Pterostilbene, a New Agonist for the Peroxisome Proliferator-Activated Receptor α-Isoform, Lowers Plasma Lipoproteins and Cholesterol in Hypercholesterolemic Hamsters

AGNES M. RIMANDO,*,[†] RANGASWAMY NAGMANI,[‡] DENNIS R. FELLER,[§] AND
WALLACE YOKOYAMA[||]

Natural Products Utilization Research Unit, ARS, U.S. Department of Agriculture, P.O. Box 8048,
University, Mississippi 38677, Department of Pharmacology, School of Pharmacy, University of
Mississippi, University, Mississippi 38677, and Western Regional Research Center, ARS,
U.S. Department of Agriculture, 800 Buchanan St., Albany, California 94710

Resveratrol, a stilbenoid antioxidant found in grapes, wine, peanuts and other berries, has been reported to have hypolipidemic properties. We investigated whether resveratrol and its three analogues (pterostilbene, piceatannol, and resveratrol trimethyl ether) would activate the peroxisome proliferator-activated receptor α (PPARα) isoform. This nuclear receptor is proposed to mediate the activity of lipid-lowering drugs such as the fibrates. The four stilbenes were evaluated at 1, 10, 100, and 300 μM along with ciprofibrate (positive control), for the activation of endogenous PPARα in H4IIEC3 cells. Cells were transfected with a peroxisome proliferator response element-AB (rat fatty acyl CoA β-oxidase response element)−luciferase gene reporter construct. Pterostilbene demonstrated the highest induction of PPARα showing 8- and 14-fold increases in luciferase activity at 100 and 300 μM, respectively, relative to the control. The maximal luciferase activity responses to pterostilbene were higher than those obtained with the hypolipidemic drug, ciprofibrate (33910 and 19460 relative luciferase units, respectively), at 100 μM. Hypercholesterolemic hamsters fed with pterostilbene at 25 ppm of the diet showed 29% lower plasma low density lipoprotein (LDL) cholesterol, 7% higher plasma high density lipoprotein (HDL) cholesterol, and 14% lower plasma glucose as compared to the control group. The LDL/HDL ratio was also statistically significantly lower for pterostilbene, as compared to results for the control animals, at this diet concentration. Results from in vitro studies showed that pterostilbene acts as a PPARα agonist and may be a more effective PPARα agonist and hypolipidemic agent than resveratrol. In vivo studies demonstrate that pterostilbene possesses lipid and glucose lowering effects.

KEYWORDS: Pterostilbene; PPARα; cholesterol; lipoprotein

INTRODUCTION

The peroxisome proliferator-activated receptor (PPAR) isoforms are members of the nuclear receptor superfamily of ligand-activated transcription factors. They were first identified in *Xenopus* frogs as receptors that induce the proliferation of peroxisomes (*1*). Three PPAR isoforms are known as follows: PPARα, PPARγ, and PPARδ. The PPARs control gene expression by interaction with specific response elements in the promoter region of target genes (*2*). The PPARs play a central role in carbohydrate and lipid homeostasis and govern other biological processes such as energy metabolism, cell proliferation and differentiation, and inflammation (*3−5*). The PPARα isoform is predominantly involved in fatty acid and lipid catabolism and import and activation of genes involved in fatty acid oxidation in the liver, heart, kidney, and skeletal muscles (*6, 7*). In the liver, activation of PPARα leads to increased β-oxidation of fatty acids and decreased triglyceride and very low density lipoprotein (VLDL) synthesis (*8*). Activation of PPARα also leads to reduction of triglyceride because of repression of hepatic apolipoprotein C-III and an increase in lipoprotein lipase gene expression (*9*). Furthermore, PPARα activation causes induction of hepatic apoliporotein A-I and A-II expression, in humans, leading to increased plasma HDL cholesterol. PPARα agonists are also known to slow the progression of premature coronary atherosclerosis (*6*) and have

* To whom correspondence should be addressed. Tel: 662-915-1037. Fax: 662-915-1035. E-mail: arimando@msa-oxford.ars.usda.gov.
  [†] Natural Products Utilization Research Unit, ARS, U.S. Department of Agriculture.
  [‡] Current address: Enzymatic Therapy, Inc., Product Development, 825 Challenger Drive, Green Bay, WI 54311.
  [§] University of Mississippi.
  [||] Western Regional Research Center, ARS, U.S. Department of Agriculture.

been demonstrated to regulate metabolism of amino acids in the liver (*10*).

Resveratrol is a well-known antioxidant (*11*, *12*) and cancer chemopreventive (*13*) compound present in grapes and wine. Its occurrence in wine has been linked to a low incidence of fatal coronary heart disease among populations consuming wine moderately (*14*, *15*). Dietary resveratrol at 50 parts per million (ppm) suppressed blood serum lipid peroxidase levels in rats and dose-dependently suppressed serum triglyceride and VLDL and low density lipoprotein (LDL) cholesterol levels (*16*). Pterostilbene is another grape compound that was also found to have antioxidant (*11*, *17*) and cancer chemopreventive properties similar to resveratrol (*17*). Pterostilbene has antidiabetic (*18*) properties and is cytotoxic to a number of cancer cell lines in vitro (*19*). A cardiotonic Ayurvedic preparation, Darakchasava, which has *Vitis vinifera* as the main ingredient, was shown to contain resveratrol and pterostilbene (*20*). Like resveratrol and pterostilbene, piceatannol has cancer chemopreventive properties (*21*) and is a stronger antioxidant than resveratrol and a potent antiarrhythmic agent (*22*, *23*). Piceatannol showed antiallergic effects in experimental models of type I allergy (*24*). Resveratrol trimethyl ether was found to be more cytotoxic than resveratrol in cultured human lung and colon cancer cells (*25*).

In view of the above-mentioned reports on the hypolipidemic property and a recent report on the activation of PPAR isoforms by resveratrol (*26*) and cardioprotective effects of drinks and preparations containing resveratrol and pterostilbene, we propose that these structurally related compounds may be activators of PPARα. Piceatannol and resveratrol trimethyl ether were of interest because their biological activity profiles are similar to that of resveratrol and pterostilbene and in some assays are reported to be more potent. We therefore also investigated whether these analogues would activate PPARα isoform.

EXPERIMENTAL SECTION

General Experimental Procedures. Resveratrol and piceatannol were commercial samples obtained from Sigma-Aldrich (St. Louis, MO) and Calbiochem (San Diego, CA), respectively. H4IIEC3 cells were purchased from the American Type Culture Collection (Rockville, MD). The PPRE-AB luciferase (LUC) gene reporter construct was provided by Dr. Daniel J. Noonan (Department of Biochemistry, University of Kentucky, Lexington, KY). The LUC assay kit was obtained from Promega Corporation (Madison, WI). NMR experiments were carried out on a Bruker Avance DRX (500 MHz) instrument. For thin-layer chromatography (TLC), aluminum-backed silica gel F_{254} plates, 10 cm × 20 cm, 0.2 mm thick (EM Science, Gibbstown, NJ) were used. For preparative layer chromatography (PLC), Merck silica gel F_{254} 20 cm × 20 cm, 1.0 mm thick plates (VWR Scientific, Atlanta, GA) were used. All solvents used were high-performance liquid chromatography grade (Fisher Scientific, Suwanee, GA).

Preparation of Pterostilbene and Resveratrol Trimethyl Ether. Pterostilbene and resveratrol trimethyl ether were prepared by partial methylation of *trans*-resveratrol. To a solution of *trans*-resveratrol (150 mg in 3.0 mL of MeOH), diazomethane was added dropwise, and the reaction was monitored by TLC for the methylated products. The reaction solution was dried under vacuum. The partially methylated products were purified by PLC (developing solvent, hexane:EtOAc, 8:2; R_f 0.6 and 0.8 for pterostilbene and resveratrol trimethyl ether, respectively). The identity and structure of these compounds were confirmed by comparison with published spectroscopic data.

Activation of PPARα in Rat Liver Cells. The conditions for these experiments are essentially as described (*27*). Briefly, H4IIEC3 (rat hepatoma cell line) cells were grown in 150 mm × 25 mm Petri dishes with 1:1 mixture of Dulbecco's modified Eagle's medium:Ham's F12 medium (DMEM/F12) supplemented with 10% (v/v) fetal bovine serum and penicillin G sodium (100 U/mL) or streptomycin (100 μg/mL).

When the cultures were 75% confluent, the cells were detached and transfected with the firefly LUC reporter gene construct containing PPRE-AB (peroxisome proliferator response element with rat fatty acyl CoA β-oxidase AB promoter region sequence). Detached cells were electroporated in 400 μL of medium containing the plasmid at 190 V and a single 70 ms pulse using BTX disposable cuvettes (model 640; 4-mm gap) with a BTX model T 820 electroporator (BTX Instrument Division, Harvard Apparatus, Inc., Holliston, MA). The transfected cells were plated at a density of 100000 cells/well in 96 well microtiter plates (Cultureplate, Packard) and allowed to grow for 24 h. After 24 h, the cells were treated with ciprofibrate at 10 and 100 μM and resveratrol and its three analogues at 1, 10, 100, and 300 μM concentrations. After incubation with the test compounds for 30 h, the cells were lysed and the LUC activity was measured using a LucLite assay kit (Packard). Light output was detected by a TopCount microplate scintillation counter in the single photon counting mode luminometer (Packard Instrument Company, Meriden, CT).

Data Analysis. Data are expressed as relative light or LUC units, and the changes in LUC response (counts per minute) are plotted vs the analogue concentration. Results are also expressed as fold-induction over the corresponding control value. Each analogue treatment was done in triplicate or quadruplicate. Data were analyzed using GraphPad Prism 3 software.

Hamster Feeding. Male golden Syrian hamsters (Charles River, Wilmington, MA), 7−8 weeks old and 34−41 g, were housed individually in wire bottom cages in a room kept at 20−22°C, 60% relative humidity, and 12 h light and dark cycle. The hamsters were fed a powdered stock diet (Rodent Lab Chow 5001, Purina Mills, St. Louis, MO) for 7 days. Following the initial 7 day period, 8−10 animals were randomly assigned from a weight-sorted list to each of two test diets (α-cellulose control and pterostilbene-fortified diet). The diet consisted of 80 g of anhydrous butterfat, 100 g of corn oil, 20 g of Menhaden fish oil, 1.5 g of cholesterol, 50 g of microcrystalline cellulose, 200 g of casein, 497.5 corn starch, 3 g of methionine, 3 g of choline bitartrate, 35 g of mineral mix, and 10 g of vitamin mix. The diets were prepared by dissolving powdered cholesterol into warmed butterfat, followed by the addition of corn and fish oil. The liquid fat was also added to the dry ingredients while stirring. The diet for the test group was fortified with pterostilbene at 25 mg/kg of diet. Pterostilbene was dissolved in about 50 mL of ethanol and added to the stirring dry ingredients.

Food intake was measured twice each week, and body weights were monitored once a week. After 21 days, the animals were anesthesized with isofluorane, and then, 4−5 mL of blood was taken by heart puncture. Blood was analyzed for total cholesterol by the cholesterol oxidase method and lipoprotein cholesterol by size exclusion chromatography as previously described (*28*). All animal procedures were approved by the Animal Care and Use Committee, Western Regional Research Center, U.S. Department of Agriculture (Albany, CA) and conformed to the principles in Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals 1985). Plasma blood glucose was determined using a blood glucose meter (Fast Glucose Meter-Precision Xtra, MediSense, Bedford, MA).

RESULTS AND DISCUSSION

Of the four stilbene analogues (**Figure 1**) tested, pterostilbene demonstrated the highest induction of LUC activity at 100 and 300 μM in H4IIEC3 cells transfected with the PPRE-AB-LUC reporter gene construct (**Figure 2**). The maximal LUC responses to pterostilbene at 100 and 300 μM were about 8- and 14-fold, respectively, when compared to the corresponding control values. The maximal LUC activity responses to 100 μM ciprofibrate were about 5-fold as compared to control. These results showed that PPARα activation by pterostilbene (33910 ± 788 relative LUC units) is higher than that of ciprofibrate (19460 ± 1466 relative LUC units) at 100 μM. However, at a lower concentration (10 μM), PPARα activation by pterostilbene was lower than that of ciprofibrate. These results indicate that ciprofibrate, a potent hypolipidemic agent of the fibrate class,
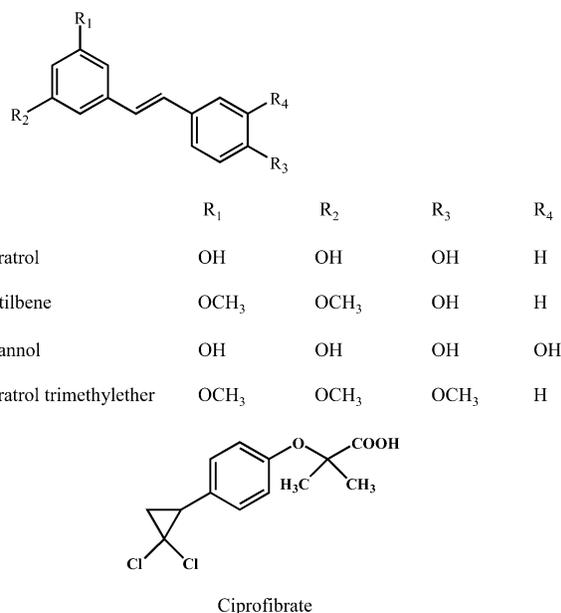
Pterostilbene as a New PPARα Agonist

J. Agric. Food Chem., Vol. 53, No. 9, 2005 **3405**

Figure 1. Structures of compounds tested for activation of PPARα in rat liver cells.

	R₁	R₂	R₃	R₄
Resveratrol	OH	OH	OH	H
Pterostilbene	OCH₃	OCH₃	OH	H
Piceatannol	OH	OH	OH	OH
Resveratrol trimethylether	OCH₃	OCH₃	OCH₃	H

Table 1. Effect of Pterostilbene on Plasma Cholesterol and Glucose Levels and Liver and Body Weights in Hamsters

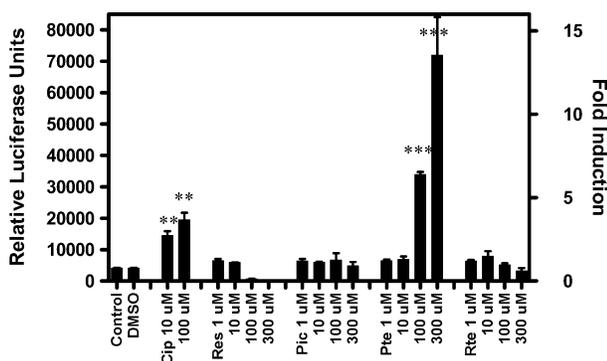|	control	pterostilbene
plasma lipoprotein cholesterol (mg/dL)[a]		
VLDL	99.3 ± 15.3	82.7 ± 15.7
LDL	320.9 ± 4.9	228.1 ± 4.2
HDL	127.4 ± 1.1	137.0 ± 2.1
total	547.6 ± 6.7	447.8 ± 5.6
LDL/HDL ratio	2.6 ± 0.37	1.8 ± 0.39
final weights (g)[a]		
body weight	100.9 ± 2.0	110.5 ± 2.7
liver weight	6.6 ± 0.18	7.9 ± 0.28
liver/body weight ratio	6.6 ± 0.09	7.1 ± 0.12
plasma glucose (mg/dL)[a]	216.5 ± 10.1	185.1 ± 8.7
animals per group (*n*)	10	8

[a] Values are expressed as the means ± SEM.

Figure 2. Effect of stilbenes on PPARα in H4IIEC3 cells transfected with PPRE-AB LUC reporter gene plasmid. Cip, ciprofibrate; Res, resveratrol; Pic, piceatannol; Pte, pterostilbene; and Rte, resveratrol trimethyl ether. **Significantly different from control, $p = 0.001$; ***highly significantly different from control, $p = 0.0001$; $n = 4$. Control is media only; DMSO is solvent control without test compounds.

resveratrol and the three other stilbenes investigated in this study did not activate PPARγ using a MCF-7 breast tumor cell line (data not shown). The difference in responses to resveratrol may be due to the difference in experimental protocols and conditions under which the tests were performed. In our study, endogenous PPARα population was used and the rat hepatoma cells were transfected with only the PPRE-AB-LUC reporter gene plasmid. In the published study, the PPARα receptor was cotransfected with the PPRE-AB response elements resulting in a higher density of PPARα receptor in the cell-based assays. The difference in cell type and species may also explain the discrepancy between our result and those of Inoue's group. It has been shown that clofibrate induced PPARα expression greater than linoleic acid in a murine hepatoma cell line; however, in a human hepatoma cell line, linoleic acid was the most effective ligand (*29*). In their study, Inoue et al. (*26*) tested resveratrol for the activation of PPARα in the absence of a positive control. In our study, we have determined the activity of resveratrol together with its three analogues and ciprofibrate as a positive control. Our results indicate that pterostilbene, like ciprofibrate, acts as an agonist of PPARα in H4IIEC3 cells, whereas the three other stilbene analogues are not activators of PPARα.

Several PPARα specific agonists have been shown to reduce hyperlipidemia and insulin resistance in hamsters fed normal chow, high fat chow, or high fructose and fat augmented chow diets (*30–32*, respectively). We conducted an animal feeding study on high fat fed hamsters to investigate the effect of pterostilbene on plasma levels of cholesterol and lipoproteins, having found this compound to activate PPARα in our in vitro assay. Hamsters were fed a single dose of 25 mg pterostilbene per kg of diet. This dose was decided based upon published studies on resveratrol feeding using rats, i.e., 10 and 50 ppm of the diet (*16*), and is much lower than the dose of methylclofenapate used in a study, i.e., 25 mg/kg animal body weight (*30*), or of fenofibrate, 30–300 mg/kg (*31*), fed to hamsters. Pterostilbene intake is estimated to be about 2.5 mg/kg body weight of animal based on approximately 10 g/day feed intake in a 100 g animal. As compared to the control group, plasma LDL cholesterol was 29% lower and plasma high density lipoprotein (HDL) cholesterol was 7% higher in the pterostilbene-fed group (**Table 1**). The total plasma cholesterol was 18% lower as compared with control animals. The LDL cholesterol and total plasma cholesterol values for the pterostilbene-fed hamsters are statistically significantly different from those of the control animals (as analyzed by two-tailed *t*-test; $p < = 0.05$). The total cholesterol was lowered about 25% by fenofibrate in the high fructose- and fat-fed hamster model and lowered by about

is more potent than pterostilbene and that higher concentrations of pterostilbene ($>100 \mu$M) are required to produce a concentration-dependent increase in PPARα activation as measured by increased LUC activities. Piceatannol and resveratrol trimethyl ether did not significantly increase LUC activity at all of the concentrations tested. Pterostilbene, as well as resveratrol, at 1 and 10 μM, produced no significant change in LUC activity, when compared to the control value. Resveratrol was toxic to H4IIEC3 cells at 100 and 300 μM. In summary, it is noteworthy that the presence of two methoxy groups (as in pterostilbene) but not three methoxy groups (as in resveratrol trimethyl ether) or three or four unmasked hydroxyl groups (as in resveratrol and piceatannol, respectively) was important as a structural requirement for PPARα activation in H4IIEC3 cells within this series of compounds.

Our result on resveratrol differs from that of a published study (*26*), where resveratrol was shown to activate PPARα in assays using five different cell lines: monkey kidney CV-1 cells, murine primary cortical cultures, bovine brain microvessel vascular endothelial cells, human umbilical venous endothelial cells, and bovine arterial endothelial cells. Inoue's group also found resveratrol to activate PPARγ in these cells. In contrast,

3406 *J. Agric. Food Chem.,* Vol. 53, No. 9, 2005

Rimando et al.

30% in the high fat chow-fed hamsters. Methylclofenapate had no effect on plasma cholesterol in hamsters fed chow (*30*). The LDL/HDL ratio was also statistically significantly lower for pterostilbene, as compared to results for the control animals at this diet concentration. Weight gain was similar for both groups. The plasma glucose was 14% lower in the pterostilbene-fed group as compared to the control group. This result is in agreement with an earlier study that showed pterostilbene to lower plasma blood glucose in hyperglycemic rats (*18*) and fenofibrate in high fat-fed hamsters (*31, 32*). It is worth mentioning that at the same time the feeding experiment with pterostilbene was done, feeding studies with two tomato varieties were also conducted. Freeze-dried tomatoes were fed at 10% of the total diet. Plasma cholesterol lowering was greater for pterostilbene (results not presented). The plasma lipid profile of the pterostilbene-fed hamsters showed improved cholesterol levels after 21 days in this diet, as compared to the hypercholesterolemic (control) animals. The plasma glucose levels were also lowered. These results are similar to results obtained with fenofibrate, a known PPARα specific agonist, in hamsters fed high fat diets. While results are from a single dose feeding study only, the data on lipid and glucose lowering effects appear to support the results obtained in vitro on pterostilbene's activation of PPARα. In summary, the comparable potency of pterostilbene to the known PPARα specific agonist, fenofibrate, at about 1/10 the dose warrants further studies of pterostilbene in animal models of hyperlipidemia and diabetes.

With regard to a comparison of results for related analogues present in blueberries, resveratrol has been shown to have hypolipidemic properties in rat feeding studies causing lowering of triglyceride (*16*) and serum cholesterol levels (*16, 33*). On the contrary, a study in rabbits showed no difference in lipoprotein−cholesterol concentration between the control group and the group that received oral resveratrol (*34*). Additionally, resveratrol promoted atherosclerotic development in these animals. Whether or not resveratrol is a cholesterol lowering agent, the mechanism by which it does so appears not to be via activation of PPARα, as shown in this in vitro study. On the basis of results of studies on the effect of resveratrol in human aortic cells, it was hypothesized that it may confer cardiovascular protection by functioning as a pleiotropic cellular effector (*35*). Results from studies in Donyu rats showed the hypocholesterolemic activity of resveratrol to be due to increased excretion of neutral sterols and bile acids in the feces (*16*).

"PPAR ligands or agonists" evolved as a group of structurally diverse compounds that activate these transcription factors and emerged as an important class of therapeutic agents as PPARs have become an important molecular target to treat human metabolic disorders. The fibrate drugs, which include ciprofibrate that was used in this study, were found to be ligands for PPARα, and their activation of PPARα provided a mechanistic explanation for their clinical efficacy to treat cardiovascular diseases (*36*). The finding that pterostilbene, a natural product, is an agonist for PPARα, which possesses an activity comparable to a clinically used hypolipidemic fibrate drug, provides a possible alternative for the treatment of dyslipidemias. Pterostilbene has been reported in some small fruits (*37, 38*) as well as in woody plants (*39, 40*). Results from our in vitro study suggest that pterostilbene may be a more effective hypolipidemic agent than resveratrol. In this paper, we also have demonstrated that pterostilbene is effective as a lipid/lipoprotein lowering agent in hypercholesterolemic hamsters. This is consistent with the proposal that PPARα activation by pterostilbene is a mediator of this in vivo lipid/liporotein lowering response. More

experiments will be required to determine if pterostilbene is acting primarily as a PPARα agonist.

LITERATURE CITED

(1) Dreyer, C.; Krey, G.; Keller, H.; Givel, F.; Helftenbein, G.; Wahli, W. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* **1992**, *68*, 879−87.

(2) Tugwood, J. D.; Aldridge, T. C.; Lambe, K. G.; Macdonald, N.; Woodyatt, N. J. Peroxisome proliferator-activated receptors: Structures and function. *Ann. N. Y. Acad. Sci.* **1996**, *804*, 252−265.

(3) Chakrabarti, R.; Rajagopalan, R. The role of PPARs in obesity and other insulin resistance associated disorders. *Curr. Med. Chem.: Immunol. Endocr. Metab. Agents* **2004**, *4*, 67−73.

(4) Escher, P.; Wahli, W. Peroxisome proliferator-activated receptors: Insight into multiple cellular functions. *Mutat. Res*. **2000**, *448*, 121−138.

(5) Mudaliar, S.; Henry, R. R. PPAR agonists in health and disease: A pathophysiologic and clinical overview. *Curr. Opin. Endocrinol. Diabetes* **2002**, *9*, 285−302.

(6) Fruchart, J.-C.; Staels, B.; Duriez, P. PPAR-alpha in lipid and lipoprotein metabolism, vascular inflammation and atherosclerosis. *Prog. Exp. Cardiol*. **2003**, *8*, 3−16.

(7) Gilde, A. J.; Van Bilsen, M. Peroxisome proliferator-activated receptors (PPARS): Regulators of gene expression in heart and skeletal muscle. *Acta Physiol. Scand.* **2003**, *178*, 425−434.

(8) Fruchart, J.-C.; Duriez, P. Anti-cholesterol agents, new therapeutic approaches. *Ann. Pharm. Fr.* **2004**, *62*, 3−18.

(9) Gervois, P.; Torra, I. P.; Fruchart, J.-C.; Staels, B. Regulation of lipid and lipoprotein metabolism by PPAR activators. *Clin. Chem. Lab. Med.* **2000**, *38*, 3−11.

(10) Kersten, S.; Mandard, S.; Escher, P.; Gonzalez, F. J.; Tafuri, S.; Desvergne, B.; Wahli, W. The peroxisome proliferator-activated receptor α regulates amino acid metabolism. *FASEB* **2001**, *15*, 1971−1978.

(11) Stivala, L. A.; Savio, M.; Fedarico, C.; Perucca, P.; Bianchi, L.; Magas, G.; Forti, L.; Pagnoni, U. M.; Albini, A.; Prosperi, E.; Vannini, V. Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol. *J. Biol. Chem.* **2001**, *276*, 22586−22594.

(12) Teguo, P. W.; 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.

(13) Jang, M.; Cai, L.; 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. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218−220.

(14) Hegsted, D. M.; Ausman, L. M. Diet, alcohol and coronary heart disease in man. *J. Nutr*. **1988**, *118*, 1184−1189.

(15) Renaud, S.; De Lorgeril, M. Wine, alcohol, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523.

(16) Miura, D.; Miura, Y.; Yagasaki, K. Hypolipidemic action of dietary resveratrol, a phytoalexin in grapes and red wine, in hepatoma-bearing rats. *Life Sci.* **2003**, *73*, 1393−400.

(17) Rimando, A. M.; Cuendet, M.; Desmarchelier, C.; Mehta, R. G.; Pezzuto, J. M.; Duke, S. O. Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. *J. Agric. Food Chem.* **2002**, *50*, 3453−3457.

Pterostilbene as a New PPARα Agonist

J. Agric. Food Chem., Vol. 53, No. 9, 2005 **3407**

(18) Manickam, M.; Ramanathan, M.; Jahromi, M. A. F.; Chansouria, J. P. N.; Ray, A. B. Antihyperglycemic activity of phenolics from *Pterocarpus marsupium. J. Nat. Prod.* **1997**, *60*, 609−610.

(19) Rimando, A. M.; Pezzuto, J. M.; Farnsworth, N. R.; Santisuk, T.; Reutrakul, V. Revision of the NMR assignments of pterostilbene and dihydrodehydro-diconiferyl alcohol: Cytotoxic constituents from *Anogeissus acuminata. Nat. Prod. Lett.* **1994**, *4*, 267−272.

(20) Paul, B.; Masih, I.; Deopujari, J.; Charpentier, C. Occurrence of resveratrol and pterostilbene in age-old Darakchasava, an Ayurvedic medicine from India. *J. Ethnopharmacol.* **1999**, *68*, 71−76.

(21) Waffo-Teguo, P.; Hawthorne, M. E.; Cuendet, M.; Merillon, J.-M.; Kinghorn, A. D.; Pezzuto, J. M.; Mehta, R. G. Potential cancer chemopreventive activities of wine stilbenoids and flavans extracted from grape (*Vitis vinifera*) cell cultures. *Nutr. Cancer* **2001**, *40*, 173−179.

(22) Hung, L. M.; Chen, J.-K.; Lee, R.-S.; Liang, H.-C.; Su, M.-J. Beneficial effects of astringinin, a resveratrol analogue, on the ischemia and reperfusion damage in rat heart. *Free Radical Biol. Med.* **2001**, *30*, 877−883.

(23) Lee, S. K.; Mbwambo, Z. H.; Chung, H.; Luyengi, L.; Gamez, E. J. C.; Mehta, R. G.; Kinghorn, A. D.; Pezzuto, J. M. Evaluation of the antioxidant potential of natural products. *Comb. Chem. High Throughput Screen.* **1998**, *1*, 35−46.

(24) Matsuda, H.; Tomohiro, N.; Hiraba, K.; Harima, S.; Ko, S.; Matsuo, K.; Yoshikawa, M.; Kubo, M. Study on anti-oketsu activity of rhubarb II. Anti-allergic effects of stilbene components from *Rhei undulati* rhizoma (dried rhizome of *Rheum undulatum* cultivated in Korea). *Biol. Pharm. Bull.* **2001**, *24*, 264−267.

(25) Lee, S. K.; Nam, K. A.; Hoe, Y. H.; Min, H.-Y.; Kim, E.-Y.; Ko, H.; Song, S.; Lee, T.; Kim, S. Synthesis and evaluation of cytotoxicity of stilbene analogues. *Arch. Pharm. Res.* **2003**, *26*, 253−257.

(26) Inoue, H.; Jiang, X.-F.; Katayama, T.; Osada, S.; Umesono, K.; Namura, S. Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator-activated receptor alpha in mice. *Neurosci. Lett.* **2003**, *352*, 203−206.

(27) Jaradat, M. S.; Noonan, D. J.; Wu, B.; Feller, D. R. Pseudolaric acid analogues as a new class of peroxisomal proliferator-activated receptor agonists. *Planta Med.* **2002**, 68, 667−671.

(28) German, J.; Xu, R.; Walzem, R.; Kinsella, J.; Nakamura, M.; Yokoyama, W. Effect of dietary fats and barley fiber on total cholesterol and lipoprotein cholesterol distribution in plasma of hamsters. *Nutr. Res.* **1996**, *16*, 1239−1249.

(29) Akbiyik, F.; Ray, D.; Bozkaya, H.; Demirpence, E. Ligand- and species-dependent activation of PPARalpha. *Cell Physiol. Biochem.* **2004**, *14*, 269−276.

(30) Choudhury, A. I.; Sims, H. M.; Horley, N. J.; Roberts, R. A.; Tomlinson, S. R.; Salter, A. M.; Bruce, M.; Shaw, P. N.; Kendall, D.; Barrett, D. A.; Bell, D. R. Molecular analysis of peroxisome proliferation in the hamster. *Toxicol. Appl. Pharmacol.* **2004**, *197*, 9−18.

(31) Wang, P.-R.; Guo, Q.; Ippolito, M.; Wu, M.; Milot, D.; Ventre, J.; Doebber, T.; Wright, S. D.; Chao, Y.-S. High fat fed hamster, a unique animal model for treatment of diabetic dyslipidemia with peroxisome proliferator activated receptor alpha selective agonists. *Eur. J. Pharm.* **2001**, *427*, 285−293.

(32) Rizvi, F.; Puri, A.; Bhatia, G.; Khanna, A. K.; Wulff, E. M.; Rastogi, A. K.; Chander, R. Antidyslipidemic action of fenofibrate in dyslipidemic-diabetic hamster model. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 215−222.

(33) Kollar, P.; Kotolova, H.; Necas, J.; Karpisek, M.; Bartosikova, L.; Karesova, P. Experimental study of resveratrol and flavonoids in red wine with regard to their possible hypolipidemic effect. *Vnitrni Lekarstvi* **2000**, *46*, 856−860.

(34) Wilson, T.; Knight, T. J.; Beits, D. C.; Lewis, D. S.; Engen, R. L. Resveratrol promotes atherosclerosis in hypercholesterolemic rabbits. *Life Sci.* **1996**, *59*, 15−21.

(35) Wu, J. M.; Lu, X.; Guo, J.; Hsieh, T.-C. In *Phytochemicals: Mechanisms of Action*; Meskin, M. S., Bidlack, W. R., Davies, A. J., Lewis, D. S., Randolph, R. K., Eds.; CRC Press LLC: Boca Raton, FL, 2003; pp 145−161.

(36) Roberts, R. A.; Moffat, G. J. Biology and toxicology of PPARα ligands−The hypolipidemic fibrate peroxisome proliferators. *Commun. Toxicol.* **2001**, *7*, 259−273.

(37) Adrian, M.; Jeandet, P.; Douillet-Breuil, A. C.; Bessis, R. Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. *J. Agric. Food Chem.* **2000**, *48*, 6103−6105.

(38) Rimando, A. M.; Magee, J.; Kalt, W.; Ballington, J. Resveratrol, pterostilbene and piceatannol in *Vaccinium* berries. *J. Agric. Food Chem.* **2004**, *52*, 4713−4719.

(39) Maurya, R.; Ray, A. B.; Duah, F. K.; Slatkin, D. J.; Schiff, P. L., Jr. Constituents of *Pterocarpus marsupium. J. Nat. Prod.* **1984**, *47*, 179−81.

(40) Arnone, A.; Camarda, L.; Merlini, L.; Nasini, G.; Taylor, D. A. H. Coloring matters of the West African red woods *Pterocarpus osun* and *P. soyauxii*. Structures of santarubins A and B. *J. Chem. Soc., Perkins Trans. 1* **1977**, *19*, 2116−2118.