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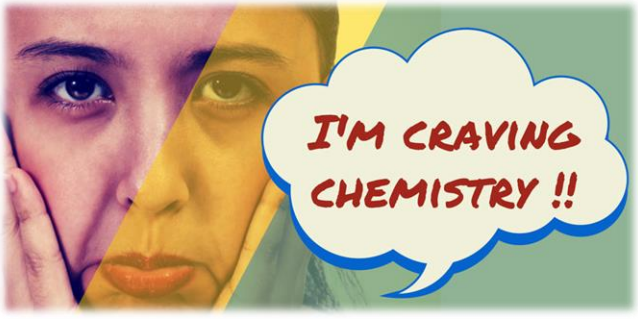

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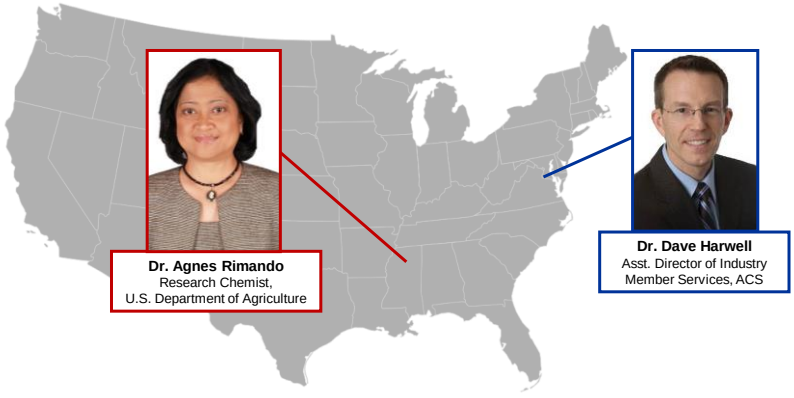
"Natural Product Chemistry:
Benefits of Pterostilbene on Health, Memory, and Anxiety"



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Natural Product Chemistry:
Benefits of Pterostilbene on Health, Memory, and Anxiety

Agnes M. Rimando, Ph.D.

Research Chemist
U.S. Department of Agriculture, Agricultural Research Service
Natural Products Utilization Research Unit
P.O. Box 1848
University, MS 38677 U.S.A.
agnes.rimando@ars.usda.gov

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Audience Survey Question

ANSWER WITH THE CORRECT LETTER IN THE QUESTIONS BOX

How familiar are you with the grape compound resveratrol?

a) **reserva-who?**
b) I have **heard of it** but know it by name only.
c) I have **read** scientific papers on resveratrol.
d) I have **written** scientific papers on resveratrol.

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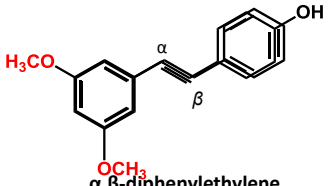
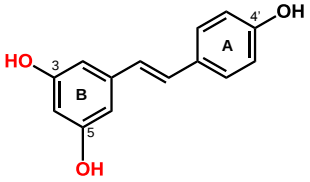
Pterostilbene



α,β-diphenylethylene
Pterostilbene
3,5-Dimethoxy-4'-hydroxystilbene



Resveratrol
3, 4',5-Trihydroxystilbene

- **First isolated from red sandalwood (*Pterocarpus santalinus*)** (Späth and Schläger, *Chem. Ber.* 73:881-884, 1940)
- **Phytoalexin**
- **Has diverse pharmacological properties**

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Pterostilbene Has Multiple Health-beneficial Properties

- Analgesic
- Antiallergy
- **Antianxiety**
- **Antiobesity**
- Antiinflammatory
- Anticancer
- Antidiabetic
- Antiinfective
- Asthma
- Arthritis
- Antioxidant
- **Hypolipidemic**
- **Improves cognitive function**
- **Potent neuromodulator in Alzheimer's disease**
- UV-A and UV-B protectant

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Peroxisome Proliferator-Activated Receptors

- Members of the nuclear receptor superfamily of ***ligand-activated*** **transcription factors that play essential roles in the regulation of carbohydrate and lipid-lipoprotein homeostasis, cell proliferation and differentiation, and inflammation.**
- **Three PPAR isoforms: PPARα, PPARβ/δ and PPARγ**
- **PPARα agonists (fibrates) have been extensively studied for their clinical use as hypolipidemic drugs for treatment of atherosclerosis.**
- **PPARα is mainly expressed in liver and predominantly involved in fatty acid and lipid-lipoprotein catabolism and import.**

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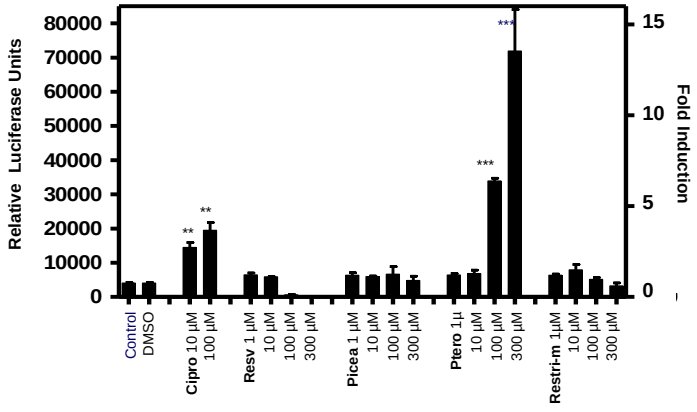
J. Agric. Food Chem. 2005, 53, 3403−3407

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||



* In H4IIEC3 rat hepatoma cells transfected with PPRE-AB-luciferase reporter gene plasmid.
** Significantly different from Ctrl, $p = 0.001$.
*** Highly significantly different from Ctrl, $p = 0.0001$ (n=4).

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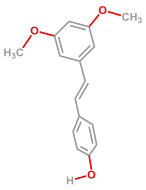
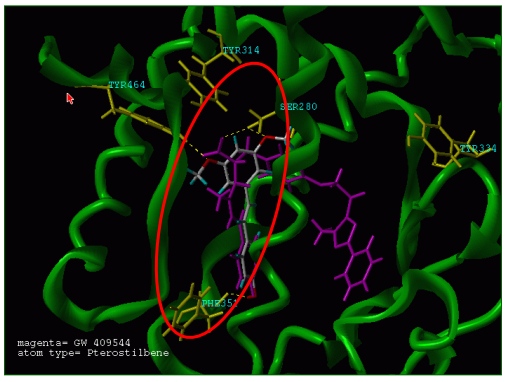
Docking of Pterostilbene in the PPARα Ligand-Binding Domain





Binding pose of pterostilbene inside PPARα ligand binding domain, superimposed on the co-crystal structure of GW409544 (magenta).

Pterostilbene fits in the hydrophobic binding cavity, and has H-bond interactions with the amino acids Tyr464, Ser280, and Phe351 that are involved in PPARα activation.

Mizuno et al., *Bioorg. Med. Chem.* 16:3800-3808, 2008.

Effect of Pterostilbene on Plasma Cholesterol and Glucose Levels in Hypercholesterolemic Hamster[a]

	Control	Pterostilbene
Plasma Lipoprotein Cholesterol (mg/dL)[b]		
VLDL	99.3 ± 15.3	82.7 ± 15.7 (17% ↓)
LDL	320.9 ± 4.9	228.1 ± 4.2 (29% ↓)
HDL	127.4 ± 1.1	137.0 ± 2.1 (7%↑)
Total	547.6 ± 6.7	447.8 ± 5.6 (18% ↓)
LDL/HDL ratio	2.6 ± 0.37	1.8 ± 0.39
Plasma glucose (mg/dL)	216.5 ± 10.1	185.1 ± 8.7 (14% ↓)
Animals per group	10	8

[a]Based on a single dose of 25 mg/kg of diet; 21-day feeding; pterostilbene intake is ~2.5 mg/kg BW.
[b]Values are expressed as the mean ± SEM.

Rimando et al., *J. Agric. Food Chem.* **2005**, *53*, 3403-3407.

Final body weight, food intake, and adipose tissue weights of rats fed pterostilbene-supplemented diets for 6 weeks

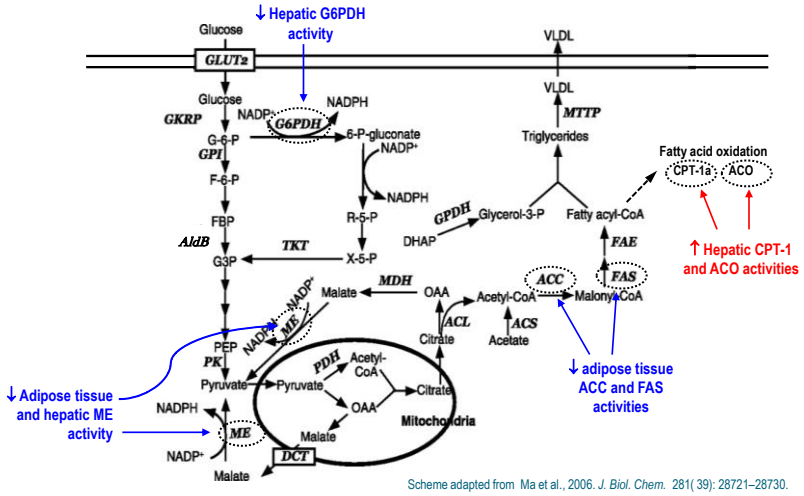
	Control	PT15	PT30	ANOVA
Body Weights (g)	202 ± 10	206 ± 9	200 ± 6	NS
Food intake (g/day)	17.6 ± 0.3	17.7 ± 0.5	17.5 ± 0.3	NS
Adipose tissue weights (g)				
Epididymal	11.8 ± 0.9	10.5 ± 0.9	10.5 ± 1.1	NS
Perirenal	13.5 ± 0.8^{a}	12.0 ± 1.1^{ab}	11.2 ± 1.0^{b} ↓	P<0.05
Mesenteric	6.0 ± 0.42^{a}	5.2 ± 0.4^{ab}	4.8 ± 0.5^{b} ↓	P<0.05
Subcutaneous	16.8 ± 1.1^{a}	12.6 ± 1.2^{b} ↓	12.7 ± 1.6^{b} ↓	P<0.05
Total fat depots	47.5 ± 3.0^{a}	40.3 ± 3.0^{b} ↓	36.6 ± 3.5^{b} ↓	P<0.05

Values are means ± SEM (n=9). Values in the same row with different superscript are significantly different at P<0.05 as determined by Newman-Keuls test. NS, not significant. PT15=15 mg/kg BW/day; PT30=15 mg/kg BW/day.

Gómez-Zorita et al., *J. Agric. Food Chem.* **2014**, *62*, 8371−8378.

21

Pterostilbene decreased the activities of key lipogenic enzymes in adipose tissue and liver, and increased the activities of hepatic fatty acid oxidation rate-limiting enzymes



Scheme adapted from Ma et al., 2006. *J. Biol. Chem.* 281(39): 28721–28730.

ACL, ATP citrate lyase; **ACC**, acetyl-CoA carboxylase 1; **ACO**, acyl-CoA oxidase; **CPT-1a**, carnithine palmitoyl transferase 1a; **DCT**, dicarboxylate transporter; **FAE**, fatty acid elongase 2; **FAS**, fatty acid synthase; **G6PDH**, glucose-6-phosphate dehydrogenase; **ME,** malic enzyme; **MTTP**, microsomal triglyceride transfer protein; **PDH**, pyruvate dehydrogenase subunit β; **TKT**, transketolase.

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Pterostilbene Clinical Trial

(University of Mississippi Medical Center in Jackson, MS)

- **Patients on pterostilbene exhibited minor weight loss**

	50 mg x 2 per day		125 mg x 2 per day	
	Effect (95% CI)	P value	Effect (95% CI)	P value
BMI*	-0.27 (-0.74, 0.20)	P = 0.268	-0.26 (-0.70, 0.18)	P = 0.250

*Compared to placebo.

- **Patients on the 250 mg/day dose achieved significant reduction in blood pressure compared to the placebo group: 7.8 mm HG in systolic (p,0.01) and 7.3 mm HG in diastolic (p , 0.001).**

Riche et al., Evidence-Based Complementary and Alternative Medicine. Volume 2014, Article ID 459165, 8 pages.

23

Metabolic Syndrome

- Also known as **Syndrome X**, is a cluster of cardiovascular risk factors.

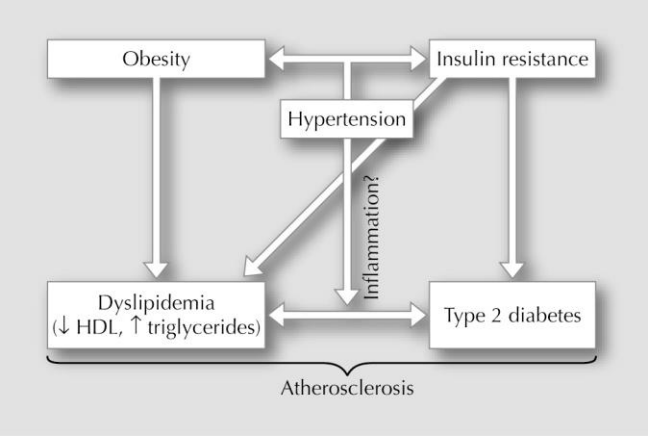

Figure from Akiyama et al. *Curr. Diabetes Rep*. 5:45-52, 2005.

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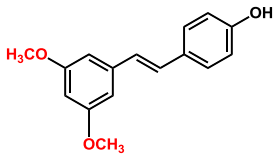
Beneficial Effect of Pterostilbene on Mind Function



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Audience Survey Question

ANSWER WITH THE CORRECT LETTER IN THE QUESTIONS BOX

A behavioral task to test hippocampal-dependent learning and memory is?

a) Mickey Mouse Maze
b) Morris Water Maze
c) Morris Wood Maze
d) Wisconsin Cheese Maze

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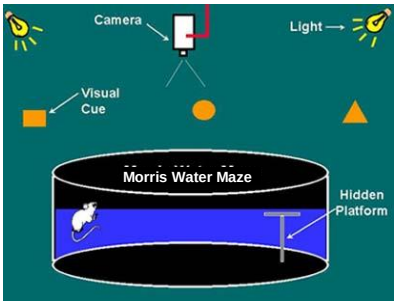
Pterostilbene Reversed Cognitive Deficits in Aged Mice

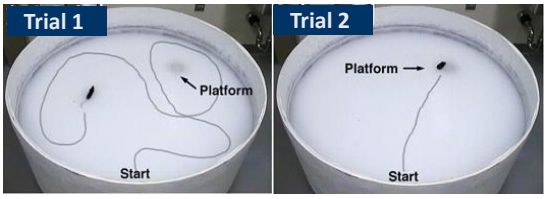


Downloaded from http://www.mcg.edu/Core/Labs/sabc/Morriswatermaze.html

Description: The Morris Water Maze test involves placing the rodent in a pool of water where it must use external visual cues to remember the location of a hidden platform just below the water's surface.

Purpose: The MWM test measures spatial learning and memory. This is one of the most popular tasks in behavioral neuroscience and is sensitive to both the amnestic and memory-enhancing effects of drugs.

Reference memory or acquisition trial



Working memory or retrieval trial

Downloaded from https://neuroamer.wordpress.com/2011/11/02/mpa-the-hormone-used-in-the-depo-provera-birth-control-shot-causes-memory-problems-in-rats/

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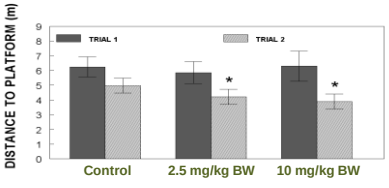
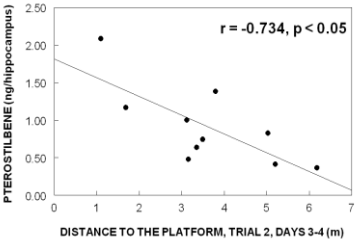
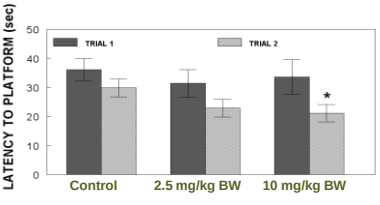
Working Memory was Correlated with Hippocamapal Levels of Pterostilbene

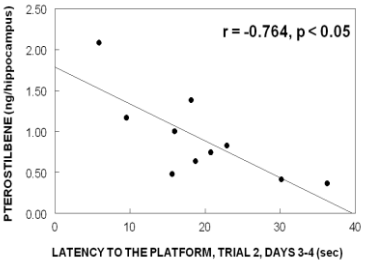


MWM performance assessed as **distance** to find the hidden platform. *Indicates a difference bet. Trial 1 and Trial 2 performance ($p < 0.05$); *i.e.*, **these groups had improved working memory**. This improvement was not seen in the control rats.





MWM performance assessed as **latency** to find the hidden platform. *Indicates a difference between Trial 1 and Trial 2 performance for the **high dose group** ($p < 0.05$); *i.e.*, this group **had improved working memory**. Improvement was not seen in the control rats; improvement approached significance in the low dose group ($p = 0.09$).



Joseph et al., *J. Agric. Food Chem.* 2008, 56,10544

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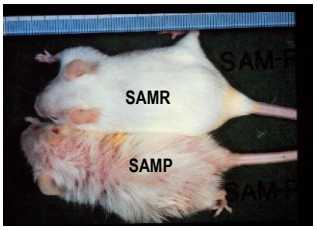
Neurobiol. Aging. 33, 2062-71, 2012

Low-dose pterostilbene, but not resveratrol, is a potent neuromodulator in aging and Alzheimer's disease

Jaewon Chang[a], Agnes Rimando[b], Merce Pallas[c], Antoni Camins[c], David Porquet[c], Jennifer Reeves[a], Barbara Shukitt-Hale[d], Mark A. Smith[e], James A. Joseph[d], Gemma Casadesus[a,*]

- Resveratrol or pterostilbene at 120 mg/kg of diet, 8-week feeding to 5-month old SAMP8. The dose is equated to content of resveratrol in 2 glasses of wine.
- Pterostilbene, but not resveratrol, significantly improved radial arm water maze function.
- Markers of cellular stress, inflammation, and Alzheimer's disease pathology were positively modulated by pterostilbene but not resveratrol.
- Improvements in behavior and cellular markers were associated with up-regulation of hippocampal PPARα.



Downloaded from http://www.ortodonziafunzionale.it/sofia/index.php/2008/05/05/sintropia-occlusione-il-ruolo-del-trigemino/

SAMR = Senescence accelerated mouse resistant

SAMP = Senescence accelerated mouse prone

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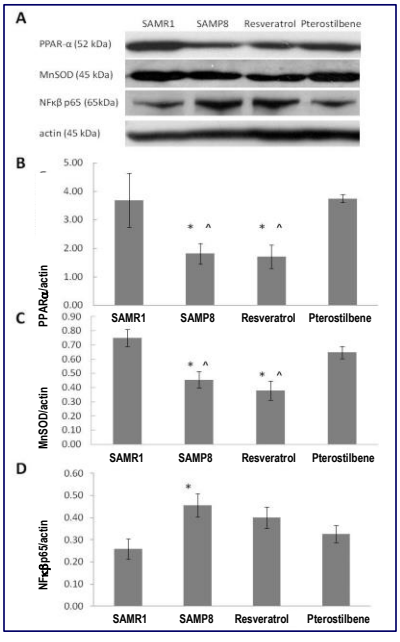
Pterostilbene Positively Modulated Changes in Antioxidant and Inflammatory Systems in SAMP8



*Significant from control. ^Significant from pterostilbene group.

A) Western blot of peroxisome proliferator-activated receptor(PPAR-α) and manganese superoxide dismutase (MnSOD), with β-actin control) in mouse hippocampus.

B) PPAR-α/actin ratio. There was a significant decrease of protein expression in SAMP8 compared with SAMR1; this was reversed by pterostilbene treatment but not resveratrol.

C) MnSOD/actin ratio. There was a significant decrease of protein expression in SAMP8 compared with SAMR1; this was increased by pterostilbene treatment.

D) NFκβ p65/actin ratio. NFκβ p65 levels in SAMP8 were significantly higher than those in the control SAMR1 group. Pterostilbene treatment showed a strong trend toward significance in inhibiting NFκβ p65 levels.

Chang et al. *Neurobiol. Aging* **2012**, *33*, 2062-71, 2012.

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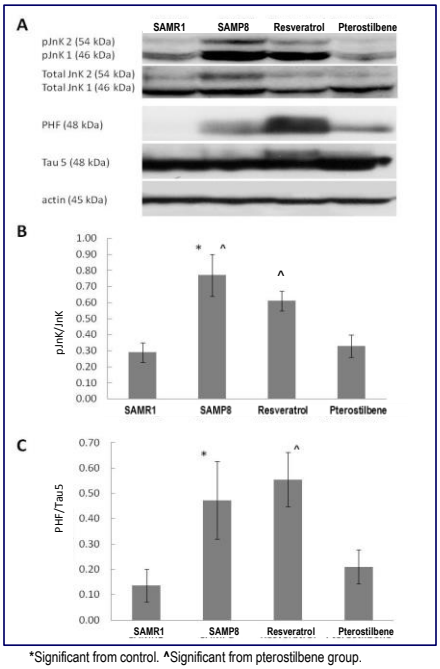
Pterostilbene Positively Modulated Changes in Stress Markers in SAMP8



*Significant from control. ^Significant from pterostilbene group.

A) Western blot of phosphorylated jun NH_2-terminal kinases (pJNK) and phosphorylation of tau (PHF) in mouse hippocampus. Hyperphosphorylation of tau is intimately associated with cellular stress processes including JNK phosphorylation.

B) pJNK/total JNK ratio showed a reduction in JNK phosphorylation levels compared with SAMP8 groups, similar to levels of SAMR1 control animals.

C) PHF/tau 5 ratio. Pterostilbene was effective at downregulating PHF-1 expression and was able to restore levels to SAMR1 controls.

Chang et al. *Neurobiol. Aging* **2012**, *33*, 2062-71, 2012.

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Bioavailability of Pterostilbene vs. Resveratrol

Other studies on bioavailability and pharmacokinetic properties of pterostilbene:

Azzolini et al., Pharmacokinetics and tissue distribution of pterostilbene in the rat. *Molec. Nutr. Food Res.* **2014**, *58*, 2122-2132.

Lin, et al., Determination of pterostilbene in rat plasma by a simple HPLC-UV method and its application in pre-clinical pharmacokinetic study. *Biomed. Chromatogr.* **2009**, *23*, 1308-1315.

Remsberg et al., Pharmacometrics of Pterostilbene: Preclinical Pharmacokinetics and Metabolism, Anticancer, Antiinflammatory, Antioxidant and Analgesic Activity. *Phytother. Res.* **2008**, *22*, 169–179.

Yeo et al., Pharmacokinetics of pterostilbene in Sprague-Dawley rats: The impacts of aqueous solubility, fasting, dose escalation, and dosing route on bioavailability. *Molec. Nutr. Food Res.* **2013**, *57*, 1015-1025.

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Physico-chemical Properties of Pterostilbene Favoring Good Membrane Permeability

- Moderate lipophilicity
  - Pterostilbene logP = 3.15 ± 0.001
  - Resvetatrol logP = 1.35 ± 0.000
    (Sobolev et al., 2011, *J. Agric. Food Chem.* 59:1673-1682)
- **Low** number of hydrogen bond donor and acceptor
- **Low** number of conjugation site
- **Low** polar surface area (38.7 A^2)
- **Low** number of rotatable bonds

Yeo et al., *Mol. Nutr. Food Res.* **2013**, *57*, 1015-1025.

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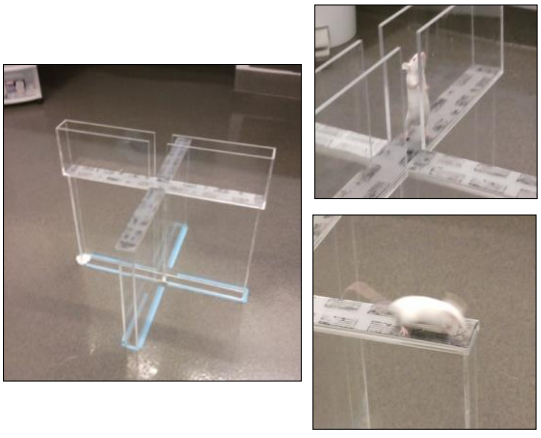
Antianxiety Effect of Pterostilbene



Pictures from Dr. Abir El-Alfy.

Elevated Plus Maze Test - one of the most widely validated tests for assaying new antianxiety agents. The rodent is placed in the center of the apparatus and allowed to freely explore for 5 min. The distance traveled and time spent on both open and enclosed arms, and the number of open- and enclosed-arm entries are quantified using a computer-assisted video tracking system.

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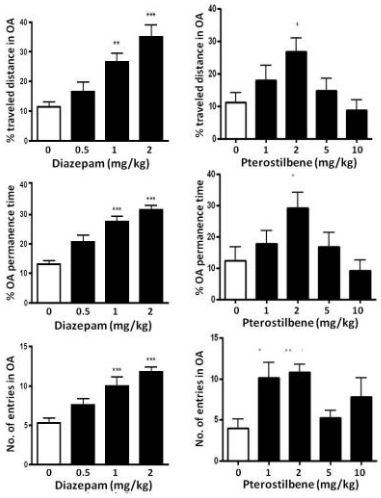
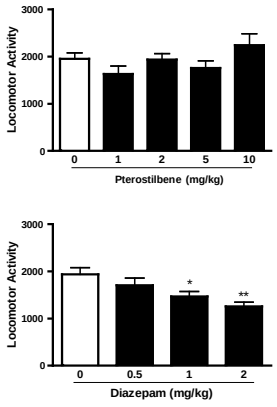
Pterostilbene Manifested Antiaxiety Activity Similar to Diazepam in the EMP Test



Pterostilbene animals (right panels) at 1 and 2 mg/kg BW dose caused increases in % permanence time and number of entries in the open arms similar to the Diazepam animals (left panels).



The locomotor activity of the pterostilbene animals (top) was unaffected at all doses while the Diazepam animals (bottom) showed decreases in locomotor activity.

Rahim, M.D.; Rimando, A.M. et al., *Planta Medica*, **2013**, *79*, 723.

Audience Survey Question

ANSWER WITH THE CORRECT LETTER IN THE QUESTIONS BOX

Pterostilbene has been reported in these fruits?

a) blueberries
b) cranberries
c) grapes
d) strawberries
e) All of the above

Vaccinium Berries from Mississippi, North Carolina and Oregon

Twenty-four varieties representng five *Vaccinium* species were analyzed for pterostilbene content by GC-MS.

V. arboretum
(Sparkleberry)

V. ashei
(Rabbiteye blueberry)

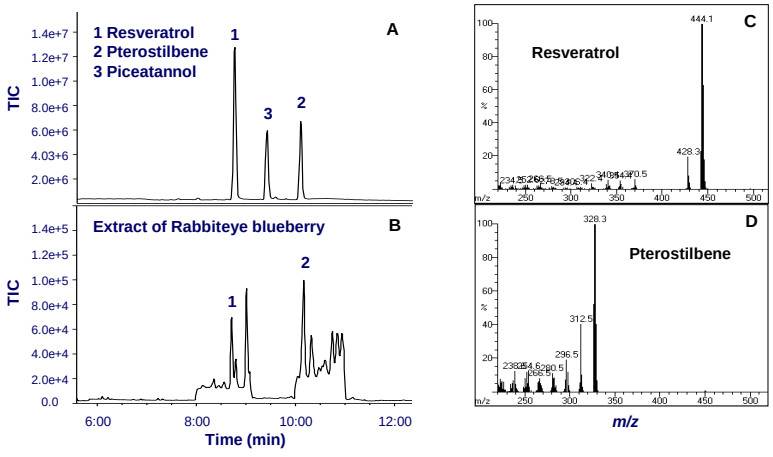
V. corymbosum
(Highbush blueberry)

V. elliottii
(Elliott's blueberry)

V. stamineum
(Deerberry)

Rimando et al., *J. Agric. Food Chem.* **2004**, *52*, 4713.

GC-MS Analysis of Pterostilbene in *Vaccinium species*



Chromatograms of standards **(A)** and extract of *Vaccinium ashei* **(B)**. The extract was monitored by selected ion monitoring for resveratrol (*m/z* 444, 428, 370) and pterostilbene (*m/z* 328, 312, 296). Mass spectra of resveratrol **(C)** and pterostilbene **(D)**.

Rimando et al., *J. Agric. Food Chem.* **2004**, *52*, 4713.

Pterostilbene in Blueberries*

Scientific Name (Common name)		Pterostilbene# (ng/g dry extract)
Vaccinium ashei (Rabbiteye blueberry)	(1)	151
	(2)	99
Vaccinium corymbosum (Highbush blueberry)	(1)	128
	(2)	124
	(3)	475
V. corymbosum* x *V. angustifolium (Half-highbush blueberry)	(1)	302
	(2)	142
	(3)	353
Vaccinium ovalifolium (Oval-leafed blueberry)		214

From: Rimando et al., *J. Agric. Food Chem.* **2004**, *52*, 4713-4719; Rimando and Barney, *Acta Hort.* **2005**, *680*, 137-143.

* • 110 ng/g dry extract of blueberries reported by Rodríguez-Bonilla et al., *J. Chromatogr. B.* 2011, *879*, 1091–1097.
  • 9.9 - 15.3 ug/kg fresh blueberries reported by Aiyer et al., *J. Agric. Food Chem.* 2012, *60*, 5693−5708.

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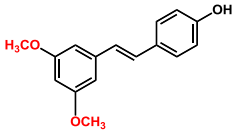
Lessons Learned

Pterostilbene…



- **demonstrates lipid lowering and anti-obesity effects; activation of PPARα contributes to these effects**
- **is effective in improving cognitive function in aging**
- **is a more potent modulator of cognition and cellular stress, inflammation, and Alzheimer's disease pathology than resveratrol**
- **as effective as Diazepam as an antianxiety agent, without untoward effect on locomotor activity**
- **exemplifies a natural compound that is as efficacious as synthetic clinically-used drugs**

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Acknowledgments

PPARα and blueberry feeding
Wallace Yokoyama, USDA ARS WRRC
Dennis Feller, Univ. of Mississippi (retired)
Shabana Khan, Univ. of Mississippi
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Cassia Mizuno, Univ. of New England

Anti-obesity effect
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Blueberry collection
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Dan Barney, USDA ARS (retired)
Wilhelmina Kalt, Agri-Food Canada

Neurological effect
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Al Rahim, Johns Hopkins Univ. School of Medicine

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Gloria Hervey
Mark Holtan

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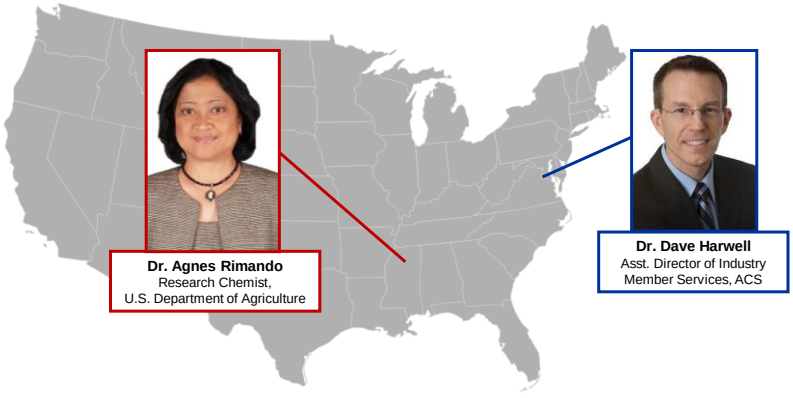

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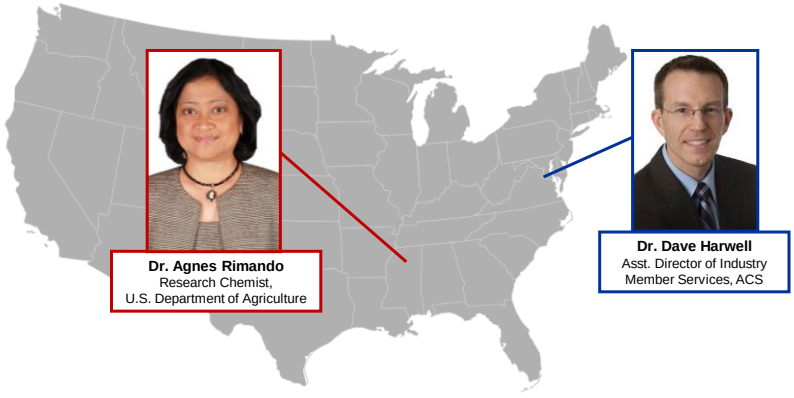

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