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Plant Foods and Herbal Sources of Resveratrol

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Stilbenes, in particular *trans*-resveratrol and its glucoside, are widely reported to be beneficial to health, having been shown to possess antioxidative, anticarcinogenic, and antitumor properties. Major dietary sources include grapes, wine, peanuts, and soy; however, they can also be introduced into the diet through Itadori tea, which has long been used in Japan and China as a traditional herbal remedy for heart disease and strokes. Analysis of grapes, peanuts, and Itadori tea shows that they contain mainly *trans*-resveratrol glucoside. In contrast, red wines are primarily a source of the aglycones *cis*- and *trans*-resveratrol. While peanuts and grapes contain low levels of the stilbenes, Itadori tea and red wine both supply relatively high concentrations of resveratrol. For people who do not consume alcohol, Itadori tea may be a suitable substitute for red wine. However, further study on the potential biological effects of other endogenous compounds in Itadori tea is required and there is also a need for more information on the absorption and in vivo biomedical actions of free and conjugated resveratrol.

KEYWORDS: Stilbene; resveratrol; wine; grape; peanut; peanut butter; Itadori root

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin, produced by plants in response to damage, particularly in grapevines (*1*), pines, and legumes (*2*). It is a member of the stilbene family and can be found in the cis or trans configurations, either glucosylated (see structures **I**−**IV** in **Scheme 1**) or in lower concentrations as the parent molecule of a family of polymers named viniferins. Stilbenes are reported to be potentially important cancer chemoprotective agents, being able to inhibit cellular events associated with carcinogenesis, including tumor initiation, promotion, and progression (*3*). *trans*-Resveratrol (**I**) and its glucoside (**III**) have also been proposed as contributors to the cardioprotective properties of red wine (*2*) as it has been shown that *trans*-resveratrol can inhibit LDL oxidation, the initial stage of the pathogenesis of atherosclerosis (*2*).

In countries with high intakes of soya-derived phytoestrogenic isoflavones, the development of osteoporosis, menopausal symptoms, and breast and prostate cancer are relatively rare (*5*). Resveratrol has also been reported to have estrogenic activity due to its structural similarity to the estrogenic agent diethylstilbestrol. Using estrogen-positive MCF-7 human breast cancer cells, *trans*-resveratrol was found to competitively inhibit binding of [^{3}H]estradiol to type 1 estrogen receptors as well as activating the receptors. This ability to antagonize estrogen binding provides a rationale for the possible use of *trans*-resveratrol in the prevention or treatment of breast cancer (*6*).

Despite the varied, yet potent, biological activities of stilbenes, little attention has focused on their presence and concentration in the diet. The major dietary sources of stilbenes include grapes, wine, soy, peanuts, and peanut products (*5*) although they may also be ingested from herbal remedies. The current study was undertaken to determine levels of *trans*- and *cis*-resveratrol (**I** and **II**) and their glucosides (**III** and **IV**) in peanuts, black grapes, red wines, and the Itadori plant (*Polygonum cuspidatum* Sieb et Zucc), which is known in the U.K. as Japanese knotweed. It is an extremely noxious weed that has invaded many areas of Europe and North America. In its native Asia, Itadori root is dried and infused to produce a tea. Itadori means "well-being" in Japanese, and Itadori tea has been used for centuries in Japan and China as a traditional herbal remedy for many diseases including heart disease and stroke (*7*). The active agent is believed to be *trans*-resveratrol and its glucoside (*2*).

MATERIALS AND METHODS

Chemicals. *trans*-Resveratrol (**I**) was obtained from Sigma (Poole, Dorset, U.K.) and *trans*-resveratrol-*O*-*β*-D-glucoside (**III**) was supplied by Apin (Abingdon, Oxford, U.K.). Compound **III** was also isolated and crystallized from the root of *P. cuspidatum* supplied by the Weed

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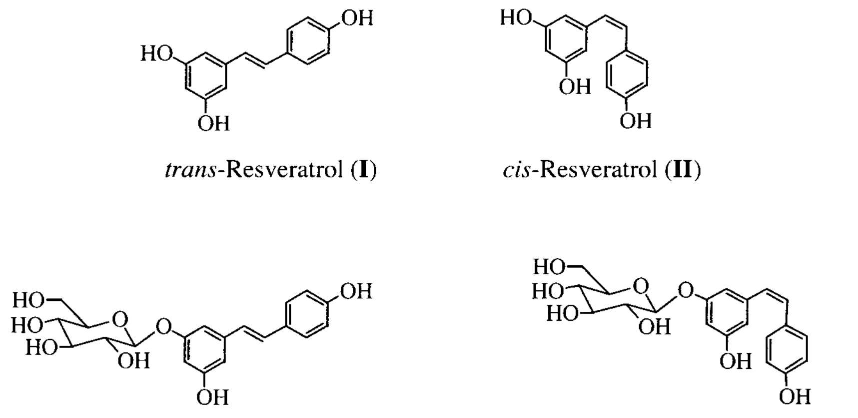
Scheme 1. Structures of the Major Stilbenes



trans-Resveratrol (**I**)
cis-Resveratrol (**II**)
trans-Resveratrol-3-*O*-glucoside (**III**)
cis-Resveratrol-3-*O*-glucoside (**IV**)

Table 1. Content of *trans*-Resveratrol and *trans*-Resveratrol Glucoside in Selected Tissues[a]

sample	*trans*-resveratrol	*cis*-resveratrol	*trans*-resveratrol glucoside	total
		Black Grapes		
Merlot	0.5 ± 0.0	ND	7.3 ± 0.4	7.8 ± 0.4
Merlot	ND	ND	5.5 ± 0.3	5.5 ± 0.3
Cabernet Sauvignon	0.5 ± 0.0	ND	2.2 ± 0.4	2.7 ± 0.4
Cabernet Sauvignon	ND	ND	1.5 ± 0.1	1.5 ± 0.1
		Itadori Root		
commercial root	523 ± 1	ND	1653 ± 2	2170 ± 9
young leaf	ND	ND	867 ± 17	867 ± 17
young stem	ND	ND	497 ± 4	497 ± 4
old leaf	ND	ND	370 ± 9	370 ± 9
old stem	ND	ND	83 ± 3	83 ± 3
		Peanut Products[b]		
peanuts (boiled)	5.1 ± 2.8			5.1 ± 2.8
peanut butter	0.3 ± 0.1			0.3 ± 0.1

[a] Data expressed as μg/g fresh weight ± SE ($n = 3$). *cis*-Resveratrol was quantified as *trans*-resveratrol equivalents; ND, not detected. [b] From Sobolev et al. (*15*).

Research Institute, Utsunomiya, Japan. The fresh root (ca. 1 kg) was chopped into small pieces and extracted first with methanol and then 80% aqueous methanol. The extracts were combined and reduced to an aqueous solution in vacuo. After the precipitated material by was removed filtration, the filtrate was partitioned against diethyl ether after which the aqueous phase was kept in darkness at 4 °C for 4 days, allowing light brown crystals to form. The crude crystals were repeatedly recrystallized from aqueous methanol to give **III** in a pure state (ca. 1.5 g). The authenticity of this compound was confirmed by ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR. *cis*-Resveratrol and *cis*-resveratrol-*O*-glucoside were obtained by isomerization of *trans*-resveratrol and *trans*-resveratrol-*O*-glucoside, respectively, in methanol during 12 h of exposure to high white light as described by Sato et al. (*9*). Methanol (high-performance liquid chromatography (HPLC) Grade) and acetonitrile (HPLC Grade) were from Rathburn Chemicals (Walkerburn, U.K.). All other chemicals and reagents were obtained from Sigma-Aldrich.

Extraction of Stilbenes. Red wines, provided by Safeway Stores plc (Hayes, Middlesex, U.K.) were untreated prior to analysis. A weighed aliquot (ca. 10 g) of frozen grapes, obtained from Viña San Pedro, Lontúe, Chile, was defrosted at room temperature prior to homogenization with 30 mL of methanol containing 2% formic acid. Samples were centrifuged at 10 000*g* for 10 min, and the supernatant was stored at −80 °C until analysis. Approximately 33 mg of peanuts (Original Salted Peanuts, KP Foods U.K., Ashby-de-la-Zouche, Leicestershire, U.K.) and 75 mg of Smooth Peanut Butter (Safeway Stores plc) were extracted overnight with 1 mL of 50% methanol. After centrifugation, the aqueous supernatant was stored at −80 °C until analysis. Samples of *P. cuspidatum* stem and leaf tissue, collected in the Botanic Garden, Glasgow, were lyophilized prior to overnight extraction of ca. 10 mg aliquots with 1 mL of 50% methanol. Samples of dried commercial Itadori root (Kojoukon) purchased from the Kinokuniya Herbal Medicine Co. (Kanda, Tokyo, Japan) were similarly processed. In addition, 1 g samples of the commercial root were used to prepare Itadori tea by infusion with 100 mL of boiling water for 5 min, after which they were filtered and 1 mL aliquots of the tea were stored at −80 °C until analysis.

HPLC Analysis of Samples. *trans*- and *cis*-Resveratrol in samples were analyzed on a 250 mm × 4.6 mm i.d., 5 μm ODS Hypersil (Shandon, Astmoor, U.K.) column, eluted at a flow rate of 1 mL/min with 25% acetonitrile in 0.5% aqueous formic acid using a photodiode array detector at 307 nm and a fluorimeter operating at excitation 298 nm and emission 385 nm. *trans*-Resveratrol-*O*-β-glucoside and its cis isomer were separated using a mobile phase of 17% acetonitrile in 0.5% aqueous formic acid (*10*) although subsequently only the trans isomer was detected in extracts that were analyzed.

RESULTS AND DISCUSSION

Levels of Stilbenes in Wines and Grapes. Grapes were found to contain mainly *trans*-resveratrol glucoside in concentrations ranging from 1.5 to 7.3 μg/g (**Table 1**). The aglycone *trans*-resveratrol was also present in two of the four grape samples at a concentration of 0.5 μg/g, while *cis*-resveratrol was not present in detectable amounts in any of samples that were analyzed. The stilbene content of grapes is dictated by three factors: cultivar, disease pressure, and time. The *trans*-resveratrol content of Californian table grapes, for instance, exhibits a ca. 20-fold variation between different clones. Levels of 0.16 μg/g have been reported in Crimson seedless grapes, as compared with 3.0 μg/g in Fantasia seedless grapes (*11*).

Table 2. Content of *trans*-Resveratrol and *trans*-Resveratrol Glucoside in Selected Beverages[a]

sample		*trans*-resveratrol	*cis*-resveratrol	*trans*-resveratrol glucoside	total
red wines					
	Pinot Noir, 1994 (California)	1057 ± 60	746 ± 9	ND	1803 ± 71
	Cabernet Sauvignon, 1996 (Bulgaria)	672 ± 10	520 ± 16	189 ± 5	1380 ± 25
	Merlot, 1994 (Chile)	48 ± 1	152 ± 5.3	ND	200 ± 3
	Cabernet Sauvignon, 1995 (California)	53 ± 1	45 ± 1	ND	98 ± 3
Itadori tea		68 ± 1	ND	906 ± 3	974 ± 2

[a] Data expressed as μg/100 mL ± SE ($n = 3$); ND, not detected. *cis*-Resveratrol was quantified as *trans*-resveratrol equivalents.

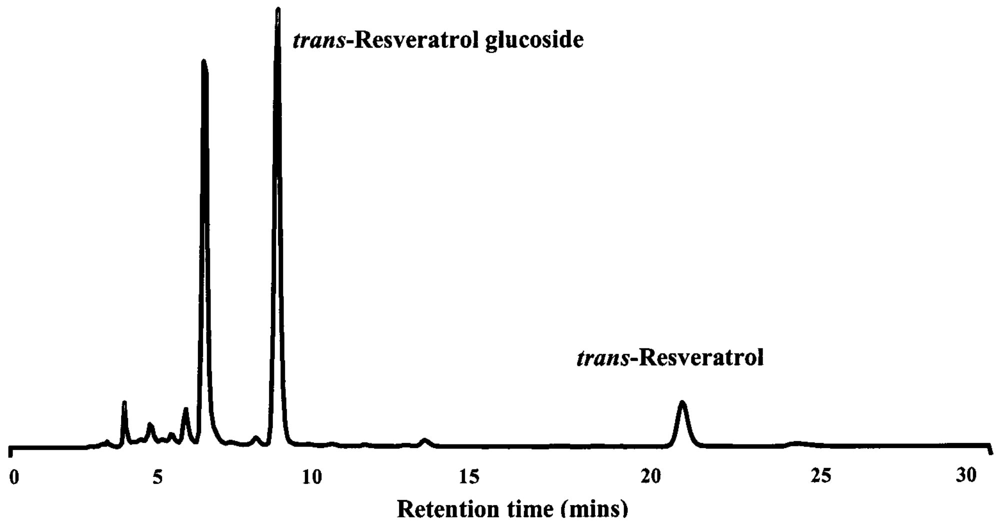


Figure 1. Reversed phase HPLC analysis of *trans*-resveratrol and *trans*-resveratrol glucoside in a 10 μL aliquot of Itadori tea. Sample analyzed on a 250 mm × 4.6 mm i.d., 5 μm ODS Hypersil column, eluted at a flow rate of 1 mL/min with 25% acetonitrile in 0.5% aqueous formic acid using a photodiode array detector at 307 nm.

In addition to *trans*-resveratrol and its glucoside, *cis*-resveratrol was detected in red wines (**Table 2**). The total stilbene content of red wines ranged from as little as 98 μg/100 mL to over 1803 μg/100 mL, with *cis*- and *trans*-resveratrol each contributing a similar proportion. *trans*-Resveratrol glucoside was detected in only one sample (Cabernet Sauvignon, Bulgaria). Levels of stilbenes in red wine can vary depending on grape variety, vinification approach, and climate (*12*). In red wines, the majority of the stilbenes are present as aglycones rather than glucosides due to sugar cleavage presumably occurring during vinification (*10*). However, while many studies have confirmed the presence of *cis*-resveratrol, *trans*-resveratrol, *trans*-resveratrol glucoside, and other related stilbenes in red wine, they are generally found in lower levels than many other phenolics (*10*, *13*−*15*). For instance, total flavonols in red wine can range from 550 to 6020 μg/100 mL, as compared with 5030−18 800 μg/100 mL for total catechins and 9210−65 000 μg/100 mL for total hydroxycinnamates (*10*).

Levels of Stilbenes in Peanuts and Peanut Butter. Analysis of one sample of peanuts and another of peanut butter in the present study found that *trans*-resveratrol and its glucoside were present but in very small amounts below the 200 ng/g limit of quantification. Trace levels of *trans*-resveratrol and related stilbenes have, however, been quantified in peanuts and in peanut butter by other investigators (*16*, *17*), and these data are presented in **Table 1**.

Levels of Stilbenes in Itadori Plants and Tea. Leaf and stem tissue from both young and old Itadori plants collected in Glasgow were analyzed (**Table 1**). Only *trans*-resveratrol glucoside was detected, with higher concentrations found in young stem (497 μg/g) as compared to old stem tissue (83 μg/g). A similar pattern was noted with young and old leaf tissues. A commercial root sample from Japan had an even higher stilbene content, one-quarter of which was attributable to the aglycone *trans*-resveratrol (**Table 1**).

Itadori tea was prepared by infusing 1 g of the commercial root preparation with 100 mL of boiling water for 5 min. The tea was found to contain 974 μg total resveratrol/100 mL, principally in the form of *trans*-resveratrol glucoside (**Figure 1**), which is within the range found in an average red wine (**Table 2**). This is perhaps surprising considering that Merlot and Cabernet Sauvignon grapes that were analyzed had a stilbene content of 1.5−55 μg/g as compared to 2170 μg/g for the Itadori root used to produce the tea (**Table 1**). This apparent discrepancy is because ca. 100 g of grapes are used to produce 100 mL of wine while 100 mL of tea is made from only 1 g of Itadori root powder.

Absorption of Stilbenes. It is noteworthy that while Itadori tea contains predominately *trans*-resveratrol glucoside, the cis and trans aglycones predominate in red wines (**Table 2**). This difference may be important when the absorption and potential bioavailability of the stilbenes is considered. Despite the increasing interest surrounding *trans*-resveratrol and the other stilbenes, few studies have been carried out with animals or humans to investigate their absorption. *trans*-Resveratrol has been detected in rat plasma after the administration of red wine (*18*) and pure *trans*-resveratrol (*19*). Considerably higher levels of *trans*-resveratrol were detected in kidney and liver tissue (*18*). Following a study on the absorption of *trans*-resveratrol by isolated sections of rat intestine, it has been proposed that the aglycone is converted to a glucuronide conjugate during transport across the wall of the small intestine and that only the glucuronide conjugate passes into the blood stream (*20*). This hypothesis is not in keeping with other reports on the presence of *trans*-resveratrol in kidneys, liver, and plasma of rats (*18*, *19*). Further work is required on the absorption and metabolism of *trans*-resveratrol and its glucoside to clarify matters.

Speculation that *trans*-resveratrol is a biomedically active agent in red wine has led to the production of red wine extracts and stilbene capsules (www.newu.com, www.myvitanet.com). These have been marketed as a safe, alcohol-free source of resveratrol. Like red wine, Itadori tea contains high concentrations of stilbenes in the form of *trans*-resveratrol glucoside and for those who do not consume alcoholic beverages, it too may be a suitable source of resveratrol. However, before widespread use of the tea in the Western world can be recommended, the biological effects of other components present in the brew requires investigation. While there are many reports on the anticancer effects of resveratrol derivatives in in vitro test systems (*3*), little is known about their absorption, metabolism, bioavailability, and, more important, biological effects in vivo.

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LITERATURE CITED

(1) Langcake, P.; Pryce, R. J. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.* **1976**, *9*, 77−86.

(2) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Resveratrol: a molecule whose time has come and gone? *Clin. Biochem.* **1997**, *30*, 91−113.

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

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

(5) Cassidy, A.; Hanley, B.; Lamuela-Raventós, R. M. Isoflavone, lignans and stilbenes−origins, metabolism and potential importance to human health. *J. Sci. Food Agric.* **2000**, *80*, 1044−1062.

(6) Williams, R. L.; Elliott, M.; Perry, R.; Greaves, B. The estrogenic activity of the polyphenolic resveratrol, benefits of moderate consumption of red wine. *Polyphenols Communications 96*, *Bordeaux France* **1996**, *P210*, 489−490.

(7) Kimura, Y.; Okuda, H.; Arichi, S. Effects of stilbenes on arachidonate metabolism in leukocytes. *Biochim. Biophys. Acta* **1985**, *834*, 275−278.

(8) Arichi, H.; Kimura, Y.; Okuda, H.; Baba, M.; Kozawa, M.; Arichi, S. Effects of stilbene components of the roots of *Polygonum cuspidatum* Sieb. et Zucc. on lipid metabolism. *Chem. Pharm. Bull.* **1982**, *30*, 1766−1770.

(9) Sato, M.; Suzuki, Y.; Okuda, T.; Okotsuka, K. Contents of resveratrol, piceid, and their isomers in commercially available wines made from grapes cultivated in Japan. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1800−1805.

(10) Burns, J.; Gardner, P. T.; O'Neil, J.; Crawford, S.; Morecroft, I.; McPhail, D. B.; Lister, C.; Matthews, D.; MacLean, M. R.; Lean, M. E. J.; Duthie, G. G.; Crozier, A. Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *J. Agric. Food Chem.* **2000**, *48*, 220−230.

(11) Creasy, L. L.; Coffee, M. Phytoalexin production potential of grape berries. *J. Am. Soc. Hortic. Sci.* **1988**, *113*, 230−234.

(12) Burns, J.; Gardner, P. T.; Matthews, D.; Duthie, G. G.; Lean, M. E. J.; Crozier, A. Extraction of phenolics and changes in antioxidant activity of red wine during vinification. *J. Agric. Food Chem.* **2001**, *49*, 5797−5808.

(13) Siemann, E. H.; Creasy, L. L. Concentration of the phytoalexin resveratrol in wine. *Am. J. Enol. Vitic.* **1992**, *43*, 49−52.

(14) Jeandet, P.; Bessis, R.; Maume, B. F.; Sbaghi, M. Analysis of resveratrol in Burgundy wines. *J. Wine Res.* **1993**, *4*, 79−85.

(15) Lamuela-Raventós, R. M.; Waterhouse, A. L. Occurrence of resveratrol in selected Californian wines by a new HPLC method. *J. Agric. Food Chem.* **1993**, *41*, 521−523.

(16) Sobolev, V. S.; Cole, R. J. *trans*-Resveratrol content in commercial peanuts and peanut products. *J. Agric. Food Chem.* **1999**, *47*, 1435−1439.

(17) Sanders, T. H.; McMichael, R. W.; Hendrix, K. W. Occurrence of resveratrol in edible peanuts. *J. Agric. Food Chem.* **2000**, *48*, 1243−1246.

(18) Bertelli, A. A. E.; Giovannini, L.; Stradi, R.; Urien, S.; Tillment, J.-P.; Bertelli, A. Kinetics of *trans*-and *cis*-resveratrol (3,4′,5-trihydroxystilbene) after red wine oral administration in rats. *Int. J. Clin. Pharm. Res.* **1996**, *XVI*, 77−81.

(19) Juan, M. E.; Lamuela-Raventós, R. M.; de la Torre-Boronat, M. C.; Planas, J. M. Determination of *trans*-resveratrol in plasma by HPLC. *Anal. Chem.* **1999**, *71*, 747−750.

(20) Kuhnle, G.; Spencer, J. P. E.; Chowrimootoo, G.; Schroeter, H.; Debnam, E. S.; Srai, S. K. S.; Rice-Evans, C. A.; Hahn, U. Resveratrol is absorbed in the small intestine as resveratrol glucuronide. *Biochem. Biophys. Res. Commun.* **2000**, *272*, 212−217.

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