperoxide TBHP was also assessed in rat liver microsomes, or in cultured human fibroblasts, respectively. The effects on cell proliferation were studied by analyzing the cell clonogenic efficiency and cell cycle progression. In addition, the recruitment of proliferating cell nuclear antigen (PCNA) and replication protein A (RPA) to the DNA replication sites were investigated. These proteins are required for the initiation and elongation steps of DNA replication, respectively. Finally, the ability of resveratrol and its derivatives to inhibit replicative DNA polymerases was also assessed with *in vitro* assays.

The results have shown that the hydroxyl group at the 4' position is not the sole determinant for the antioxidant activity. Similarly, the 4'-hydroxyl group is necessary for the antiproliferative activity and the DNA polymerase inhibition, but the *trans* conformation is absolutely required for these effects.

EXPERIMENTAL PROCEDURES

Reagents

trans-Resveratrol (99% purity) and DPPH were obtained from Sigma; aphidicolin was obtained from Roche Molecular Biochemicals. Monoclonal antibodies anti-bromodeoxyuridine (BrdUrd clone BU20) and anti-PCNA (clone PC10) were obtained from Dako, while the anti-RPA (32 kDa subunit) 9H8 monoclonal antibody was kindly provided by M. Wold (Iowa University). The fluorescein isothiocyanate (FITC)-conjugated anti-mouse antibody was purchased from Sigma.

[³H]dATP (40 Ci/mmol) and [³H]dTTP (40 Ci/mmol) were from Amersham Pharmacia Biotech. Activated calf thymus DNA was prepared as described (40). Unlabeled dNTPs and poly(dA) and oligo(dT)₁₂₋₁₈ homopolymers were from Amersham Pharmacia Biotech. Whatman was the supplier of the GF/C filters. All other reagents were of analytical grade and purchased from Merck, Fluka, and Aldrich.

Poly(dA)/oligo(dT)₁₂₋₁₈ primer-template was prepared according to the manufacturer's protocol. Briefly, poly(dA) template oligonucleotide was mixed with the complementary oligo(dT)₁₂₋₁₈ oligonucleotide in a 10:1 molar ratio (w/w, nucleotides) in 20 mM Tris-HCl (pH 8.0), containing 20 mM KCl and 1 mM EDTA, heated at 90 °C for 5 min then incubated at 65 °C for 2 h and slowly cooled at room temperature.

Calf thymus DNA polymerase α (pol α) and δ (pol δ) were purified as described (40). The pol δ used in this study was 2,200 units/ml (0.08 mg/ml). Pol α was 250 units/ml (0.2 mg/ml). 1 unit of polymerase activity corresponds to the incorporation of 1 nmol of total dTMP into acid-precipitable material in 60 min at 37 °C in a standard assay containing 0.5 μ g (nucleotides) of poly(dA)/oligo(dT)_{10:1} and 20 μ M dTTP. Recombinant human wt PCNA was prepared as described (41).

Resveratrol Derivatives Synthesis

The *cis* form (II) was obtained by photoisomerization. The *trans*-isomer (95 mg) was dissolved in 20 ml of acetonitrile, flushed with nitrogen for 20 min, capped, and irradiated by means of 4 external 15 W phosphor-coated lamps until the photostationary state (about 1 to 1) was reached (40 min). Repeated separation by column chromatography eluting with cyclohexane/ethyl acetate (1:1) gave a sample (20% yield) of 98% pure (HPLC) *cis* derivative. ¹H NMR (CD₃CN, 300 MHz): δ 6.15 (dd, 1H, $J_{4,2} = J_{4,6} = 2.0$ Hz, H-4); 6.25 (d, 2H, $J_{2,4} = J_{6,4} = 2.0$ Hz, H-2 and H-6); 6.38 and 6.50 (AB system, 2H, $J = 12.0$ Hz, CH=CH); 6.62 (d, 2H, $J_{3,2'} = J_{5,6'} = 8.5$ Hz, H-3' and H-5'); 6.75 (bs, 2H, 2xOH); 6.90 (bs, 1H, OH); 7.13 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.5$ Hz, H-2' and H-6').

trans-3,5-Dihydroxy-4'-methoxystilbene (4'-O-methylresveratrol) (compound III) was obtained as previously described (44) by Wittig reaction between the phosphonium salt of the commercially available 4-methoxybenzyl chloride and 3,5-bis-(*tert*-butyldimethylsilyloxy)benzaldehyde. Butyllithium (2.3 ml, 1.6 M in hexane, 3.6 mmol) was added dropwise to a suspension of (4-methoxybenzyl)triphenylphosphonium chloride (3.6 mmol) in tetrahydrofuran (50 ml) at -15 °C. The resulting reddish solution was allowed to warm at room temperature and stirred for 30 min. 3,5-Bis-(*tert*-butyldimethylsilyloxy)benzaldehyde (3.6 mmol) was then added and the reaction mixture was stirred for 1 h, diluted with ice-cold water (2 \times 25 ml), and extracted with ethyl acetate (3 \times 30 ml). The organic extracts were washed with water and the solvent was removed under reduced pressure to obtain a mixture of *trans*- and *cis*-3,5-di-(*tert*-butyldimethylsilyloxy)-4'-methoxystilbene, which were desilylated in tetrahydrofuran at room temperature with tetrabutylammonium fluoride. After addition of ethyl ether the solution was washed

with water and the solvent was removed under reduced pressure. The residue was filtered over silica gel to obtain 3,5-dihydroxy-4'-methoxystilbene as a 2:1 mixture of the (*trans*/*cis*)-isomers in 75% yield. After crystallization from CHCl₃/pentane, the pure *trans*-isomer (III) was obtained (33% yield). ¹H NMR (Me₂SO-d₆, 200 MHz): δ 3.78 (s, 3H, OCH₃); 6.14 (dd, 1H, $J_{4,2} = J_{4,6} = 2.05$ Hz, H-4); 6.42 (d, 2H, $J_{2,4} = J_{6,4} = 2.05$ Hz, H-2 and H-6); 6.90 and 7.00 (AB system, 2H, $J = 16.28$ Hz, CH=CH); 6.94 (d, 2H, $J_{3,2'} = J_{5,6'} = 8.77$ Hz, H-3' and H-5'); 7.53 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.77$ Hz, H-2' and H-6'); 9.23 (s, 2H, 2 \times OH).

trans-3,5-Dimethoxy-4'-hydroxystilbene (IV) was obtained by Perkin condensation between 4-hydroxybenzaldehyde and 3,5-dimethoxyphenylacetic acid (Aldrich), as reported by Pezet and Pont (45). An alternative synthetic procedure was attempted, consisting in the Wittig reaction between the phosphonium salt of 3,5-dimethoxybenzyl bromide (synthesized from the commercially available 3,5-dimethoxybenzyl alcohol) and 4-acetoxybenzaldehyde on 3.6 mmol scale, as reported for the compound III. In this case, a 1:1 mixture of the *trans*/*cis*-isomers was obtained in 48% yield. The 4'-hydroxyl function was deprotected by treatment with K₂CO₃ in methanol at room temperature. After addition of ethyl acetate, the solution was washed with water and the solvent removed under reduced pressure: the residue was then chromatographed over silica gel using petroleum ether/diethyl ether gradient, giving *trans*-3,5-dimethoxy-4'-hydroxystilbene in 20% yield. ¹H NMR (Me₂SO-d₆, 200 MHz): δ 3.78 (s, 6H, 2 \times OCH₃); 6.38 (dd, 1H, $J_{4,2} = J_{4,6} = 2.22$ Hz, H-4); 6.73 (d, 2H, $J_{2,4} = J_{6,4} = 2.22$ Hz, H-2 and H-6); 6.78 (d, 2H, $J_{3,2'} = J_{5,6'} = 8.61$ Hz, H-3' and H-5'); 6.94 and 7.17 (AB system, 2H, $J = 16.42$ Hz, CH=CH); 7.43 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.61$ Hz, H-2' and H-6'); 9.56 (s, 1H, OH).

3,4',5'-*trans*-Trimethoxystilbene (derivative V) was synthesized by direct methylation, refluxing a mixture of *trans*-resveratrol (0.44 mmol) and CH₃I (9.24 mmol) in acetone (15 ml) in the presence of anhydrous potassium carbonate (6.6 mmol). After addition of ethyl ether, the solution was washed with water and the solvent was removed under reduced pressure: the residue was then chromatographed over silica gel using petroleum ether/diethyl ether gradient, giving *trans*-3,4',5'-trimethoxystilbene in quantitative yield. ¹H NMR (CDCl₃, 200 MHz): δ 3.87 (s, 9H, 3 \times OCH₃); 6.42 (dd, 1H, $J_{4,2} = J_{4,6} = 2.26$ Hz, H-4); 6.69 (d, 2H, $J_{2,4} = J_{6,4} = 2.26$ Hz, H-2 and H-6); 6.93 and 7.08 (AB system, 2H, $J = 16.26$ Hz, CH=CH); 6.934 (d, 2H, $J_{3,2'} = J_{5,6'} = 8.71$ Hz, H-3' and H-5'); 7.48 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.71$ Hz, H-2' and H-6').

α,β -Dihydro-3,4',5'-trihydroxystilbene (α,β -dihydroresveratrol) (derivative VI) was obtained by catalytic hydrogenation of *trans*-resveratrol (0.15 mmol) with 10% Pd/C catalyst in methanol (5 ml) at room temperature and atmospheric pressure. The catalyst was filtered off through Celite and washed with methanol; the solvent was then removed under reduced pressure and the residue chromatographed over silica gel (7:3, hexane/ethyl acetate) to obtain α,β -dihydro-3,4',5'-trihydroxystilbene (87% yield). ¹H NMR (Me₂SO-d₆, 200 MHz): δ 2.55-2.77 (m, 4H, CH₂CH₂); 6.03 (dd, 1H, $J_{4,2} = J_{4,6} = 2.11$ Hz, H-4); 6.07 (d, 2H, $J_{2,4} = J_{6,4} = 2.11$ Hz, H-2 and H-6); 6.66 (d, 2H, $J_{3,2'} = J_{5,6'} = 8.52$ Hz, H-3' and H-5'); 7.00 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.52$ Hz, H-2' and H-6'); 8.99 (s, 2H, 2 \times OH); 9.08 (s, 1H, OH). The products synthesized were further purified by crystallization to reach a final purity > 98%, as determined by high performance liquid chromatography.

Stock solutions of each substance were prepared in *N,N*-dimethylformamide and final dilution was performed in chlorobenzene for the *in vitro* oxidation test. For cell culture experiments, stock solutions were prepared in Me₂SO and diluted directly in cell culture medium.

Cell Culture and Treatments

Normal human embryonic fibroblasts and HT1080 fibrosarcoma cells (Istituto Zooprofilattico, Brescia, Italy) were cultured in Earle's minimal essential medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Life Technologies, Inc.), 100 IU/ml penicillin, and 100 μ g/ml streptomycin. Normal fibroblasts were used between the 5th and 20th passages. Cell treatments were performed by adding *trans*-resveratrol or its derivatives in culture medium at final concentrations ranging from 7 to 100 μ M. For resveratrol, these concentrations are comparable with doses found in red wine and grapes (39). Untreated cultures received the same amount of the solvent alone (Me₂SO < 0.1%). Aphidicolin (60 nM) was used as positive control for cell cycle arrest in S phase. For the antioxidant activity determination, normal fibroblasts were incubated for 30 min with 60 μ M *trans*-resveratrol or its derivatives, before the oxidative treatment. For cell cycle analysis, normal fibroblasts and HT1080 cells were incubated for 24, 48, and 72 h with *trans*-resveratrol at the concentration of 15, 30, and 90 μ M, then cells were washed twice with phosphate-buffered saline (PBS), and

detached with a standard trypsinization procedure.

Antioxidant Activity

The antioxidant activity of *trans*-resveratrol and its derivatives was evaluated *in vitro* both by the citronellal thermo-oxidation inhibition test (46), and the DPPH method (47, 48). In addition, antioxidant activity was assessed in rat liver microsomes by measuring lipid peroxidation inhibition after Fe^{2+} /ascorbate treatment, and in human fibroblast cultures after TBHP treatment.

Citronellal Thermo-oxidation Method

In this test, the aldehyde (–)–citronellal is used as the oxidation substrate: it is subjected to heating and intensive oxygenation in chlorobenzene, and its disappearance with the consequent formation of its degradation products are monitored by gas chromatography. Chlorobenzene was selected as the reaction solvent because of (i) its stability to oxidation; (ii) the ability to dissolve both polar and nonpolar compounds, better than dimethylformamide; and (iii) the boiling point is higher than 80 °C (temperature test). Fifteen ml of a chlorobenzene solution, containing 150 μl of dodecane (Aldrich) and 150 μl of tridecane (Aldrich) as internal standards, were poured into a two-necked flask equipped with a condenser to prevent evaporation. Resveratrol, or its derivatives, dissolved in dimethylformamide, were added to the chlorobenzene solution to reach final concentrations ranging from 60 to 120 μM . The mixture was then heated at 80 °C and intensively oxygenated by bubbling in O_2 at a flow rate of 10 ml/min. At time 0, 300 μl of (–)–citronellal (Fluka) were added to the reaction medium. Immediately and at periodic intervals, 0.1- μl samples were withdrawn and analyzed by gas chromatography. The antioxidant power of resveratrol and the derivatives was measured by determining the efficient quantity (EQ), *i.e.* the concentration required for each compound to double the half-life with respect to control reaction (citronellal without antioxidant).

DPPH Reduction Method

Antioxidant solution in methanol (0.1 ml) was added to 3.9 ml of a 6×10^{-5} M DPPH solution in methanol (48). The exact initial DPPH concentration in the reaction medium was calculated from a calibration curve. The decrease in absorbance was determined at 515 nm at 0 min, every 5 min for 1 h, and every 60 min until the reaction reached a plateau (about 6 h). Antiradical activity was expressed as the EC_{50} , *i.e.* the antioxidant concentration necessary to decrease the initial amount of DPPH by 50%.

Lipid Peroxidation in Rat Liver Microsomes

Rat liver microsomes were prepared from Wistar rats by tissue homogenization with 5 volumes of ice-cold 0.25 M sucrose containing 5 mM Hepes, 0.5 mM EDTA (pH 7.5). Briefly, microsomal fractions were isolated by removal of the nuclear fraction at $8,000 \times g$ for 10 min, removal of mitochondrial fraction at $18,000 \times g$ for 10 min, and sedimentation at $105,000 \times g$ for 60 min. Microsomal fractions were diluted in phosphate buffer, 0.1 M (pH 7.5), at the final protein concentration of 1 mg/ml. The microsomes were preincubated in a shaking water bath at 37 °C for 10 min with varying concentrations (0.01–100 μM) of each compound before starting lipid peroxidation with 100 μM Fe^{2+} , 500 μM ascorbate. After 60 min incubation, the inhibitory effect on lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) by the method of Yagi *et al.* (49). Briefly, 500 μl of microsomal fraction were added to 500 μl of 20% trichloroacetic acid to stop the lipid peroxidation reaction, and then 500 μl of 0.74% thiobarbituric acid was added. The mixture was then heated in a boiling water bath for 15 min. After centrifugation, 200 μl of supernatant was transferred to the microtiter plate, the absorbance was measured at 535 nm and compared with standards prepared from the acid hydrolysis of malonaldehyde tetraethylacetyl (Sigma). Inhibition of lipid peroxidation was expressed as percentage, and the effective concentration giving 50% inhibition (EC_{50}) was calculated from the inhibition curve.

Lipid Peroxidation in Cell Cultures

Lipid peroxidation was induced in cultured normal human fibroblasts by TBHP (Sigma). Cells were preincubated for 30 min with 60 μM *trans*-resveratrol or its analogues in PBS, and then 250 μM TBHP was added for 60 min. The production of TBARS was assessed, as above described. In both experimental models, microsomes and cells, TBHP-treated and untreated control samples received the same amount of the solvent ($\text{Me}_2\text{SO} < 0.02\%$).

Clonogenic Efficiency Assay

The clonogenic efficiency was determined after incubation of cells in culture medium containing *trans*-resveratrol or its derivatives. Briefly, the cells were diluted in complete Earle's minimal essential medium to ~4000 cells/ml, and volumes of this suspension containing 200 cells were transferred to 30-mm dishes. After 24 h of treatment with *trans*-resveratrol and the different derivatives, the cells were washed twice and 5 ml of fresh medium was added. After 10 days, the colonies were stained with crystal violet and counted, and the clonogenic efficiency was calculated as the mean percentage with respect to control cells. Clonogenic efficiency of untreated control cultures was about 35%.

Cell Cycle Analysis

Cell cycle distribution was assessed by determining BrdUrd incorporation *versus* DNA content. Normal fibroblasts and HT1080 cells were incubated with 30 μM BrdUrd (Sigma) during the last hour of culture, harvested, and fixed in cold 70% ethanol. Fixed cells were washed in PBS, resuspended in 2 N HCl for 30 min at room temperature, pelleted, and then resuspended in 0.1 N sodium tetraborate for 15 min. The samples were then washed in PBS, incubated for 15 min in PBS containing 1% bovine serum albumin and 0.2% Tween 20 (PBT), and then for 60 min in 100 μl of anti-BrdUrd monoclonal antibody diluted 1:20 in PBT. After two washes with PBT, cells were incubated for 30 min with 100 μl of FITC-conjugated anti-mouse antibody diluted 1:50 in PBT, then washed twice, and resuspended in PBS containing 5 $\mu\text{g}/\text{ml}$ propidium iodide and 2 mg/ml RNase A. Cells were analyzed with a Coulter Epics XL (Coulter Corp.) flow cytometer. Ten thousand cells were measured for each sample. Computer statistical analysis of mean fluorescence intensity (MFI) and graphic representation were performed with the XL2 software (Coulter).

Immunofluorescence Determination of Nuclear-bound PCNA and RPA (32 kDa)

In order to determine the nuclear-bound (detergent-insoluble) fraction of protein involved in the DNA replication complex, a hypotonic lysis was performed according to the following protocol (50). Briefly, cells were resuspended in hypotonic lysis buffer 10 mM Tris-HCl (pH 7.4), containing 2.5 mM MgCl_2 , 1 mM phenylmethylsulfonyl fluoride (BDH), and 0.5% Nonidet P-40 (Sigma). After the lysis was completed, cells were resuspended in saline, fixed in cold ethanol to a final 70% concentration, and stored at –20 °C until analysis. Fixed cells were pelleted by centrifugation, washed in PBT, and incubated for 60 min with 100 μl of monoclonal antibodies anti-PCNA or anti-RPA, diluted 1:100 in PBT. At the end of incubation, cells were washed twice with PBT and incubated for 30 min with 100 μl of FITC-conjugated anti-mouse antibody, diluted 1:100. After the secondary antibody incubation, cells were washed twice in PBT, and resuspended in PBS. Negative controls consisted of cells incubated only with the secondary FITC-conjugated antibody, or cells in which the primary antibody was replaced by an isotypic irrelevant antibody (Sigma). For biparametric analysis of immunofluorescence *versus* DNA content, cells were stained with propidium iodide as described above.

DNA Polymerase Assays

Enzymatic Assays—Pol α activity on activated DNA was assayed in a final volume of 25 μl containing: 50 mM Tris-HCl (pH 7.6), 0.25 mg/ml bovine serum albumin, 1 mM dithiothreitol, 6 mM MgCl_2 , 10 μM each [^3H]dATP (5 Ci/mmol), dGTP, dCTP, and [^3H]dTTP (5 Ci/mmol), 0.05 units of pol α and 3 μg of activated DNA. All reactions were incubated for 15 min at 37 °C unless otherwise stated and the DNA precipitated with 10% trichloroacetic acid. Insoluble radioactive material was determined as described (51). Enzymes and proteins were added as indicated in the figure legends. Pol δ activity was assayed on poly(dA)/oligo(dT) in a final volume of 25 μl containing: 50 mM BisTris-HCl (pH 6.6), 0.25 mg/ml bovine serum albumin, 1 mM dithiothreitol, 6 mM MgCl_2 , 10 μM [^3H]dTTP (5 Ci/mmol), 50 ng of PCNA, 0.035 units of pol δ , and 200 nM (3'-OH ends) of poly(dA)/oligo(dT). All reactions were incubated for 15 min at 37 °C unless otherwise stated and the DNA precipitated with 10% trichloroacetic acid. Insoluble radioactive material was determined as described (51).

Inhibition Assays—Assays were performed under the conditions described above. Different concentrations of the inhibitors to be tested were added as indicated in figure legends to the reaction mixture in the absence of DNA template and nucleotides. After 5 min of incubation at room temperature, the reaction was started by addition of the missing reagents and incubation was as described above. K_i values were calculated by Dixon plot of the experimental data.

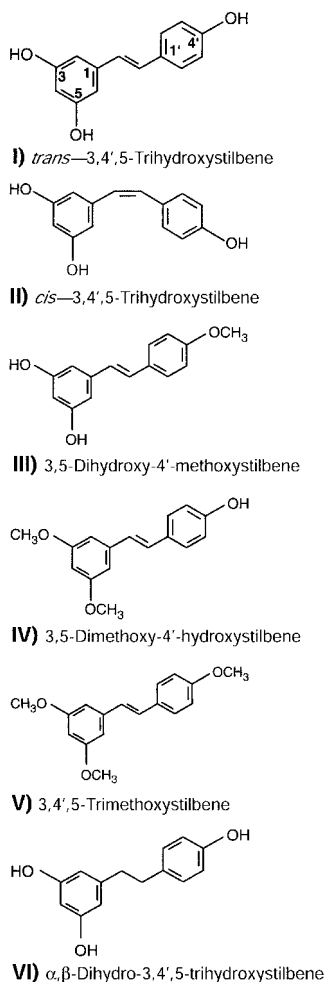


FIG. 1. Chemical structures of *trans* (I), *cis*-resveratrol (II), and derivatives III-VI. *trans*-Resveratrol derivatives were synthesized, as described under "Experimental Procedures," in order to change the stereoisomery in the *cis* conformation (II), to selectively substitute the hydroxylic functions with methoxy (OCH₃) groups (III, IV, and V), or to reduce the stilbenic double bond (VI).

Electronic Structure and Thermodynamic Stability of the Phenoxyl Radicals from Resveratrol

The electronic structure and the formation enthalpy of the three different phenoxyl radicals arising from the loss of hydrogens at the 3-, 5-, and 4'-OH groups in resveratrol were determined by semiempirical Molecular Orbital calculations using the MNDO-PM3 semiempirical method. Full optimization of molecular geometries were achieved using a gradient best fit procedure with an energy convergence criterium of 10⁻⁴ kcal/mol. The input geometries were determined by best fit using a Molecular Mechanics method (all methods implemented in the Hyperchem package, release 5.1).

Statistics

All experiments were performed at least three times. Experimental data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's multiple range test for significant differences.

RESULTS

Fig. 1 shows the chemical structures of *trans* (I) and *cis*-resveratrol (II), of three *trans*-form derivatives in which each hydroxylic group is protected by methyl groups (III, IV, and V), and a derivative with reduced double bond (VI).

Antioxidant Activity of *trans*-Resveratrol and Derivatives II-VI—The comparison of antioxidant activity of *trans*-resveratrol and the derivatives, as estimated in *in vitro* tests, is reported in Table I. The results are expressed as the EQ (citronellal test) or the EC₅₀ (microsomes and DPPH assays) of each compound

TABLE I

Antioxidant activities of *trans*-resveratrol and derivatives II-VI

The antioxidant power is expressed by EQ and EC₅₀, as described under "Experimental Procedures." Each value is the mean of at least three independent experiments \pm S.D.

Compound	Citronellal EQ	Microsomes EC ₅₀	DPPH EC ₅₀
		μM	
I	135 \pm 8.81	0.77 \pm 0.08	24.5 \pm 1.5
II	241 \pm 38.0	1.10 \pm 0.09	24.1 \pm 1.2
III	661 \pm 20.3	2.40 \pm 0.34	48.6 \pm 4.2
IV	355 \pm 7.43	1.22 \pm 0.18	30.1 \pm 2.1
V	>1000	>1000	>1000
VI	260 \pm 11.2	1.57 \pm 0.17	106.8 \pm 6.3

used. The stronger the antioxidant, the smaller the EQ or EC₅₀ value. In all three tests, *trans*-resveratrol (I) showed the highest antioxidant activity, whereas compound V did not exert any significant effect. Increasing values of EQ and EC₅₀ were observed for derivatives II, IV, and VI, and compound III reaching values about 5 and 3 times higher ($p < 0.01$) than *trans*-resveratrol, in the citronellal and microsome test, respectively. With the DPPH assay, the *trans* (I) and the *cis* (II) forms showed a similar EC₅₀, and derivative IV gave a comparable value. Derivatives III and VI were less efficient, showing EC₅₀ values about 2 ($p < 0.05$) and 4 times ($p < 0.01$) higher than *trans*-resveratrol, respectively. Fig. 2 shows the effects of *trans*-resveratrol (I) and the derivatives on TBARS production induced by TBHP in normal human fibroblasts. Incubation of the cells for 1 h in the presence of 250 μM TBHP significantly increased membrane lipid peroxidation, raising the TBARS production to 4.04 nmol/5 \times 10⁵ cells, from the level of 0.92 nmol/5 \times 10⁵ cells measured in untreated control samples. *trans*-Resveratrol (I) inhibited the TBARS production by about 67% ($p < 0.01$). Among the derivatives, compounds II, IV, and VI exhibited significant antioxidant activity, by reducing TBARS production by about 42% ($p < 0.01$), 61% ($p < 0.01$), and 33% ($p < 0.01$), respectively. Derivatives III and V did not exert any statistically significant activity in this assay. The concentration of Me₂SO used (<0.02%) did not induce any significant protective effect against lipid peroxidation (data not shown). Bybenzil (compound without double bond and OH groups) and *trans*-stilbene (compound with double bond, without OH groups) did not show any detectable antioxidant activity, with any of the three *in vitro* methods (results not shown).

Effect of *trans*-Resveratrol and Derivatives II-VI on the Clonogenic Efficiency of Normal Fibroblasts—The clonogenic efficiency was studied in normal fibroblasts treated for 24 h with *trans*-resveratrol or the derivatives at concentrations ranging from 0 to 90 μM . Fig. 3 shows that only *trans*-resveratrol and derivative IV induced a dose-dependent reduction in clonogenic efficiency, with an estimated IC₅₀ of about 60 μM for both. All other derivatives failed to induce a significant inhibition in cell growth.

Effects of *trans*-Resveratrol and Derivatives II-VI on Cell Cycle Progression of Normal and Tumor Cell Lines—To further investigate the effect of the various derivatives on cell proliferation, the distribution in each phase of the cell cycle was analyzed by determining the DNA content with flow cytometry. Fig. 4 compares the effects in normal fibroblasts treated for 24 h with *trans*-resveratrol or derivatives II-VI, at the 30 μM concentration, because significant effects could be already observed in these conditions. A significant accumulation ($p < 0.01$) in S phase, and a consequent reduction in the number of cells in G₁ phase, was observed with *trans*-resveratrol (G₁ = 44.5%, S = 42.5%, G₂ + M = 13.0%), and with derivative IV (G₁ = 48.0%, S = 40.1%, G₂ + M = 11.9%), with respect to the cell cycle distribution of control cells (G₁ = 61.3%, S = 22.3%, G₂ +

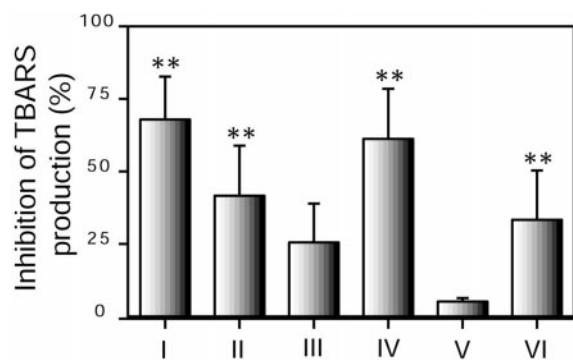


FIG. 2. Antioxidant activity of *trans*-resveratrol (I) and derivatives II-VI. Antioxidant activity of 60 μM *trans*-resveratrol (I) or derivatives II-VI, expressed as the percentage of inhibition of TBARS production in normal human fibroblasts treated with TBHP. Mean values \pm S.D. of at least five independent experiments are shown. *, = $p < 0.05$; **, = $p < 0.01$ significantly different as compared with control by one way ANOVA-Tukey's test.

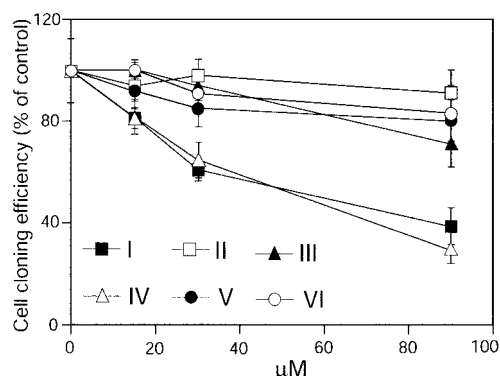


FIG. 3. Effect of *trans*-resveratrol (I) and derivatives II-VI on the clonogenic efficiency of normal human fibroblasts. Cells were incubated 24 h after plating with the different compounds, and grown for 10 days before counting colonies, as described under "Experimental Procedures." Each point was assayed in triplicate dishes, and each experiment was repeated at least three times. Values are expressed in percentage and referred to untreated control cultures. Clonogenic efficiency of untreated control cultures was about 35%.

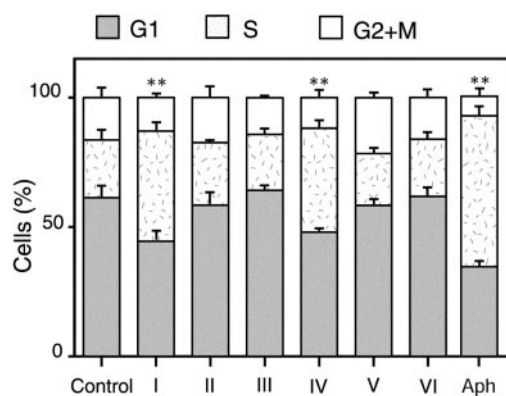


FIG. 4. Cell cycle analysis of normal human fibroblasts treated with *trans*-resveratrol (I) and the derivatives II-VI. Determination of cell cycle distribution was performed in samples incubated for 24 h with 30 μM of the above compounds, stained with propidium iodide, and measured by flow cytometry, as described under "Experimental Procedures." Aphidicolin (60 nM) was used as positive control for cell cycle arrest in S phase. Mean values of the percentage of cells in each phase of cell cycle were obtained from three independent experiments. **, $p < 0.01$ significantly different from to control by one way ANOVA-Tukey's test.

M = 16.4%). The cell cycle distribution of samples treated with the other derivatives (II, III, V, and VI) was comparable to that observed in the controls. Aphidicolin, a well known inhibitor of

DNA polymerases α and δ , used as a positive control, induced a significant accumulation of cells in S phase ($G_1 = 34.5\%$, $S = 58\%$, $G_2 + M = 7.5\%$), comparable to that induced by compounds I and IV. Cells incubated with the solvent alone ($\text{Me}_2\text{SO} < 0.1\%$) did not show any alteration of cell cycle progression. The inhibitory effect on cell growth induced by *trans*-resveratrol and derivative IV was reversible, since 48 h after removal of the compounds, the percentage of cells in S phase returned to the control value (not shown).

To study whether the observed cell cycle imbalance induced by *trans*-resveratrol and derivative IV was consequent upon a change in DNA synthesis, DNA replication was assessed by BrdUrd incorporation and determined with immunofluorescence and flow cytometric analysis. Fig. 5 shows the dot plots of BrdUrd immunofluorescence versus DNA content in control cells and in fibroblasts treated with 30 μM *trans*-resveratrol for 24, 48, and 72 h. The results showed that cells progressed through S phase at a slower rate than control cells and incorporated significantly lower amounts of BrdUrd incorporation. Quantitative analysis of BrdUrd immunofluorescence in the region corresponding to S-phase cells indicated that *trans*-resveratrol inhibited BrdUrd incorporation by about 55% at 24 h (see Figs. 5B and 7A), and by about 70 and 80% at 48 and 72 h (Fig. 5, C and D), respectively. Similar results were obtained in HT1080 fibrosarcoma cells (not shown).

Effects of *trans*-Resveratrol on the Recruitment of PCNA and RPA Proteins to DNA Replication Sites—To explore the basis underlying the inhibition of DNA synthesis, the recruitment of PCNA and RPA (32-kDa subunit) proteins to DNA replication sites was investigated next. To this aim, cells were lysed in hypotonic buffer to separate the detergent-insoluble forms of the two proteins. Fig. 6A shows the dot plots of PCNA immunofluorescence versus DNA content in control cells (a) and in fibroblasts treated for 24 h with 15 (b), 30 (c), or 90 μM (d) *trans*-resveratrol. Accumulation of cells in S phase was evident at the lowest concentrations, while with 90 μM *trans*-resveratrol, cells were blocked at the G_1/S phase transition, as indicated by the high PCNA immunofluorescence levels typical of cells entering S phase (52). Quantitative analysis of immunofluorescence intensity in S phase indicated that the amount of PCNA assembled in replication foci in treated samples, was about 12% higher (not significant) than that of control cells. The amount of RPA protein (32 kDa) assembled at the replication foci was also not significantly modified after treatment with *trans*-resveratrol (Fig. 6B).

Effects of *trans*-Resveratrol and Derivatives II-VI on DNA Synthesis—The above results indicated that BrdUrd incorporation was inhibited at a step following to the recruitment of RPA and PCNA to the replication foci, thus suggesting that the inhibition of DNA synthesis occurred at the level of DNA polymerase activity, as also suggested by the reduction in BrdUrd incorporation. For this purpose, the ability of *trans*-resveratrol to inhibit DNA synthesis was compared with the other derivatives, both in cells and in *in vitro* assays testing the activity of replicative DNA pol α and pol δ . The results reported in Fig. 7A show that among the derivatives, only compound IV inhibited BrdUrd incorporation to a similar extent of *trans*-resveratrol. Comparable results were obtained in the *in vitro* assays on DNA polymerase activity (Fig. 7B). *trans*-Resveratrol inhibited both pol α and pol δ with similar potencies, whereas the derivative IV showed a 2-fold preference for pol α ($p < 0.01$) with respect to pol δ ($p < 0.05$). It must be noted that this inhibition was enhanced by the preincubation with the enzyme, suggesting a slow-binding mode of inhibition. All the other compounds were 3–10-fold less active than the *trans*-resveratrol. The *cis*-form (II) of resveratrol was also tested and found to be inactive,

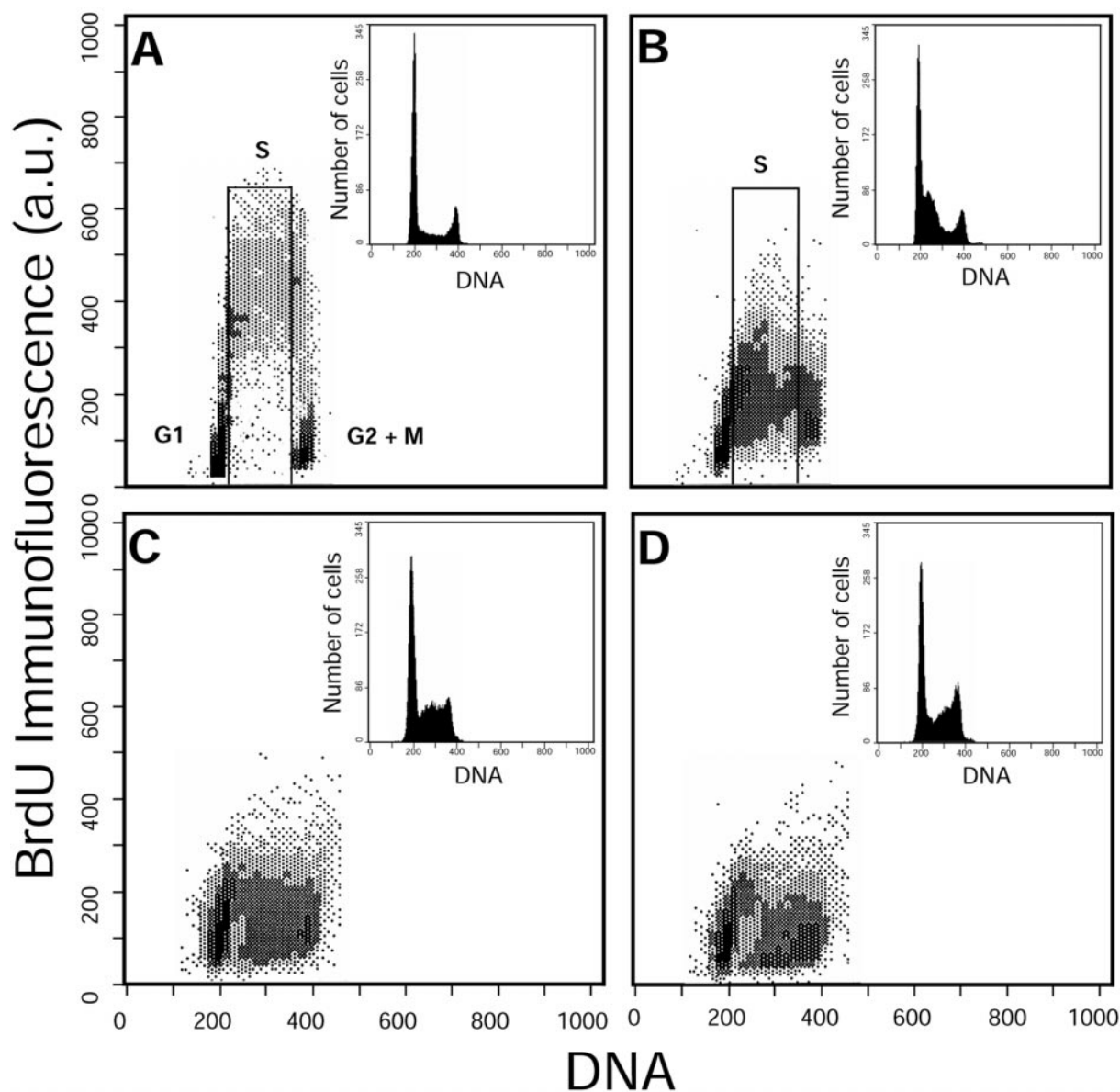


FIG. 5. Effect of *trans*-resveratrol on DNA synthesis in normal human fibroblasts. Two-parameter dot plots of BrdUrd incorporation versus DNA content in control cells and in samples treated with 30 μM *trans*-resveratrol for 24, 48, and 72 h. During the last hour of *trans*-resveratrol treatment, 30 μM BrdUrd was added to each sample. Immunostaining and flow cytometric analysis of BrdUrd incorporation were performed as described under "Experimental Procedures." Results shown are from one out of three independent experiments. Control cells (A) and fibroblasts treated with 30 μM *trans*-resveratrol for 24 h (B), 48 h (C), and 72 h (D).

confirming that for DNA synthesis inhibition, the active configuration of the compound was in the *trans*-form. The corresponding K_i values for the active compounds are listed in Table II. None of the derivatives inhibited *Escherichia coli* pol I (Klenow fragment), HIV-1 reverse transcriptase, and HSV-1 DNA polymerase (data not shown), confirming the specificity of the above inhibition observed.

Electronic Structure and Thermodynamic Stability of the Phenoxy Radicals from *trans/cis*-Resveratrol—Fig. 8 shows the limit surface diagrams for the singly occupied molecular orbitals in the three phenoxy-type radicals of both the *trans* and *cis* conformations, as were obtained from PM3. In all cases a delocalization of the unpaired spin is observed. However, in the *trans* configuration, the 4'-phenoxy (picture A) is extended also to the adjacent ring through the stilbene double bond, whereas in the 3- and 5-phenoxy species (picture B) the delocalization is confined to the aromatic ring bound to the oxygen radical center. This different electronic structure leads to a greater resonance stabilization energy for the 4'-phenoxy rad-

ical, the formation of which is accordingly predicted to be more exothermic, as indicated by ΔH_f° reported in Fig. 8.

The rationale for these results is obtained by considering that in mononuclear phenoxy radicals the maximum unpaired spin densities are at the ring *meta* and *para* positions. In the resveratrol 4'-phenoxy radical the spin density can flow to the adjacent ring since the stilbene double bond acts as a bridge being bound to a maximum spin density center. This is not the case for the other two radical species where the double bond is bound at positions of minimum spin density. As a consequence of the greater resonance stabilization energy, the hydrogen abstraction from the 4'-OH bond is expected to be favored being more exothermic with respect to the analogue reactions at the 3-OH and 5-OH positions. According to the above arguments, the absence of the olefinic double bond in α,β -dihydro-3,4',5'-trihydroxystilbene is expected to cause a decrease in the 4'-phenoxy radical resonance stabilization energy and, consequently, a decrease in the overall inhibition efficiency.

Based on molecular orbitals calculations, all the phenoxy

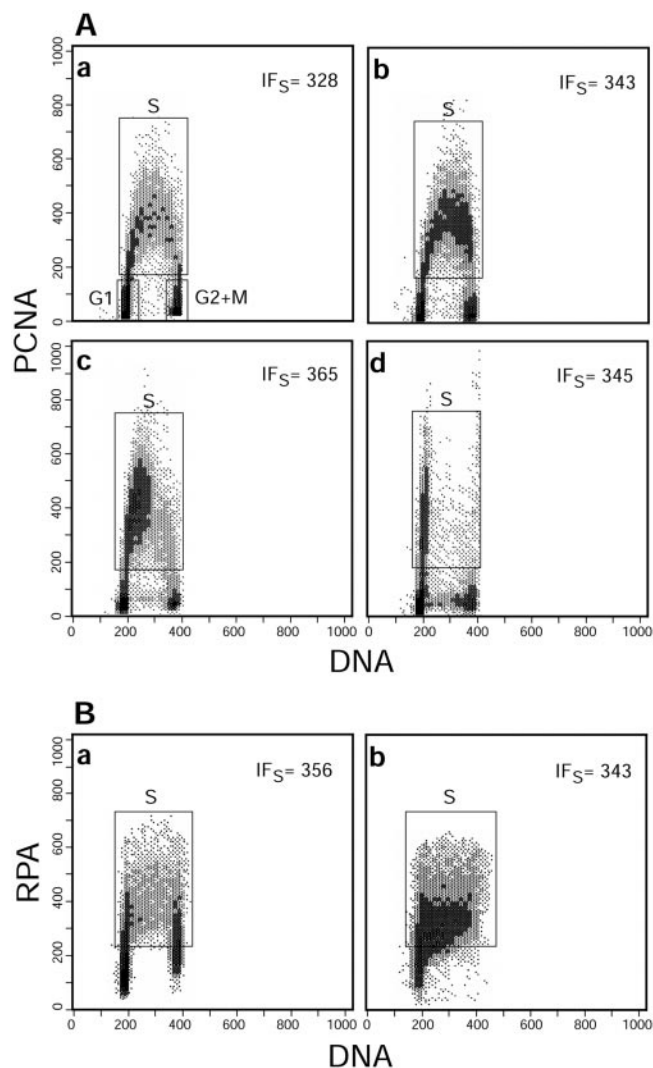


FIG. 6. Effect of *trans*-resveratrol on the recruitment of PCNA and RPA to DNA replication sites in normal human fibroblasts. A, two-parameter dot plots of PCNA immunofluorescence versus DNA content in control cells (a) and in fibroblasts treated for 24 h with *trans*-resveratrol 15 (b), 30 (c), and 90 (d) μM . B, two parameter dot plots of RPA (32 kDa) immunofluorescence versus DNA content in control cells (a) and in fibroblasts treated for 24 h with *trans*-resveratrol 30 μM (b). In both experiments (A and B), determination of detergent-insoluble form of PCNA or RPA was performed by immunostaining and flow cytometric analysis, as described under "Experimental Procedures." Results shown are from one out of three independent experiments. IF_S , immunofluorescence value of S-phase.

radicals of resveratrol in the *cis* conformation (Fig. 8C and D) show an intrinsic thermodynamic stability lower than that of corresponding radicals in the *trans*-form (Fig. 8, A and B), since the formation enthalpies are less exothermic by 7.1 kcal/mol for the 4'-phenoxy radical (ΔH_f° *trans* (-55.8 kcal/mol) - ΔH_f° *cis* form (-48.7 kcal/mol)), and by 6.4 kcal/mol, for the 5- and 3-phenoxy radicals. Furthermore, within the *cis* configuration framework, the 3- and 5-phenoxy radicals (Fig. 8D) are also found to be less stable ($\Delta H_f^\circ = -46.77$ kcal/mol) than the 4'-phenoxy analogue (Fig. 8C, $\Delta H_f^\circ = -48.71$ kcal/mol). In fact, in the *cis* 4'-phenoxy radical, the spin delocalization to the phenyl ring via the double bond is partially hindered by the lack of coplanarity of the π system. This effect is witnessed by the higher resonance stabilization energy of the *trans*, with respect to the *cis* analogue, which is obtained by this calculation ($\Delta H_f^\circ A - \Delta H_f^\circ B = -2.7$ kcal/mol; $\Delta H_f^\circ C - \Delta H_f^\circ D = -1.94$ kcal/mol). The difference between these values leads to 0.76 kcal/mol, which represents a 30% decrease in the resonance stability.

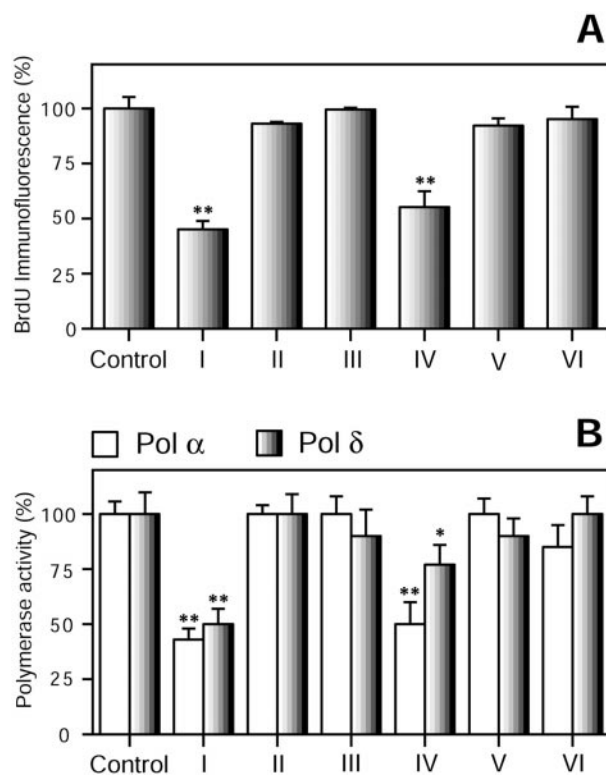


FIG. 7. Effect of *trans*-resveratrol (I) and derivatives II-VI on DNA synthesis in cell cultures and *in vitro*. A, quantitative evaluation of BrdUrd incorporation. Data are BrdUrd immunofluorescence values expressed as the percentage of samples treated with 30 μM of each derivative versus untreated control cells. B, DNA synthesis performed *in vitro* by purified pol α and pol δ in the absence (control) and presence of *trans*-resveratrol and derivatives II-VI. Assays were performed as described under "Experimental Procedures" in the presence of 5 μM of each inhibitor to be tested. DNA polymerase activity was expressed as percentage of the control reaction without inhibitor which was 0.8 pmol \times min⁻¹ for pol α and 0.6 pmol \times min⁻¹ for pol δ and taken as 100%. *, $p < 0.05$; **, $p < 0.01$ significantly different from to control by one way ANOVA-Tukey's test.

TABLE II
Inhibition constants (K_i) for *trans*-resveratrol and derivatives against DNA polymerase α and δ

Compound	DNA polymerase K_i	
	DNA polymerase α , K_i	DNA polymerase δ , K_i
	μM	
<i>trans</i> -Resveratrol	3.3 (± 0.5) ^a	5 (± 1)
III	NA ^b	30 (± 3)
IV	5 (± 0.5)	10 (± 1)
VI	29 (± 3)	NA

^a Number in parentheses indicate \pm S.D. from three independent experiments.

^b NA, not active; $K_i > 100$ μM .

For the sake of comparing the radical reactivities of *cis*- and *trans*-resveratrol, the reaction enthalpies for the hydrogen abstraction reaction have been calculated using the molecular orbitals method (Table III). The hydrogen abstraction reactions from *trans*-resveratrol are predicted to be more exothermic by 2–3 kcal/mol.

DISCUSSION

In agreement with abundant evidence obtained on other systems *in vitro* (14, 17, 39, 53, 54) the work presented here has documented a significant antioxidant activity of resveratrol. The results have shown that the hydroxyl group in the 4' position is required for the antioxidant activity, but acts synergistically with the 3- and 5-OH groups. In fact, the derivative IV, which has a free hydroxyl group in the 4' position, exert a

significant antioxidant activity in all the tests used. The *trans*-isomery and the double bond in the stilbenic skeleton also play a role, at least as indicated by the citronellal and microsome tests, since *cis*-resveratrol and compound VI, in which the double bond is reduced, are significantly less effective than the *trans*-resveratrol. A similar conclusion is suggested by the cell culture assay, based on the inhibition of TBHP-induced lipid peroxidation. The major role played by the 4'-OH group in the radical scavenging and antioxidant activity of *trans*-resveratrol could be related to the electronic structure and the formation enthalpy of the three different phenoxy radicals arising from the loss of hydrogens at the 3-, 5-, and 4'-OH groups in res-

veratrol (Fig. 8). The hydrogen abstraction from 4'-OH bond is expected to be favored, being more exothermic than the analogue reactions at the 3-OH and 5-OH positions, as a consequence of the greater resonance stabilization energy. In addition, the absence of the double bond is expected to cause a decrease in the 4'-phenoxy radical resonance stabilization energy. This would induce a decrease in the antioxidant activity of compound VI, as observed in our experimental models. Finally, on comparing the radical reactivity of *trans*- and *cis*-resveratrol (Table III), the hydrogen abstraction reactions from the *trans*-form are predicted to be more exothermic. This provides a thermodynamic rationale for the experimental observation in two *in vitro* methods and in cell culture that the antioxidant power of *cis*-resveratrol is lower than that of the *trans*-analogue. DPPH measurements differ from the above results in that *trans*- and *cis*-form have the same effect, while again both methylation and, more markedly, reduction of the olefinic double bond, decrease the antioxidant activity. Thus, in this assay, hydrogen transfer to the radical is equally efficient from both isomers. It may be inferred from these results that the calculated decrease in the stabilization energy for the phenoxy radical in the *cis* configuration does not significantly influence the reaction kinetic activation parameters. This in turn might be considered diagnostic of the activated complex along the reaction path being closer to reactants than the products.

In agreement with studies by others (15, 16, 25–28, 30), *trans*-resveratrol has been found to inhibit in a dose- and time-dependent manner cell growth both in normal fibroblasts and in fibrosarcoma cells. An antiproliferative activity comparable to that of *trans*-resveratrol has been observed only for derivative IV. Importantly, their effect was cytostatic and reversible since no evidence of cell death was obtained by a number of tests such as visual inspection for detached cells, trypan blue exclusion, and annexin V staining (data not shown).

The cytostatic effect may be attributed to decreased DNA synthesis given that a significant inhibition of BrdUrd incorporation was previously found in other cell lines (19, 29, 30, 37), and also observed in our study on normal fibroblasts and in fibrosarcoma cells. The results described here suggest that inhibition of DNA synthesis occurred at the level of DNA polymerase activity, since the recruitment of PCNA and RPA (32 kDa) proteins to DNA replication sites was not affected by *trans*-resveratrol.

The *in vitro* assays have demonstrated that only *trans*-resveratrol and derivative IV inhibited significantly DNA polymerases α and δ . Interestingly, there was an increase in specificity for the inhibition of pol α with respect to pol δ , from *trans*-resveratrol to compound IV suggesting a possible role of the -OH groups in positions 3 and 5 in the binding to the different DNA polymerases. The inhibition by resveratrol was found to be strictly specific for the B-type DNA polymerases, since neither pol I (member of the A-type family of DNA polymerases), nor HIV-1 RT (belonging to the reverse transcriptases/RNA-dependent RNA polymerases/telomerases family) were inhibited. Moreover, within the B-type family, *trans*-resveratrol discriminated between eukaryotic and viral enzymes, *i.e.* HSV-1 pol was not inhibited. These data suggest that the

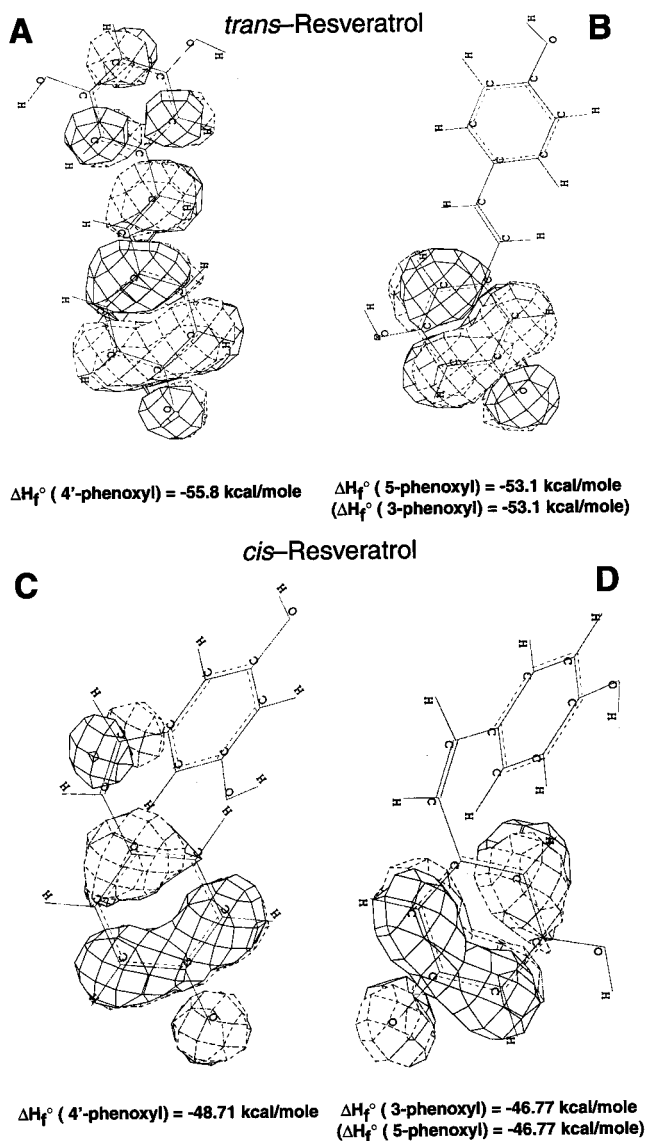
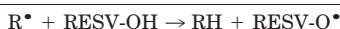


FIG. 8. Electronic structure and thermodynamic stability of the phenoxy radicals from resveratrol. Picture of the limit surface diagrams for the singly occupied molecular orbitals in the 4'-phenoxy radical (A), and 5- or 3-phenoxy radicals (B), from *trans*-resveratrol and from *cis*-resveratrol (C and D), respectively. Values of formation enthalpy (ΔH_f°) for each phenoxy radical are also shown.

TABLE III

Radical reactivities of *cis*- and *trans*-resveratrol, as determined by reaction enthalpies for the hydrogen abstraction reaction, calculated using the molecular orbital method



$\Delta H_f^\circ(\text{cis-resveratrol} \rightarrow 4'\text{-phenoxy}) - \Delta H_f^\circ(\text{trans-resveratrol} \rightarrow 4'\text{-phenoxy})$	= 3.0 Kcal/mol
$\Delta H_f^\circ(\text{cis-resveratrol} \rightarrow 3\text{-phenoxy}) - \Delta H_f^\circ(\text{trans-resveratrol} \rightarrow 3\text{-phenoxy})$	= 2.2 Kcal/mol
$\Delta H_f^\circ(\text{cis-resveratrol} \rightarrow 5\text{-phenoxy}) - \Delta H_f^\circ(\text{trans-resveratrol} \rightarrow 5\text{-phenoxy})$	= 2.2 Kcal/mol

interaction of resveratrol with the eukaryotic replicative DNA polymerases α and δ is highly specific.

In conclusion, the results of this study indicate that (i) 4'-hydroxyl group in *trans*-conformation (hydroxystyryl moiety) is not the sole determinant for antioxidant properties, while it is absolutely required for antiproliferative activity. (ii) There is a direct correlation, from a structural point of view, between the antiproliferative effect and the ability to inhibit DNA pol α and δ . Thus, a mechanism underlying the inhibition of cell cycle progression is the interaction between the 4'-hydroxystyryl moiety of *trans*-resveratrol and DNA polymerases.

The structure-activity relationship revealed by this study should be taken in account in studies aimed at synthesizing resveratrol derivatives with more selective antioxidant and/or antiproliferative activity. To this respect, the observation that the *cis* conformation of resveratrol still showed antioxidant activity but was totally inactive against DNA polymerases, is very interesting and deserves further investigation.

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