JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 1213−1217 **1213**

Antioxidant Properties of *trans*-ϵ-Viniferin As Compared to Stilbene Derivatives in Aqueous and Nonaqueous Media

CHRISTELLE PRIVAT,[†] JOÃO PAULO TELO,[‡] VANIA BERNARDES-GENISSON,[†] ABEL VIEIRA,[§] JEAN-PIERRE SOUCHARD,[†] AND FRANÇOISE NEPVEU*,[†]

Laboratoire Pharmacophores Redox, Phytochimie et Radiobiologie, EA-3030, Université Paul Sabatier, 35 Chemin des Maraîchers, 31062 Toulouse Cedex 4, France, Instituto Superior Tecnico, Avenidad Rovisco Pais, P-1096 Lisboa Codex, Portugal, and Departamento de Quimica, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825-114 Monte da Caparica, Portugal

trans-ϵ-Viniferin, the dimer of resveratrol, extracted from *Vitis vinifera*, has been evaluated for its antioxidant capacity. Its properties have been compared to those of resveratrol and synthetic stilbenic derivatives (4-hydroxystilbene, 4,4′-dihydroxystilbene, 3,5-dihydroxystilbene, and trimethylresveratrol), in regard to their liposolubility using two media with different polarity. The bleaching of β-carotene by lipoperoxyl (LOO•) radicals in an oil/water (O/W) emulsion and the scavenging of superoxide anions (O_2^-) in dimethyl sulfoxide (DMSO) using 5,5-dimethyl-1-pyrroline-*N*-oxide as a spin trap were followed using UV−visible and electron paramagnetic resonance, respectively. ϵ-Viniferin exhibits the best antioxidant capacity in the DMSO/O_2^- polar system (IC_{50} = 0.14 mM) while 4,4′-dihydroxystilbene presents the highest antioxidant capacity in the O/W/LOO• system (inhibition of β-carotene bleaching, 82%). Partition coefficients and kinetics of partition between 1-octanol and water were measured to discuss the antioxidant efficiency of the compounds in relation with their chemical structure.

KEYWORDS: Viniferin; resveratrol; antioxidant activity; hydrophilic/lipophilic partition

INTRODUCTION

ROS are implicated in oxidative damage of biological molecules and involved in the development of many pathological disturbances such as atherosclerosis, cancer, and inflammation. Moreover, epidemiological studies have shown that natural antioxidants present in food or beverages might be beneficial for health (*1*−*6*). ROS are also responsible for the deterioration of foods by lipid oxidation during processing and storage (*7*). In living systems, various endogenous (i.e., enzymes such as catalase and superoxide dismutase) and exogenous molecules (ascorbic acid and tocopherol) play a role in neutralizing these radicals. The presence of antioxidants in food, for example, is effective in preventing the development of various off-flavors and undesirable compounds that result from lipid oxidation.

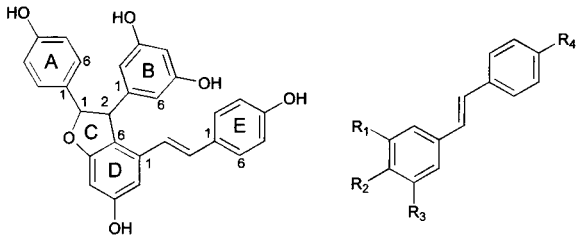
Apart from the fact that a broad spectrum of ROS-scavenging capacities is required, a good antioxidant must be able to diffuse in close proximity to its target at relatively high concentrations according to its partition between aqueous and lipid compartments (*8*). In vivo, polar antioxidants cannot cross biological membranes or move freely by diffusion between different compartments, a fact that could restrict their movement and their antioxidant efficiency. Ruiz-Larrea and co-workers (*9*) showed that the antioxidant activity of different estrogens depended not only on the hydrophilic or lipophilic nature of the scavenged radical but also on the chemical structure of the compound.

Methods for analyzing antioxidant properties of isolated molecules, extracts, or matrixes are of great interest in food and pharmaceutical industries as well as in biological studies. They are needed for comparing antioxidant properties of plant extracts and for evaluating antioxidant pools in foods depending on processing and synergistic effects between the antioxidants or the antioxidant status of living systems.

Polyphenol derivatives, which are naturally occurring antioxidants, have been demonstrated to be efficient scavengers of oxygen-centered radicals (*1*, *5*, *10*−*11*). Among them, hydroxystilbenes, including resveratrol (**2**; 3,5,4′-trihydroxystilbene), have been reported for their antioxidant properties (*12*−*13*). However, its antioxidant properties have not been studied in media with different polarities, and ϵ-viniferin (**1**), its dimer, extracted from the *Vitis vinifera* grapevine, has not yet been studied. Moreover, in contrast with **2**, **1** was not detected in wine (*14*−*16*). In this study, the antioxidant efficiency of **1** was compared to those of **2** and stilbene derivatives (**Figure 1**) in regard to the liposolubility using two media of different polarities. The bleaching of β-carotene by LOO• in an O/W emulsion was followed in the UV−visible range. Quantitative EPR analysis using DMPO as a spin trap and O_2^- as a radical was carried out in DMSO.

[†] Université Paul Sabatier.
[‡] Instituto Superior Tecnico.
[§] Universidade Nova de Lisboa.

10.1021/jf010676t CCC: $22.00
Published on Web 01/25/2002



1

	R_1	R_2	R_3	R_4
2	OH	H	OH	OH
3	H	OH	H	H
4	H	OH	H	OH
5	OH	H	OH	H
6	OCH_3	OCH_3	OCH_3	OCH_3

Figure 1. Chemical structures of **1**–**6**.

MATERIALS AND METHODS

General Procedures. UV−visible absorbances were recorded on an Uvikon 931 spectrophotometer (Kontron instruments). EPR spectra were obtained on a Bruker ER 200 D spectrometer (Bruker Spectrospin, Wissembourg, France). Standard EPR recording conditions were central field, 3500 G; scan range field, 200 G; microwave frequency, 9.71 Hz; microwave power, 4 mW; modulation frequency, 100 kHz; modulation amplitude, 1.0 G; time constant, 0.5 s; gain, variable but constant for each experiment; and scan speed, 24 G per min. ^{1}H NMR spectra were obtained on a Bruker AC200 instrument (Bruker Spectrospin, Wissembourg, France) at 200 MHz. Acetone-d_6 was used as a solvent.

Thin-layer chromatography (TLC) was performed on Aldrich silica gel TLC plates (200 μm thickness, 2−25 μm particle size; Sigma-Aldrich Chemical Co., Saint-Quentin Fallavier, France). The high-performance liquid chromatography (HPLC) equipment was from Waters (Saint Quentin en Yvelines, France) with a 717 Plus automatic sample injector, a 600 pump, and a 2487 Dual λ Absorbance UV−visible detector. A Zorbax SB-C18 5 μm (150 mm × 4.6 mm) column was used (Bios Analytique, Labege, France).

Compound **2**, DMPO, DMSO, α-tocopherol, β-carotene, 1-octanol, potassium superoxide, linoleic acid, and activated charcoal were obtained from Sigma-Aldrich Chemical Co. (Saint-Quentin Fallavier, France). Tween 40 and 18-crown-6 were purchased from Fluka (Sigma-Aldrich Chemical Co., Saint-Quentin Fallavier, France), and solvents were purchased from SDS (Peypin, France).

Synthesis of Stilbene Derivatives. *Trimethylresveratrol (**6**).* With 5 mL of triethyl phosphite, 3.7 g of 3,5-dimethoxybenzyl bromide (*17*) was heated at 130 °C until the evolution of ethyl bromide ceased. After the excess of phosphite was removed by distillation in a vacuum, pure diethyl(3,5-dimethoxybenzyl)phosphonate was distilled at 180 °C (0.05 mm Hg).

Under an argon atmosphere, 2.0 g of the phosphonate was added to 9 mL of dry dimethylformamide containing 0.4 g of sodium methoxide. The mixture was then cooled to 0 °C, 1.0 g of *p*-anisaldehyde was added, and the solution was allowed to stand at room temperature for 1 h. After the solution was heated at 100 °C for 1 h, the excess of dimethylformamide was evaporated in a vacuum. The addition of 20 mL of water/methanol 1:1 precipitated the crude product (1.7 g, 93%). Recrystallization from methanol afforded pure *trans*-**6** (mp 54−56 °C).

*Resveratrol (**2**).* In 180 mL of dry methylene chloride, 1 g of **6** was dissolved and cooled to −30 °C under argon. BBr_3 (2.4 mL) in dry methylene chloride (15 mL) was added slowly, and the mixture was allowed to reach room temperature overnight. Water was added to destroy the excess BBr_3, and the mixture was extracted twice with ethyl acetate. The organic extracts were washed with 10% sodium bicarbonate and water and dried, and the solvent evaporated. The crude product was purified by column chromatography on silica gel (2% ethyl acetate in ethyl ether) yielding 0.8 g (95%) of pure **2** (mp 267−268 °C).

*4-Hydroxystilbene (**3**).* A total of 4.0 g of phenylacetic acid, 3.7 g of *p*-hydroxybenzaldehyde, and 0.5 mL of piperidine were heated together at 120 °C for 18 h. The gummy product was digested with aqueous 5% sodium hydroxide and neutralized with aqueous hydrochloric acid to pH 6−7, and the precipitated solid was filtered. Chromatography on silica gel (toluene) afforded 4.2 g (71%) of **3** (mp 89−190 °C).

*4,4′-Dihydroxystilbene (**4**)* and *3,5-dihydroxystilbene (**5**).* Compounds **4** and **5** were prepared according to known procedures from the literature (*17*−*18*).

Purification of 1. A crude extract of vine roots containing about 20% viniferin and 1% compound **2** was first chromatographed on a preparative column packed with silica gel. A solvent gradient, starting with 100% dichloromethane and ending with 100% methanol, was used for elution. The viniferin-enriched fraction was then purified on silica gel preparative TLC (eluent: dichloromethane/methanol, 85:15). ^{1}H NMR (200 MHz, in deuterated acetone): 7.21 (2H, d, J = 9.0 Hz, H-2A and H-6A), 7.18 (2H, d, J = 9.0 Hz, H-2E and H-6E), 7.00 (1H, d, J = 15.1 Hz, H-β), 6.83 (2H, d, J = 8.0 Hz, H-3A and H-5A), 6.71 (4H, m, H-3E, 5E, 2D, α), 6.32 (1H, d, J = 1.7 Hz, H-4D), 6.24 (3H, s, H-2A and H-2B, 4B, 6B), 5.42 (1H, d, J = 5.0 Hz, H-1C), 4.49 (1H, d, J = 5.0 Hz, H-2C).

Comparison of the ^{1}H NMR spectroscopic data of purified viniferin with the data reported in the literature for oligomers of **2** led to the identification of *trans*-**1** (*19*−*20*). The purity of *trans*-**1** (98%) was evaluated by reverse phase HPLC, with a UV−visible detector working at two wavelengths, 306 and 325 nm. This analysis was performed at 37 °C using a water/acetonitrile/*ortho*-phosphoric acid solvent gradient (from 20/0/80 to 0/100/0) with a flow rate of 1 mL/min.

Determination of Antioxidant Effectiveness toward LOO• Radicals. The evaluation of the antioxidant activity of stilbene derivatives was based on the coupled oxidation of β-carotene and linoleic acid. The technique was developed by Marco (*21*) and modified by Miller (*22*) and Taga et al. (*23*). It consists of measuring the bleaching of β-carotene resulting from its oxidation by degradation products of linoleic acid such as LOO• radicals.

In a boiling flask, 100 μL of a 2 g L^{-1} β-carotene chloroform solution was mixed with 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was removed under a nitrogen flow, and 50 mL of oxygenated distilled water was slowly added to the flask with stirring to form an O/W emulsion. A 5 mL aliquot of this emulsion was then pipetted into each of a series of tubes containing 20 μL of acetone antioxidant solution. A control tube containing 20 μL of acetone alone instead of antioxidant solution was also prepared. A zero reading was taken at 470 nm on the reaction mixture in each tube immediately after adding emulsion to the antioxidant solution or acetone. The tubes were then stoppered and placed in a water bath at 50 °C. Subsequent readings were taken at regular intervals (3 h maximum) until the absorbance of the control read below 0.08, corresponding to the total bleaching of carotene. The antioxidant effectiveness of each compound tested was evaluated by the percentage of inhibition of carotene bleaching with regard to the control. All determinations were made in triplicate.

Quantification of the Antioxidative Activity Using EPR. Quantitative EPR analysis is based on the spin-trapping of O_2^- generated by KO_2 in DMSO with the addition of 18-crown-6 ether to complex K^+. In these conditions, a typical DMPO−OOH adduct is observed. DMPO, the spin trap, was purified on active charcoal as reported in the literature (*24*), aliquoted, stored frozen, and kept protected from light. As reported, 18-crown-6/potassium superoxide (1:1) was dissolved in DMSO (*25*).

A 1 mL DMSO solution was prepared in a glass tube by adding the reagents in the following order: DMPO (50 mM), test compound (× mM), and 18-crown-6/potassium superoxide (1:1, 5 μM). The reaction mixture was transferred to a flat quartz cell that was inserted into the cavity of an EPR instrument. The EPR spectra were recorded for 3 min at room temperature (about 20 °C) after the last addition. A control mixture, without test compound, gave the EPR reference signal.

The intensity of the EPR signal was calculated by adding the height of the quadruplet peaks. For each concentration of the compound tested, the percentage of inhibition of the EPR signal intensity was calculated with respect to the reference signal. The antioxidative activity of the test compounds was quantified by an IC_{50} value representing the

Table 1. Antioxidant Activity of **1** As Compared to Stilbene Derivatives against LOO· Evaluated by the Inhibition of β-Carotene Bleaching at 470 nm[a]

compd	absorbance decrease at 470 nm (SEM)[b]	inhibition of β-carotene bleaching (%) (SEM)[b]
control	0.257 (0.017)	
1	0.156 (0.012)	39.1 (2.7)
2	0.089 (0.011)	66.9 (1.9)
3	0.104 (0.009)	58.5 (1.8)
4	0.049 (0.003)	81.5 (0.4)
5	0.216 (0.017)	15.9 (6)
6	0.257 (0.017)	0 (0)

[a] The concentration of compounds was 40 μM. [b] Each value is the mean of triplicate measurements.

concentration necessary for 50% diminution of the EPR signal. This IC_{50} was deduced graphically by plotting the percentage of inhibition of the EPR signal intensity against the test compound concentration.

The antioxidative activity of each compound was investigated by three independent experiments. For each experiment, at least four concentrations giving an inhibition between 10 and 90% were assayed. Each experiment was performed with fresh solutions (except for DMPO).

Partition Coefficient Determination. Partition coefficients were determined according to Janzen et al. (*26*). In all cases, water-saturated 1-octanol and 1-octanol-saturated water were used. These solutions were made by shaking together equal volumes of each solution and then letting them separate. Stock solutions of the different compounds were made up in 1-octanol, and a full spectrum scan was run in order to determine the wavelength corresponding to maximum absorbance. (The Beer−Lambert law was verified at each maximum absorbance wavelength.) Three 10 mg samples of each compound were weighed into stoppered tubes and dissolved first in 2 mL of 1-octanol. The absorbance (A_0) of each solution was measured. Each 1-octanol solution was mixed with 2 mL of water. The tubes were shaken for 23 h at 20 °C and then separated. The absorbance of the 1-octanol phase (A) was evaluated. The partition coefficient (P) was determined from $P = A/(A_0 - A)$.

Kinetics of Stilbene Derivative Partition between 1-Octanol and Water. Water-saturated 1-octanol and 1-octanol-saturated water were used. Each compound was dissolved at a concentration of 20 μM in 1-octanol, and the absorbance (A_0) of each solution was measured at the wavelength corresponding to the maximum absorbance of the test compound. A total of 2 mL of 1-octanol solution and 2 mL of water were added to each in stoppered tubes and mixed for 23 h at 20 °C. The absorbance of the 1-octanol solution (A) was measured every 1 h. The kinetics of stilbene derivative partition was represented by plotting $(A \times 100)/A_0$ against the time.

RESULTS

Antioxidant Properties of Compounds 1−6 toward LOO• Radicals in an O/W Emulsion. The inhibitory activity against peroxidation of β-carotene in an O/W emulsion was examined for compounds **1**−**6**. Their inhibitory potency is summarized in **Table 1**.

It was found that compounds **1**−**5** were able to reduce the absorbance decrease of the β-carotene emulsion at a concentration of 40 μM in comparison with the control sample without additive. In contrast, **6** did not possess any antioxidant activity.

Among the active compounds, **4** proved to be the most efficient. It showed an absorbance decrease of 0.049 (control value, 0.257) corresponding to 81.5% inhibition of β-carotene bleaching. Its antioxidant ability was comparable to that of α-tocopherol tested in the same conditions (78.9 ± 2.7%). It is interesting to note that **2**, in these conditions, exhibited a better antioxidant activity than **3**, which in turn presented a greater activity than **1**. Compound **5** remained the weakest antioxidant of these active compounds.

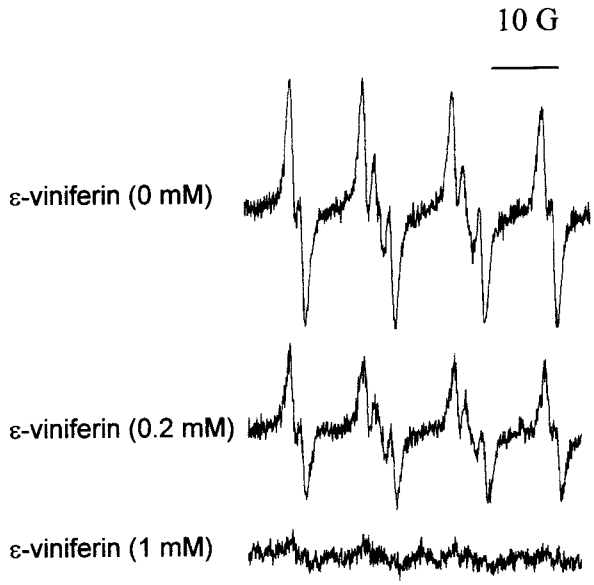


Figure 2. Inhibition of the DMPO–OOH EPR signal intensity by different concentrations of **1**.

Table 2. Antioxidant Activity (IC_{50}) of **1** As Compared to Stilbene Derivatives against Superoxide Radicals in DMSO[a]

compd	IC_{50} (mM) (SEM)[b]	compd	IC_{50} (mM) (SEM)[b]
1	0.14 (0.02)	**4**	0.82 (0.05)
2	0.95 (0.03)	**5**	1.68 (0.08)
3	1.10 (0.06)	**6**	>100

[a] IC_{50} corresponds to the stilbene concentration necessary for a 50% decrease of EPR signal intensity according to a control realized without additives. The concentration of O_2^- generated was 5 μM. [b] Each value is the mean of triplicate independent experiments.

Antioxidant Properties of Compounds 1−6 toward O_2^- Radicals in DMSO Medium. When the concentration of compounds **1**−**6** was increased, a decrease of the EPR signal intensity was observed as shown in **Figure 2** with compound **1**. The antioxidant properties of various stilbenes evaluated by their IC_{50} value are shown in **Table 2**. Compound **1** exhibited the highest activity ($IC_{50} = 0.14 \pm 0.02$ mM). It was about 25-fold more active than α-tocopherol ($IC_{50} = 3.91 \pm 0.28$ mM) in the same conditions. Although the activities of **2**−**4** were comparable, **4** had a better antioxidant ability than **3**. All of these compounds were more active than **5**. Compound **6** did not scavenge O_2^- efficiently.

Partition Coefficient of Compounds 1−5. The bar graph (**Figure 3**), which represents 1-octanol/water partition coefficients, gives an instant visual indication of stilbene derivative solubility. All of the test compounds exhibited a close partition coefficient corresponding to a hydrophobic character. In this series, **2** (Log $P = 1.53 \pm 0.01$) and **1** (Log $P = 1.58 \pm 0.02$) presented the lowest hydrophobicity. Compounds **4** and **5** had similar partition coefficients (Log $P = 1.72 \pm 0.01$), and **3** had the highest (Log $P = 1.77 \pm 0.02$).

Kinetics of Partition between 1-Octanol and Water of Compounds 1, 2, 4, and 5. Only kinetics of compounds with similar P was compared as **1** and **2** or **4** and **5**. The concentrations of **1** and **2** in the 1-octanol phase decreased with time and reached similar values after 23 h (**Figure 4**). However, the concentration lowering of **2** in organic medium was faster than that of **1**. So, it appears that **2** diffused more rapidly from the 1-octanol phase into the water phase than **1**. In the case of **4** and **5**, the kinetics was similar between them (data not shown).

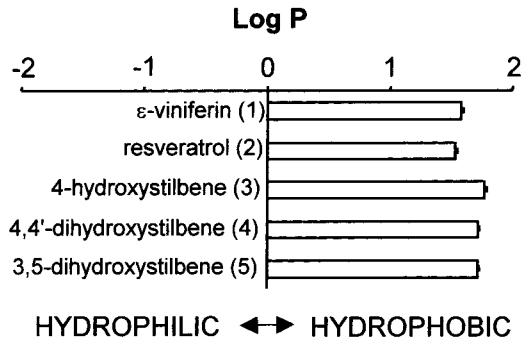


Figure 3. Bar graph displaying relative solubilities (Log P) of **1** and stilbene derivatives in 1-octanol/water mixtures on a logarithmic scale (left side is water solubility; right side is 1-octanol solubility). Each value is the mean ± SEM of three or five independent experiments.

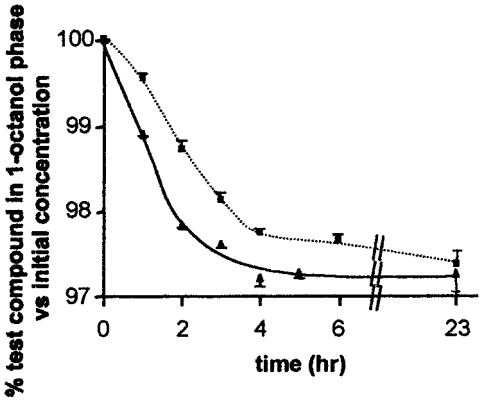


Figure 4. Kinetics of **1** (■) and **2** (▲) partition between 1-octanol and water. Each compound was initially dissolved in the 1-octanol phase at a concentration of 20 μM. The kinetics represented the average (±SEM) of three independent experiments.

DISCUSSION

In this paper, phenolic compounds exhibited an antioxidant activity, whereas **6**, which bears only methoxy groups, was not active. These results showed that, as expected, the phenolic function is involved in the mechanism of radical-scavenging. It has been reported in precedent studies that the antioxidant potential of polyphenols increases with the hydrogen-donating ability of the OH groups (*9*−*10*, *13*, *27*). It can be observed in our tests that the antioxidant potency of these compounds was highest when the OH groups occurred in the para position. Indeed, the phenoxyl radical generated in the para position can be stabilized by electronic delocalization through the stilbene framework lowering the oxygen−hydrogen bond energy (*28*). Even though the presence of a *para*-OH group is very important for a high activity, the number of OH groups on the aromatic rings also has an influence on the radical-scavenging potential. These results are in agreement with those of Wang et al. (*13*) who studied scavenging free radical processes of stilbenes against DPPH radicals.

However, it is worth noting that antioxidant efficiency of stilbene derivatives differed with the test used. This can be partly attributed to their capacity to be partitioned between lipophilic and hydrophilic phases, performed by P. Indeed, $LOO^{\bullet}$ radicals were generated in the lipophilic area of an O/W emulsion and O_2^- in a continuous and hydrophilic medium. Compound **1**, according to its P, is the least hydrophobic agent, which limits its antioxidant activity in the O/W emulsion, while **4** and **3** are in a greater concentration in the lipid phase and at the lipid/water interface allowing them to scavenge $LOO^{\bullet}$ radicals more efficiently. These observations were in agreement with the results on α-tocopherol, which is the reference antioxidant in lipophilic media: it exhibited a high activity in the O/W emulsion (inhibition of β-carotene bleaching = 78.9%) and a lower one in DMSO (IC_{50} = 3.91 mM). Talodini et al. (*29*), in a recent study, compared the antioxidant activity of **2** to α-tocopherol: they demonstrated that the capacity of these compounds to inhibit lipid peroxidation changes with the model systems used.

In the case of **2** and **1**, which have similar *P* values, it was important to observe the kinetics of the partition of these compounds between 1-octanol and water to explain the difference of antioxidant activity between the different tests. Indeed, the kinetics of partition depends on the polarity and the molecular weight of the compound in question; heavy compounds diffuse slower than light ones. Taking into account this result, it suggests that **1** is less efficient than **2** at concentrating in the lipidic phase of O/W emulsions during short times (about 3 h), limiting its antioxidant activity against $LOO^{\bullet}$ radicals.

In conclusion, among the stilbene structures studied in this series, **1**, the dimer of **2**, shows the best antioxidant capacity in the polar DMSO/O_2^- system. Compound **1** combines the required structural parameters: conjugated bonds and four OH groups with two in the para position. However, its dimeric character and steric properties seem to limit its rapidity to diffuse in the lipidic phase. It would now be interesting to compare the antioxidant properties of **2** and its dimer in in vitro and in vivo biological tests. Nevertheless, this study underscores the high antioxidant potential of **1**, which may be of interest, associated with polyphenolic compounds or not, for food industry applications.

In this paper, we also confirmed the antioxidant efficiency of **2** in both media. It is known that **2** has an antioxidant activity on copper-induced low-density lipoprotein peroxidation (*30*). Its very strong activity against lipid peroxidation depends on the chelating properties with respect to copper (*31*, *32*) and an antiradical activity by scavenging lipid peroxyl radicals within the cell membrane (*29*). Moreover, **2** is also able to scavenge O_2^- (*33*) and DPPH (*13*, *34*). However, a recent study showed that **2** is not a simple antioxidant but may act as a pro-oxidant of the DNA system through the reduction of iron or copper (*35*−*37*). It may also stimulate the reduction of iron in adenosine 5′-diphosphate—but not ethylenediaminetetraacetic acid—complexes (*37*), in contrast with stilbene derivatives such as diethylstilbestrol and stilbene itself. It could be interesting to prepare stilbenic structures as antioxidants of **2** but without any pro-oxidative effect. Compound **4** exhibits very high antioxidant capacity in both hydrophilic and lipophilic media. Moreover, it has a molecular structure close to that of diethylstilbestrol allowing it to be a potential estrogen without pro-oxidative effects. It would then be interesting to evaluate its estrogenic activities in different biological systems.

ABBREVIATIONS USED

DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picryhydrazyl; EPR, electron paramagnetic resonance; $LOO^{\bullet}$, lipoperoxyl radicals; O/W, oil/water; *P*, partition coefficient; ROS, reactive oxygen species; SEM, standard error of the mean.

ACKNOWLEDGMENT

We thank J. C. Izard (ACTICHEM, Montauban, France) for providing the crude extract of vine roots.

LITERATURE CITED

(1) Das, D. K.; Sato, M.; Maulik, G.; Engelman, R. M.; Bertelli, A. A. E.; Bertelli, A. Cardioprotection of red wine: role of polyphenolic antioxidants. *Drugs Exp. Clin. Res*. **1999**, *25*, 115−120.

(2) Kaneko, T.; Baba, N. Protective effect of flavonoids on endothelial cells against linoleic acid hydroperoxide-induced toxicity. *Biosci., Biotechnol., Biochem*. **1999**, *63*, 323−328.

(3) Renaud, S.; De Longeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

(4) St. Leger, A. S.; Cochrane, A. L.; Moore, F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet* **1979**, *1*, 1017−1020.

(5) Tesseidre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *in vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

(6) Thomas, M. J. The role of free radicals and antioxidants. *Nutrition* **2000**, *16*, 716−718.

(7) Chen, J. H.; Ho, C. T. Antioxidant properties of caffeic acid and its related hydroxycinnamic acid compounds. *J. Agric. Food Chem.* **1997**, *45*, 2374−2378.

(8) Tirosh, O.; Katzhendler, Y.; Barenholz, Y.; Ginsburg, I.; Kohen, R. Antioxidant properties of amidothionophosphates: novel antioxidant molecules. *Free Radical Biol. Med.* **1996**, *20*, 421−432.

(9) Ruiz-Larrea, M. L.; Martin, C.; Martinez, R.; Navarro, R.; Lacort, M.; Miller, N. J. Antioxidant activities of estrogens against aqueous and lipophilic radicals; differences between phenol and catechol estrogens. *Chem. Phys. Lipids* **2000**, *105*, 179−188.

(10) Chen, J. H.; Ho, C. T. Antioxidant properties of polyphenols extracted from green and black teas. *J. Food Lipids* **1995**, *2*, 35−46.

(11) Miao, J.-L.; Wang, W.-F.; Pan, J.-X.; Lu, C.-Y.; Li, R.-Q.; Yao, S.-D. The scavenging reactions of nitrogen dioxide radical and carbonate radical by tea polyphenol derivatives: a pulse radiolysis study. *Radiat. Phys. Chem*. **2001**, *60*, 163−168.

(12) Hung, L.-M.; Chen, J.-L.; Huang, S.-S.; Lee, R.-S.; Su, M.-J. Cardioprotective effect of resveratrol, a natural antioxidant derived from grape. *Cardiovasc. Res*. **2000**, *47*, 549−555.

(13) Wang, M.; Jin, Y.; Ho, C.-T. Evaluation of resveratrol derivatives as potential antioxidants and identification of a reaction product of resveratrol and 2,2-diphenyl-1-picryhydrazyl radical. *J. Agric. Food Chem.* **1999**, *47*, 3974−3977.

(14) Bavaresco, L.; Fregoni, C.; Cantu, E.; Trevisan, M. Stilbene compounds: from the grapevine to wine. *Drugs Exp. Clin. Res*. **1999**, *25*, 57−63.

(15) Breuil, A.-C.; Adrian, M.; Pirio, N.; Meunier, P.; Bessis, R.; Jeandet, P. Metabolism of stilbene phytoalexins by *Botrytis cinerea*: 1. Characterization of a resveratrol dehydrodimer. *Tetrahedron Lett*. **1998**, *39*, 537−540.

(16) Goldberg, D. A.; Tsang, E.; Kamuranchiri, A.; Diamandis, E. P.; Soleas, G.; Ng, E. Method to assay the concentrations of phenolic constituents of biological interest in wines. *Anal. Chem*. **1996**, *68*, 1688−1694.

(17) Bachelor, F. W.; Lowman, A. A.; Snowdon, L. R. Synthesis of pinosylvin and related heartwood stilbenes. *Can. J. Chem*. **1970**, *48*, 1554.

(18) Sieber, R. H., Reactionen von Chloracetaldehyd mit aromatischen Kohlenwasserstoffen, Phenolen und Phenolaethern. *Liebigs Ann. Chem.* **1969**, *730*, 31−46.

(19) Bala, A. E. A.; Kollmann, A.; Ducrot, P.-H.; Majira, A.; Kerhoas, L.; Leroux, R.; Delorme, R.; Einhorn, J. Cis ϵ-viniferin: A new antifungal resveratrol dehydrodimer from *Cyphostemma crotalarioides* roots. *J. Phytopathol*. **2000**, *148*, 29−32.

(20) Ducrot, P.-H.; Kollmann, A.; Bala, A. E.; Majira, A.; Kerhoas, L.; Delorme, R.; Einhorn, J. Cyphostemmins A−B, two new antifungal oligostilbenes from *Cyphostemma crotalarioides* (Vitaceae). *Tetrahedron Lett.* **1998**, *39*, 9655−9658.

(21) Marco, G. J. A rapid method for evaluation of antioxidants. *J. Am. Oil Chem. Soc*. **1968**, *45*, 594−598.

(22) Miller, H. E. A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc*. **1971**, *48*, 91.

(23) Taga, M. S.; Miller, E. E.; Pratt, D. E. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc*. **1984**, *61*, 928−931.

(24) Huber, K. R.; Sridhar, R.; Griffith, E. H.; Amma, E. L.; Roberts, J. Superoxide dismutase-like activities of copper(II) complexes tested in serum. *Biochim. Biophys. Acta* **1987**, *915*, 267−276.

(25) Arudi, R. L.; Allen, A. O.; Bielski, B. H. J. Some observations on the chemistry of KO_2-DMSO solutions. *FEBS Lett*. **1981**, *135*, 265−267.

(26) Janzen, E. G.; West, M. S.; Kotake, Y.; DuBose, C. M. Biological spin trapping methodology. III. Octanol−water partition coefficients of spin-trapping compounds. *J. Biochem. Biophys. Methods* **1996**, 32, 183−190.

(27) Larson, R. A. The antioxidants of higher plants. *Phytochemistry* **1988**, *27*, 969−978.

(28) Madsen, H. L.; Bertelsen, G.; Skibsted, L. H. Antioxidative activity of spices and spice extracts. In *Spices*: *Flavor chemistry and Antioxidant Properties*; Risch, S. J., Ho, C.-T., Eds.; American Chemical Society: Washington, DC, 1997; pp 176−197.

(29) Talodini, B.; Juliano, C.; Piu, L.; Franconi, F.; Cabrini, L. Resveratrol inhibition of lipid peroxidation. *Free Radical Res.* **2000**, *33*, 105−114.

(30) Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993**, *341*, 1103−1104.

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

(32) Belguendouz, L.; Frémont, L.; Linard, A. Resveratrol inhibits metal ion-dependent and independent peroxidation of porcine low-density lipoproteins. *Biochem. Pharmacol*. **1997**, *53*, 1347−1355.

(33) Zini, R.; Morin, C.; Bertelli, A.; Bertelli, A. A.; Tillement, J. P. Effects of resveratrol on the rat brain respiratory chain. *Drugs Exp. Clin. Res*. **1999**, *25*, 87−97.

(34) Waffo-Teguo, P.; Fauconneau, B.; Deffieux, G.; Huguet, F.; Vercauteren, J.; Mérillon, J.-M. Isolation, identification, and antioxidant activity of three stilbene glucosides newly extracted from *Vitis vinifera* cell cultures. *J. Nat. Prod*. **1998**, *61*, 655−657.

(35) Ahmad, A.; Asad, S. F.; Singh, S.; Hadi, S. M. DNA breakage by resveratrol and Cu(II): reaction mechanism and bacteriophage inactivation. *Cancer Lett*. **2000**, *154*, 29−37.

(36) Burkitt, M. J.; Duncan, J. 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*. **2000**, *381*, 253−263.

(37) Miura, T.; Muraoka, S.; Ikeda, N.; Watanabe, M.; Fujimoto, Y. Antioxidative and prooxidative action of stilbene derivatives. *Pharmacol. Toxicol*. **2000**, *86*, 203−208.

Received for review May 22, 2001. Revised manuscript received November 26, 2001. Accepted November 26, 2001. This research work was supported in part by French−Portuguese Grant 420 B3. We thank the Midi-Pyrénées Région and its Health-Food Network for financial support.

JF010676T