JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 1653−1657 **1653**

Oxidation of Resveratrol Catalyzed by Soybean Lipoxygenase

MARÍA DEL CARMEN PINTO, JOSÉ ANTONIO GARCÍA-BARRADO, AND PEDRO MACÍAS*

Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Extremadura, 06071 Badajoz, Spain

In this work the oxidative degradation of resveratrol catalyzed by lipoxygenase-1 (LOX-1) has been studied. The process has been characterized by spectroscopic and polarographic measurements. The oxidation of resveratrol was dependent on the concentration of resveratrol and the enzyme. When resveratrol was incubated in the presence of lipoxygenase at pH 9.0, the reaction displayed a k_M value of 18.6×10^{-6} M and a catalytic efficiency (k_{cat}/k_M) of 4.3×10^4 s^{-1} M^{-1}. These values are close to those shown by the enzyme when linoleic acid is used as the substrate. The effect of lipoxygenase inhibitors on the lipoxygenase-catalyzed resveratrol oxidation was also evaluated. The rate of resveratrol oxidation was markedly decreased by the presence of NDGA in the incubation mixture. From HPLC measurements, it can be deduced that resveratrol is oxidatively decomposed to a complex mixture of products similar to those obtained when the molecule is oxidized by hydrogen peroxide.

KEYWORDS: Resveratrol; soybean lipoxygenase; antioxidant; oxidative degration

INTRODUCTION

Resveratrol (3,4,5-trihydroxy-*trans*-stilbene) is a natural phytoalexin found in grapes and wine and shows antifungal properties and health benefits, including antioxidant and anticarcinogenic effects. Likewise, its protection against cardiovascular diseases has been reported (*1*−*3*).

Resveratrol possesses an ability to inhibit lipid peroxidation, similar to butylated hydroxyacetone and propyl gallate, compounds widely used as efficient antioxidants in foods (*4*). Although the inhibition mechanism not has been totally elucidated, it is known that resveratrol inhibits lipid peroxidation mainly by scavenging lipid peroxyl radicals in a way similar to that of α-tocopherol but with less efficiency (*5*). On the other hand, it is known that arachidonic acid metabolites are involved in the development of human cancer and that resveratrol acts as a modulator of this eicosanoid metabolic pathway with beneficial therapeutic effects, but the mechanisms of these processes are not sufficiently understood (*6*). One of the enzymes involved in the oxidative metabolization of arachidonic acid is lipoxygenase, a group of non-heme iron proteins that are ubiquitous in plant and animals. They catalyze the dioxygenation of polyunsaturated fatty acids containing a 1,4-*cis*,*cis*-pentadiene system. In animals, the resulting hydroperoxy fatty acid is a precursor to a lot of biologically potent molecules such leukotrienes and lipoxins, compounds of wide pathophysiological implications in humans, including inflammatory responses, asthma, atherosclerotic lesions, and carcinogenic processes (*7*). In plants, lipoxygenase products appear to be involved in development, growth, wound, and pest response (*8*). The modulation of lipoxygenase activity by natural compounds is also of great interest in the food industry because the enzyme products are responsible for the generation of flavor and aroma species in foods from vegetal or animal sources (*9*). In addition to dioxygenase activity, lipoxygenases from plant and animals exhibit, in the presence of a suitable hydrogen donor, a hydroperoxidase activity responsible for the cooxidation of drugs, mutagens, and a wide series of chemicals (*10*).

In previous work, we demonstrate that resveratrol is a potent inhibitor of dioxygenase activity of lipoxygenase using linoleic acid as substrate. However, hydroperoxidase activity is maintained, being that resveratrol is oxidized in the presence of lipid hydroperoxide or hydrogen peroxide (*11*). As a continuation of this study, the oxidation of resveratrol by soybean lipoxygenase in the absence of hydroperoxides has been investigated. Although it has been reported that pathogenic fungi use lacasse to degrade resveratrol (*12*) and that a tyrosinasa detected in grape juice produces a rapid degradation of resveratrol (*13*), to the best of our knowledge, resveratrol degradation by lipoxygenase in the absence of lipid hydroperoxides or hydrogen peroxide not has been previously reported.

We show in this work that resveratrol is a good lipoxygenase substrate, being oxidized to a form that is spectroscopically coincident with that obtained when it is oxidized in the presence of lipoxygenase plus hydroperoxides or only in the presence of hydroperoxides.

MATERIALS AND METHODS

Resveratrol, *trans*-stylbene, soybean lipoxygenase-1 (12 U/mg, 1 U being the amount of enzyme that forms 1 μmol of linoleic acid hydroperoxide per minute at pH 9.0 at 25 °C), NDGA (nordihydroguaiaretic acid: 1,4-bis[3,4-dihydroxyphenyl]2,3-dimethylbutane) and BHT (di-*tert*-butyl-4-methylphenol) were from Sigma-Aldrich Quimica (Madrid, Spain). All the reactives used in this work were of analytical grade.

* Corresponding author (telephone 34 924 274800, extension 9051; fax 34 924 271304; e-mail pedrom@unex.es).

10.1021/jf025818d CCC: $25.00
Published on Web 02/04/2003

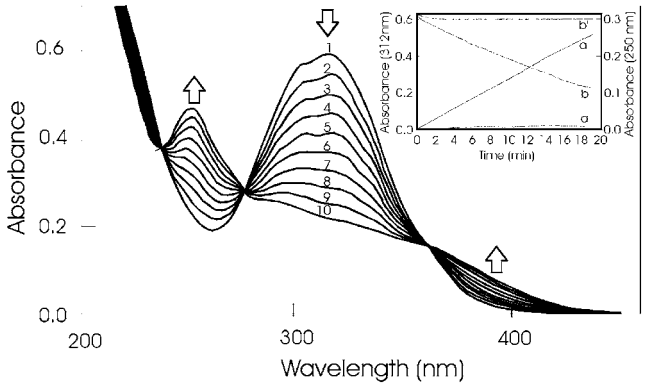

Figure 1. Oxidation of resveratrol by soybean lipoxygenase followed by repetitive scans of absorption spectra. The reaction medium contained 100 mM borate buffer, pH 9.0, 0.62 units of soybean lipoxygenase, and 2.2×10^{-5} M resveratrol. (Units of dioxygenase activity are for use of linoleic acid as substrate measured in 100 mM borate buffer, pH 9.0.) The elapsed time between scans was 2 min. The inset corresponds to the time course of the absorbance measured at 312 nm (a) and 250 nm (b). Plots a′ and b′ correspond to the same measurements but using heat-denatured lipoxygenase.

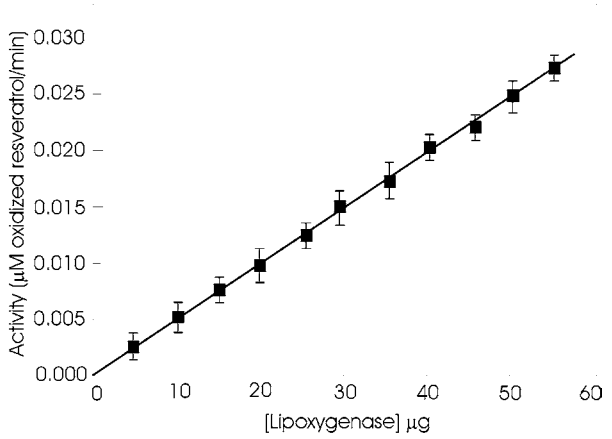

Figure 2. Effect of lipoxygenase concentration on the rate of resveratrol oxidation. The incubation medium contained 22 μM resveratrol, soybean lipoxygenase, and 100 mM borate buffer, pH 9.0, with a final volume of 1 mL. Resveratrol oxidation was measured by determination of the decrease in absorbance at 312 nm.

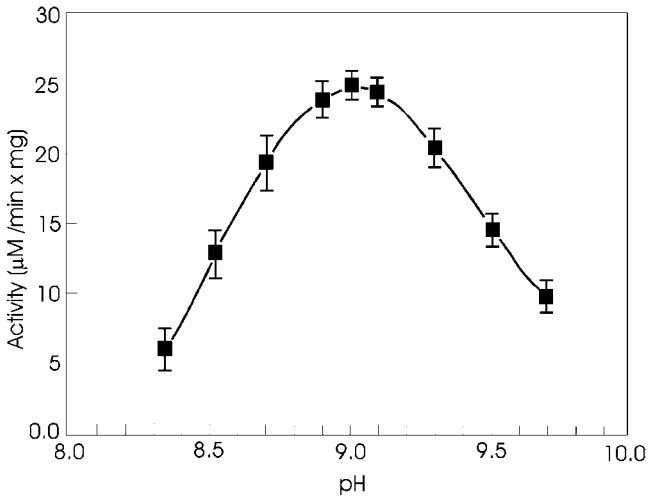

Figure 3. Determination of the pH optimum of the resveratrol oxidation by lipoxygenase. The incubation mixture was composed of 50 μM resveratrol and 0.05 mg of lipoxygenase in 100 mM borate buffer at the pH values indicated in the figure. Enzymatic activity was measured by following the decrease in absorbance at 312 nm, and 100% activity corresponds to the oxidation of 0.45 μmol of resveratrol per minute per and per milligram of protein.

The resveratrol oxidation rate was monitored as a decrease in absorbance at 312 nm. The incubation mixture contained 100 mM borate buffer, pH 9.0, soybean lipoxygenase, and resveratrol at the concentration indicated in each experiment, in a final volume of 1 mL. Resveratrol concentration was determined using an extinction coefficient of 33 400 $M^{-1}cm^{-1}$ (*11*). When linoleic acid was used as substrate, the enzymatic activity was measured by determination of the increase in absorbance at 234 nm of an incubation mixture containing 7.5×10^{-4} M linoleic acid, soybean lipoxygenase, and 100 mM borate buffer, pH 9.0, in a final volume of 1 mL.

RP-HPLC analyses of the products of the resveratrol oxidation by lipoxygenase were carried out using a Jasco equipment with a 150 mm $\times$ 4.6 mm nucleosil C18 column (5 μm particle size), (Merck, Darmstadt, Germany). Samples were eluted isocratically using a flow rate of 1 mL min^{-1}, with a solvent system of methanol/H_2O/glacial acetic acid (50:50:0.1). The effluent from the column was continuously monitored at the wavelength indicated in each experiment using a Jasco UV-1575 detector, and the chromatograms were registered and integrated using the software Biocrom 2000-3.0.

Oxygraphic measurements were performed using a Clark-type electrode (YSI 5300 biological oxygen monitor). The measurement cuvette contained 3.0 mL of 0.1 M borate buffer, pH 9.0, and substrate (resveratrol or linoleic acid). The reaction was started by adding lipoxygenase-1 (LOX-1) at the concentration indicated in each experiment.

All the results shown in the figures and tables are the mean of at least three replicates in each experiment. In the figures, the SD is indicated as error bars.

RESULTS AND DICUSSION

The oxidation of resveratrol by soybean LOX-1 was monitored by repetitive scans. **Figure 1** was obtained when lipoxygenase was incubated in the presence of resveratrol in borate buffer, pH 9.0. The reaction was started by adding LOX-1, and then the spectrum was recorded. The following can be seen: the decrease in absorbance of the band centered at 312 nm, the concomitant increase in absorbance at 250 nm and in the region of 375−395 nm, and the presence of two isosbestic points at 275 and 369 nm, respectively. From this behavior, it is often taken as evidence that only two species are involved in the process, but it is conceivable that other species generated in the medium may not have absorption in the UV−vis range. When heat-inactivated LOX-1 was used, no modification of the resveratrol spectra was observed (see inset of **Figure 1**). This fact, together with the linear correlation among the enzyme concentration in the medium and the rate of resveratrol conversion (**Figure 2**), strongly suggests the enzymatic origin of the process.

The effect of pH on the reaction rate was studied. **Figure 3** shows a pH optimum of 9.0 for resveratrol oxidation catalyzed by lipoxygenase. This value is similar to the pH optimum of the linoleic acid hydroperoxidation mediated by LOX-1. This coincidence in the pH optimum value may contribute to explain why resveratrol is a good substrate for lipoxygenase. Likewise to the described process of curcumin oxidation by lipoxygenase (*14*), the phenolic hydroxyl group of resveratrol at alkaline pH may mimic the carboxylate group of linoleic acid. This fact suggests that this phenolic group may constitute a signal for resveratrol identification as a substrate of lipoxygenase. In a previous work, we reported that resveratrol is a competitive inhibitor of dioxygenase activity of lipoxygenase when linoleic

Table 1. Kinetic Parameters of LOX-1-Catalyzed Oxygenation of Resveratrol and Linoleic Acid

substrate	assay	K_M, M	V_{Max}, μmol min^{-1} mg^{-1}	K_{cat}, s^{-1}	K_{cat}/K_M, s^{-1} M^{-1}
trans-resveratrol	absorbance	18.6×10^{-6}	0.45	0.81	4.3×10^4
trans-resveratrol	polarographic	12.6×10^{-5}	0.12	0.22	1.7×10^3
cis-resveratrol	polarographic	17.0×10^{-5}	0.21	0.38	2.2×10^3
linoleic acid	absorbance	0.15×10^{-3}	13.45	25.90	1.7×10^5
linoleic acid	polarographic	1.67×10^{-3}	74.02	133.33	8.0×10^4

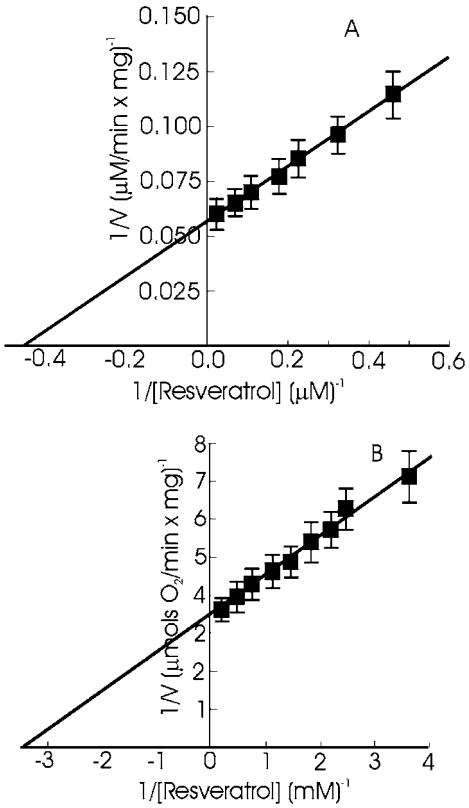

Figure 4. Lineweaver–Burk plot of the resveratrol oxidation by lipoxygenase measured by determination of the decrease in absorbance at 312 nm (A) or by determination of the oxygen consumption (B). For spectrophotometric determination of the activity, the incubation mixture contained 0.05 mg of lipoxygenase (12.4 U/mg) and resveratrol in 100 mM borate buffer, pH 9.0, with a final volume of 1 mL. For oxygraphic determination of activity, the incubation mixture contained resveratrol, 0.5 mg of soybean lipoxygenase, and 100 mM borate buffer, pH 9.0, with a final volume of 3.0 mL. The obtained values for k_M and V_{Max} are indicated in **Table 1**.

acid is used as substrate (*11*), supporting the hypothesis that resveratrol is easily recognized by the active site of lipoxygenase.

The determination of kinetic parameters of resveratrol oxidation by lipoxygenase was carried out by evaluation of the time course of the decrease in absorbance at 312 nm. The quantification was performed using an extinction coefficient for resveratrol of 33 400 M^{-1}cm^{-1} (*11*). A Lineweaver−Burk plot (**Figure 4A**) showed typical Michaelis−Menten kinetics qualitatively similar to the kinetics obtained when the substrate used is linoleic acid. It is to be noted that k_M for resveratrol oxidation is lower than that obtained for linoleic acid when the process was followed by oxygraphic or spectrophotometric measurements (**Table 1**). In both cases the ratio obtained was near 9, indicating a significant affinity of resveratrol for the lipoxygenase molecule. Although the value for K_{cat} is significantly lower for resveratrol

Table 2. Inhibition of Resveratrol Oxygenation by Lipoxygenase[a]

substrate	inhibitor (mM)	activity ± SD (%)
resveratrol		100 ± 2
linoleic acid		100 ± 3
resveratrol	NDGA (0.025)	40 ± 1
linoleic acid	NDGA (0.025)	90 ± 1
resveratrol	NDGA (0.15)	15 ± 0.2
linoleic acid	NDGA (0.15)	80 ± 1
resveratrol	BHT (0.02)	70 ± 0.5
linoleic acid	BHT (0.02)	90 ± 2
resveratrol	BHT (0.10)	40 ± 1
linoleic acid	BHT (0.10)	70 ± 2

[a] Inhibition experiments were carried out with spectrophotometric measurements, following the increase in absorbance at 234 nm or the decrease at 312 nm when linoleic acid or resveratrol, respectively, were used as substrate. A 100% activity corresponds to the generation of 13.45 μmol of hydroperoxy linoleic acid per minute and per milligram of protein or to 0.45 μmol of resveratrol oxidized per minute and per milligram of protein.

oxidation in comparison with that for linoleic acid, the catalytic efficiency is of the same order in both cases (see column K_{cat}/K_M in the **Table 1**). On the other hand, the similarity among the kinetics parameters of *cis*- and *trans*-resveratrol is to be noted, suggesting that the type of configuration is not a determinant factor for the use of resveratrol as the lipoxygenase substrate.

The effect of lipoxygenase inhibitors on the resveratrol oxidation catalyzed by LOX-1 was studied. **Table 2** shows the effect of NDGA and BHT, being remarkable that both inhibitors are more efficient in resveratrol oxidation than in linoleic acid oxidation. This behavior may be explained on the basis of the different properties of these compounds. NDGA is a more specific lipoxygenase inhibitor than BHT, which is considered to be a general antioxidant. Both compounds are oxygen radical scavengers, but NDGA additionally converts the active Fe(III) form of lipoxygenase to the inactive Fe(II) form (*15*). The strong inhibition of the resveratrol oxidation reaction shown in this study suggest that, in addition to the catalytic effect of the enzyme, the total process may be mediated by a participation of radicals in a proportion significant higher than in the catalytic reaction of linoleic acid oxidation.

The oxidative degradation of resveratrol by lipoxygenase was checked by RP-HPLC. A typical chromatogram of a sample taken from the incubation mixture immediately after the reaction was started is shown in **Figure 5A,C**. It can be seen that the peak of resveratrol (**Figure 5A**), detected at 312 nm, diminished significantly after 10 min of incubation (**Figure 5B**). **Figure 5C,D** shows the increase in the product concentration in the medium by detection at 381 nm. It is noted that the chromatographic profile of the products obtained at 381 nm (**Figure 5D**) is coincident with that obtained at 250 nm (data not shown); in both cases only a peak at a retention time of 4.81 min was obtained. These results indicate that resveratrol is oxidized probably to a unique chemical species or to a mixture of compounds with close structural and chemical properties.

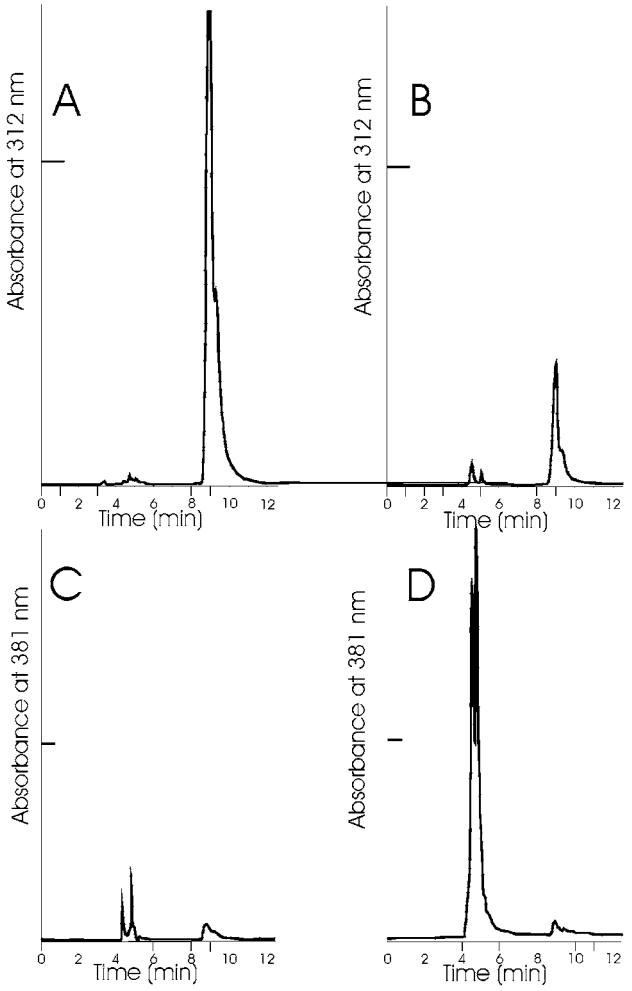

Figure 5. HPLC chromatograms of products formed by lipoxygenase-catalyzed oxidation of resveratrol. The incubation mixture contained 0.1 mg of soybean lipoxygenase and 0.1 mM resveratrol in 100 mM borate buffer, pH 9.0, with a final volume of 1 mL. Panels A and C correspond to direct injection of a sample of the incubation mixture at $t = 0$ min of incubation. Panels B and C correspond to a time of incubation of 20 min. The shoulder in panels A and B and the two peaks in panels C and D are produced by the presence of *cis*-resveratrol.

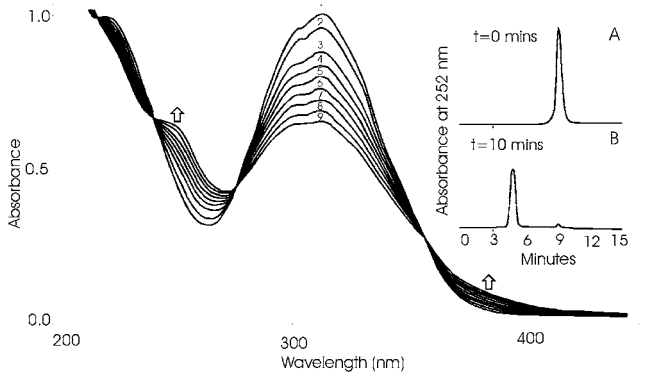

Figure 6. Resveratrol oxidation by hydrogen peroxide followed by repetitive scans of absorption spectra. The incubation mixture contained 2 M hydrogen peroxide and 2 mM resveratrol in 100 mM borate buffer, pH 9.0. The elapsed time between scans was 2 min. Inset: RP-HPLC of the incubation mixture. The sample was injected at the incubation time indicated and chromatographed using the same experimental conditions as that in the resveratrol oxidation by lipoxygenase (see Materials and Methods).

The confirmation that products from the catalytic action of lipoxygenase are derived from a degradative oxidation of resveratrol was obtained from a comparison of the above-mentioned results with the results from the chemical oxidation of resveratrol by hydrogen peroxide (**Figure 6**). The shape of the repetitive scans is coincident with that obtained for the resveratrol oxidation mediated by LOX-1 (**Figure 1**). The analysis of the reaction products by RP-HPLC reveals the elution of a single peak (**Figure 6B**) with a retention time coincident with that obtained for the oxidative process catalyzed by lipoxygenase. These results suggest that resveratrol suffers a drastically oxidative decomposition catalyzed by LOX-1 at a level comparable with that obtained when the oxidation was carried out using hydrogen peroxide in the absence of lipoxygenase. This pattern is similar to the pattern described for the oxidative degradation of quercetin (*16*). In this case, a considerable increase in absorbance in the range 260−290 nm is produced, being attributed to the formation of protocatehuic acid. In addition, an increase in absorbance in the region of 400 nm due to quinones formation has been observed when phenols are oxidized by peroxidases (*17*). These spectroscopic changes are totally coincident with the spectral shape observed when resveratrol is oxidized by lipoxygenase. This pattern has been described for the oxidative degradation of chlorogenic acid and caffeic acid, being assumed that in a lot of polyphenolic compounds the oxidation process is mediated by quinone radical species as intermediates of the final polymerization reaction products (*18*). On the other hand, spectroscopic evidence of quinonic species formation in the resveratrol oxidation process may be a consequence of an epoxidation at the ethylene bridge. This effect has been described for the process of resveratrol oxidation mediated by CytP3A4 (*19*) and fit well with the necessary structural disposition of double bonds for lipoxygenase substrates. Although additional analytic studies are necessary, the HPLC and spectroscopic data obtained in this work together with the effect of inhibitors suggest that the oxidative process studied is similar to the study previously described for other systems with peroxidase/phenolic compounds in which quinonic radical species are generated.

Finally, and with the aim of checking the molecular structural characteristic responsible for the efficiency of resveratrol as a lipoxygenase substrate, the oxidation of *trans*-stilbene by lipoxygenase was studied. By use of experimental conditions similar to the conditions used for the resveratrol oxidation by lipoxygenase, repetitive scans of an incubation mixture containing *trans*-stilbene instead resveratrol were carried out. It can be seen that *trans*-stibene is not degraded by lipoxygenase (**Figure 7**). These data suggest that the disposition of the −OH group is the main structural reason for facilitating the correct substrate−enzyme interaction, in concordance with the interpretation of the effect of pH on the lipoxygenase-mediated reaction of resveratrol oxidation (**Figure 3**).

CONCLUSIONS

This study shows that resveratrol is a good substrate for lipoxygenase, being oxidatively degraded to a product structurally complex with a spectroscopic properties similar to the properties described for the products derived from the oxidative degradation of polyphenolic antioxidants. Although more information is necessary to elucidate the complete composition of the products obtained from the oxidative degradation of resveratrol, the aim of this work has been to establish the fact

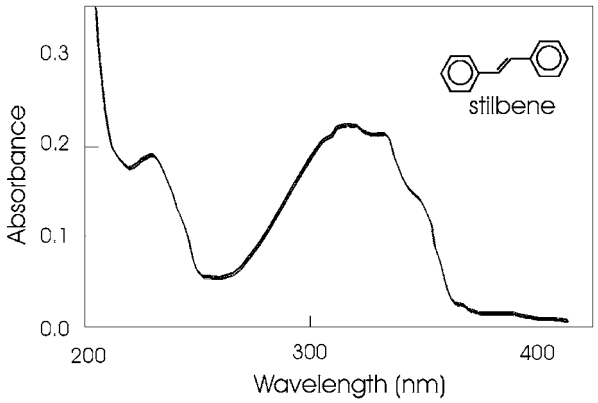

Figure 7. Repetitive scans of the incubation mixture containing 0.1 mg of lipoxygenase and 0.1 mM *trans*-stilbene in 100 mM borate buffer with a final volume of 1 mL. Ten spectra were recorded, and the elapsed time between each scan was 2 min.

that resveratrol may be enzymatically decomposed by lipoxygenase.

The results presented in this work are of application to biomedical and agrofood fields. The information obtained may be of relevance in the interpretation of the mechanism of resveratrol as an antioxidant and may contribute to the understanding of the basis of the properties of this molecule as a platelet aggregation inhibitor or as an anticarcinogenic and antiinflamatory agent. Processes in those lipoxygenase activities are involved (*20*).

Also, the results obtained here may contribute to the explanation of the changes in the composition of resveratrol detected in vegetables of great interest in the agrofood industry. It is known that, in addition to agrotechnical conditions, the content of resveratrol in wines has been related to the presence of tyrosinase activity in grapes (*13*). On the other hand, the presence of lipoxygenase in grapes produces 9- and 13-hydroperoxides, precursors of six-carbon aldehydes and compounds involved in wine organoleptic properties (*21*). In consequence, it is conceivable that the content of resveratrol in wines may be modulated by lipoxygenase activity from yeast, microorganisms, or grapes. As a result of this, and with the objective of explaining the great differences reported in the resveratrol content of different wines, our group is now studying the correlation between lipoxygenase activity and resveratrol in grape juice.

LITERATURE CITED

(1) Jang, M. S.; Cai, E. N.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218−220.

(2) Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P. Resveratrol content of wines of different ages: relationship with fungal disease pressure in the vineyard. *Am. J. Enol. Vitic.* **1995**, *46*, 1−4.

(3) Das, D. K.; Sato, M.; Ray, P. S.; 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.

(4) Murcia, M. A.; Martinez-Tome, M. Antioxidant activity of resveratrol compared with common food additives. *J. Food Prot.* **2001**, *64*, 379−384.

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

(6) Cuendet, M.; Pezzuto, J. M. The role of ciclooxygenase and lipoxygenase in cancer chemoprevention. *Drug Metab. Drug Interact.* **2000**, *17*, 109−157.

(7) Kamitani, H.; Geller, M.; Thomas, E. Expression of 15-lipoxygenase by human colorectal carcinoma Caco-2 cells during apoptosis and cell differentation. *J. Biol. Chem.* **1998**, *273*, 21569−21577.

(8) Siedow, J. N. Plant lipoxygenase: Structure and Function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 145−156.

(9) Gata, J. L.; Pinto, M. C.; Macías, P. Lipoxygenase activity in pig muscle: purification and partial characterization. *J. Agric. Food Chem.* **1996**, *44*, 2573−2577.

(10) Hu, J.; Kulkarni, A. P. Lipoxygenase-mediated demetylation of pesticides. *Pestic. Biochem. Physiol.* **1998**, *61*, 145−153.

(11) Pinto, M. C.; García-Barrado, J. A.; Macías, P. Resveratrol is a potent inhibitor of the dioxygenase activity of lipoxygenase. *J. Agric. Food Chem.* **1999**, *47*, 4842−4846.

(12) Sbaghi, M.; Jeandet, P.; Bessis, R.; Leroux, P. Degradation of stilbene-type phytoalexins in relation to the pathogenicity of *Botrytis cinerea* to grapevines. *Plant Pathol.* **1996**, *45*, 139−144.

(13) Gilly, R.; Mara, D.; Oded, S.; Zohar, K. Resveratrol and a novel tyrosinase in Carignan grape juice. *J. Agric. Food Chem.* **2001**, *49*, 1479−1485.

(14) Scheneider, C.; Amberg, A.; Feurle, J.; Rob, A.; Roth, M.; Tóth, G.; Schreier, P. 2-[(4″-Hydroxy-3′-methoxy)-phenoxy]-4-(4″-hydroxy-3″-methoxy-phenyl)-8-hydroxy-6-oxo3-oxabicyclo[3.3.0]-7-octene: unusual product of the soybean lipoxygenase-catalyzed oxygenation of curcumin. *J. Mol. Catal. B: Enzym.* **1998**, *4*, 219−227.

(15) Veldink, G. A.; Vliegenthart, J. F. G. Substrates and products of lipoxygenase catalysis. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science Publishers BV: Amsterdam, 1989; Vol. 9, pp 559−589.

(16) Makris, D. P.; Rossiter, J. T. Heat-induced, metal catalyzed oxidative degradation of quercetin and rutin (quercetin-3-*O*-rhamnosylglucoside) in aqueous mode. *J. Agric. Food Chem.* **2000**, *48*, 3830−3838.

(17) Lopez-Serrano, M.; Ros-Barcelo, A. Peroxidase mediated browning reactions in plant foods. *Recent Res. Dev. Agric. Food Chem.* **1998**, *2*, 546−563.

(18) Hotta, H.; Sakamoto, H.; Nagano, S.; Osaki, T.; Tsujuno, Y. Unusually large number of electrons oxidation of polyphenolic antioxidants. *Biochim. Biophys. Acta* **2001**, *1526*, 159−167.

(19) Chan, W. K.; Delucchi, A. B. Resveratrol, a red wine constituent, is a mechanism based inactivator of cytochrome P4503A4. *Life Sci.* **2000**, *67*, 3103−3112.

(20) Pace-Asciak, C. R.; Rounova, O.; Hahn, S. E.; Diamandis, E. P.; Goldberg, D. M. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. *Clin. Chim. Acta* **1996**, *246*, 163−182.

(21) Hsieh, R. J. Contribution of lipoxygenase pathway to food flavors. In *Lipids in Food Flavors*; Ho, C. T., Hartman, T. G., Eds.; American Chemical Society: Washington, DC, 1994; Chapter 3, pp 30−48.

Received for review July 15, 2002. Revised manuscript received December 28, 2002. Accepted January 6, 2003. This work was supported by Grant PB96-1463 from the Spanish DGES and partially by Grant IPR99CO12 from the Junta de Extremadura and Grant 1FD97-2244 from the Spanish CICYT.

JF025818D