Determination of Stilbenes (*trans*-Astringin, *cis*- and *trans*-Piceid, and *cis*- and *trans*-Resveratrol) in Portuguese Wines

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Stilbenes have been shown to have cancer chemopreventive activity and to protect lipoproteins from oxidative damage. A method is described for their direct determination in different types of wine using high-performance liquid chromatography with ultraviolet detection. In a survey of 120 commercial wines from Portugal and France, the highest concentrations of stilbenes were found in red wines. The glucosides of resveratrol were present in higher concentrations than the free isomers. Isolation from wine and characterization of *trans*-astringin in a large quantity are described for the first time.

Keywords: *Astringin; resveratrol; piceid; wine; Vitis vinifera*

INTRODUCTION

Stilbenes occur naturally in various families of plants (Hart, 1981), but grapes and related products are considered the most important dietary sources of these substances (Goldberg, 1995; Mattivi et al., 1995). The synthesis of these phytoalexins in grapevine is stimulated by stresses such as ultraviolet (UV) light and fungal infection, particularly in leaves and berry skins (Hart, 1981; Jeandet et al., 1991).

Epidemiological studies have shown that moderate wine consumption is related to a decrease in cardiovascular diseases (St Leger et al., 1979; Renaud and De Lorgeril, 1992; Gronbaek et al., 1995). It has been hypothesized that the phenolic substances of red wine may be responsible for these potential beneficial effects by potent antioxidant properties (Frankel et al., 1993a; Teissedre et al., 1996). Four resveratrol (3,5,4′-trihydroxystilbene) derivatives have been characterized in wine: the aglycon and the glucoside called piceid, in two isomeric forms cis and trans (Sieman and Creasy, 1992; Pezet et al., 1994; Lamuela-Raventos et al., 1995; Jeandet et al., 1995; Goldberg et al., 1995a). These compounds have attracted much interest for their biological properties. Indeed, they inhibit the oxidation of human low-density lipoproteins (Frankel et al., 1993b; Fauconneau et al., 1997) and the aggregation of platelets (Pace-Asciak et al., 1995; Varache-Lembège et al., 1996; Orsini et al., 1997), which may explain the decrease in coronary heart disease observed among wine drinkers.

However, this theory is subject to debate because of the very low concentration of *trans*-resveratrol (<1 mg/L) first found in wine by Sieman and Creasy (1992). Although stilbene concentrations vary depending on multiple factors including grape cultivar, fungal pressure, vinification procedures, and climate, they can reach higher concentrations (up to ∼20 mg/L) sometimes with a dominance of the glucosides. The main source of aglycons seems to be the hydrolysis of glucosides during red wine vinification (Mattivi et al., 1995). Stilbenes are thought to be stored within the cells in the form of glucosides. Moreover, unidentified stilbenes may be present in wine, such as tetrahydroxystilbenes detected in American wines but not as yet characterized (Lamikanra et al., 1996). We previously reported the isolation and characterization of stilbene glucosides from *Vitis vinifera* cell cultures, which were not previously reported to be constituents of *Vitis vinifera* plant or wine, particularly *trans*- and *cis*-piceatannol (3,5,3′,4′-tetrahydroxystilbene)-3-*O*-*β*-glucoside, named astringin (Waffo-Teguo et al., 1996b, 1998). The presence of a supplementary OH group in the B ring (catechol structure) notably increased the trapping effect and antioxidative properties of astringin and piceatannol, compared to those of piceid and resveratrol (Fauconneau et al., 1997; Waffo-Teguo et al., 1998).

Moreover, wine polyphenols have been reported to have anticarcinogenic properties, delaying tumor onset in transgenic mice (Clifford et al., 1996). These authors showed that intact catechin was absorbed. *trans*-Resveratrol has also been shown to have cancer chemopreventive activity in assays on three major stages of carcinogenesis (Jang et al., 1997). *trans*-Astringin and mainly its aglycon are active as antileukemic agents (Mannila and Talvitie, 1992; Mannila et al., 1993), and *trans*-piceatannol is a known inhibitor of protein-tyrosine kinases, which are positive regulators of cell proliferation (Gealhen and McLaughlin, 1989; Oliver et al., 1994).

Owing to the potential biological significance of tetrahydroxystilbenes, we have undertaken to isolate these substances from wine. We report the NMR character-

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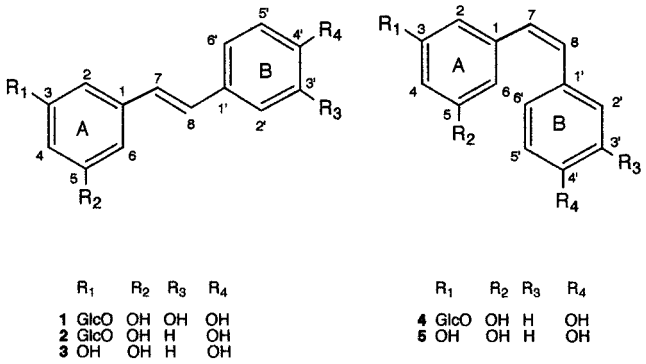

Figure 1. Structures of *trans*-astringin (**1**), *trans*-piceid (**2**), *trans*-resveratrol (**3**), *cis*-piceid (**4**), and *cis*-resveratrol (**5**).

Table 1. Areas and Vintages of White and Red Wine Samples from Continental Portugal, Azores Islands, and France

varieties (no. of wines)	vintages	area
white and red monovarietal Portuguese and Azores wines (84)	1994, 1995, 1996, 1997	Vinhos Verdes, Douro, Bairrada, Bucelas, Palmela, Evora, Pico, Bisoitos, Terceira
Portuguese and Azores blended red and white wines (14)	1996, 1997	Vinhos Verdes, Palmela, Pico, Graciosa
white fortified wines from Azores Islands (15)	1993, 1994, 1995, 1996, 1997	Pico, Bicoitos, Terceira
French red samples (7)	1996, 1997	Montpeyroux, Béziers

ization of *trans*-astringin (**1**) (Figure 1) and its concentration in a wide range of commercial red and white wines from Portugal and France. The contents of *trans*- and *cis*-piceid (**2** and **4**) and their aglycons (**3** and **5**) were also determined using a high-performance liquid chromatography (HPLC) method coupled with UV detection.

MATERIALS AND METHODS

Wine Samples. Stilbene concentrations (*trans*-astringin, *trans*-piceid, *cis*-piceid, *trans*-resveratrol, and *cis*-resveratrol) were determined for 120 samples of white and red varieties and vintage wines from continental Portugal, Azores Islands, and the south of France (Table 1).

Seventy-four Portuguese wines were white and 39 red. Fifty white and 34 red wines were monovarietal wines, 14 wines were blends from different varieties (5 red and 9 white), and 15 white wines were fortified "licoroso". All of the French wines were red. All of the samples (120) were analyzed during the year 1998.

Sample Preparation for HPLC Analysis. Stilbene extraction was done with 30 mL of wine by adding three times 10 mL of EtOAc. After solvent evaporation, 100 μL of methanol was added to the residue. The extract was diluted in 1 mL of distilled water. The solution was passed through a short column of cation-exchange resin (6 mm × 40 mm). The column was then rinsed by distilled water (2 mL). Elution was then done with 5 mL of a solution MeOH/H_2O (3:2) to obtain stilbenes. The eluate was evaporated to a dry residue, which was dissolved in 200 μL of MeOH/H_2O (1:1).

HPLC Analysis. Stilbene compounds (*trans*-astringin, *trans*-piceid, *cis*-piceid, and *cis*-resveratrol) were isolated from *V. vinifera* cell cultures and unambiguously characterized by spectrometric methods (Waffo-Teguo et al., 1996a,b). *trans*-Resveratrol was obtained from Sigma.

Separation and quantification of stilbenes were done by HPLC as described by Waffo-Teguo et al. (1996b). A Hewlett-Packard model 1090 with three low-pressure pumps and a diode array UV−visible detector coupled to an HP Chem Station was used for solvent delivery and detection. A Nucleosil 100 C_{18} column (4.0 × 250 mm) from Hewlett-Packard thermostated at 30 °C was used as the stationary phase with a flow of 0.5 mL/min.

The solvents used for the separation were as follows: solvent A, acetic acid in H_2O, pH 2.4; solvent B, 20% phase A with 80% MeCN. The gradient solvent system was as follows: 0 min, 100% A, 0% B; 10 min, 100% A, 0% B; 20 min, 90% A, 10% B; 30 min, 80% A, 20% B; 40 min, 60% A, 40% B; 45 min, 0% A, 100% B. Twenty-five microliters of extract was injected into the HPLC system after filtration on a 0.45 μm Millipore membrane. After each analysis, the column was re-equilibrated with phase A for 10 min. The detection was at 286 and 306 nm for cis and trans isomers, respectively. Measurements were done in duplicate. Analytical characteristics of stilbene determinations are given in Table 2 for the five compounds. An HPLC chromatogram (Figure 2) at 286 nm of a stilbene standard solution indicated retention time and concentration for each compound.

Table 2. Analytical Characteristics for the Determination of Stilbenes in Wine Samples by HPLC−UV

stilbene	detection limit,[b] ng	calibration range[a] mg/L	*r*	accuracy recovery,[c] %	precision RSD,[d] %
trans-astringin	8	0.0−50	0.9993	99.1 ± 3.8	1.5−3.4
cis-resveratrol	8	0.0−50	0.9995	99.5 ± 3.5	1.3−3.1
trans-resveratrol	5	0.0−50	0.9992	99.0 ± 3.9	1.8−2.6
trans-piceid	5	0.0−50	0.9991	99.5 ± 2.5	1.3−3.3
cis-piceid	8	0.0−50	0.9990	99.3 ± 2.8	1.8−3.8

[a] Calibration range in mg/L and coefficient of correlation (*r*) obtained for five points. [b] Detection limit calculated according to IUPAC rules (25 μL). [c] Mean value ± SD of determinations in two different samples. [d] Relative standard deviation (RSD) (%) of six determinations in five different samples.

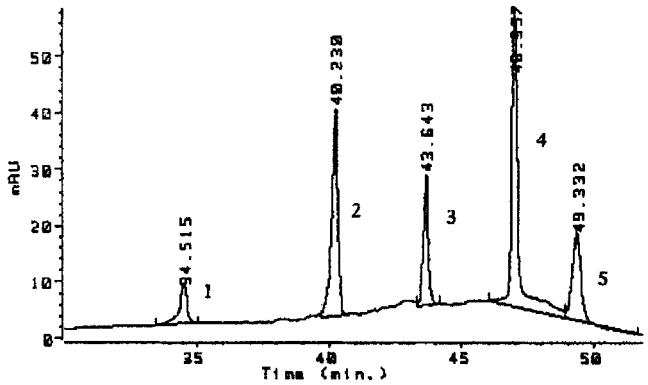

Figure 2. HPLC chromatogram at 286 nm of a stilbene standard solution. Peaks 1−5 are *trans*-astringin (5 mg/L), *trans*-piceid (5 mg/L), *cis*-piceid (4 mg/L), *trans*-resveratrol (5 mg/L), and *cis*-resveratrol (6.5 mg/L), respectively.

Isolation and Identification of *trans*-Astringin from Wine. Stilbenes were purified from French red wine (500 mL) that had been freed of all ethanol. Isolation from wine was carried out with EtOAc (4 × 200 mL). The EtOAc extract was chromatographed over a cation-exchange resin column and eluted by H_2O/MeOH gradient. Stilbenes were eluted by 75% MeOH. For further fractionation, the crude stilbenes were divided into fractions on a Sephadex LH20 column. Two main fractions were obtained. The mixture of *cis*-stilbenes was eluted by 20% MeOH and the mixture of *trans*-stilbenes by 30% MeOH. Extracts were constantly protected from light to avoid trans/cis isomerization. *trans*-Astringin (t_R = 16 min) in the mixture of *trans*-stilbenes was obtained by semi-preparative HPLC (Ultrasep RP 18 column, 6 μm particle size, column dimensions 250 × 8 mm, with column guard, monitored at dual mode 286 and 306 nm using a UV detector). The elution program at 3 mL/min was done as described by Waffo-Teguo et al. (1996b).

Table 3. Stilbene Levels (in Milligrams per Liter) for Different Wines from Portugal and France

wine[a]	*trans*-astringin		*trans*-piceid		*cis*-piceid		*trans*-resveratrol		*cis*-resveratrol		total
	min−max	av	min−max	av	min−max	av	min−max	av	min−max	av	stilbenes av
monovarietal white wines, Portugal (50)	nd[b]−15.6	5.4	nd−5.8	2.6	nd−4.0	1.3	nd−2.1	0.6	nd−1.7	0.4	10.2
blended white wines, Portugal (9)	2.4−11.3	5.5	1.2−4.7	2.9	nd−2.8	1.2	nd−0.5	0.5	nd−0.9	0.2	10.4
fortified wines, Portugal (15)	nd−13.4	8.0	1.6−7.0	4.5	nd−3.9	1.6	0.3−2.1	0.8	nd−0.6	0.1	15.1
monovarietal red wines, Portugal (34)	nd−35.9	10.4	nd−50.8	11.8	nd−17.9	5.0	nd−5.7	1.0	nd−9.5	2.6	30.6
blended red wines, Portugal (5)	nd−24.8	10.3	nd−17.1	8.0	nd−8.1	3.3	nd−3.9	1.5	0.7−4.0	2.1	25.1
red wines, France (7)	2.5−26.1	13.1	3.1−32.8	13.0	nd−5.3	1.9	0.6−6.8	3.0	1.1−5.3	2.6	33.7
total white wines (74)	nd−15.6	5.9	nd−7.0	3.0	nd−4.0	1.4	nd−2.1	0.6	nd−1.7	0.3	11.2
total red wines (46)	nd−35.9	10.8	nd−50.8	11.6	nd−17.9	4.3	nd−6.8	1.3	nd−9.5	2.5	30.5
total wines (120)	nd−35.9	7.8	nd−50.8	6.3	nd−17.9	2.5	nd−6.8	0.9	nd−9.5	1.1	18.6

[a] Number of samples in parentheses. [b] nd, not detected.

^{1}H NMR spectra were recorded at 500 MHz, in CD_3OD. The resonance of the methyl group of CD_3OD was used as reference for the δ value: $\delta = 3.3$ ppm.

RESULTS AND DISCUSSION

Stilbene Concentrations in Wines. The amounts of stilbene compounds varied considerably in the different types of wine, depending on the grape variety, environmental factors in the vineyard, and wine-processing techniques. The concentrations of total stilbenes determined by the HPLC method varied from 0.6 to 73 mg/L (mean = 18.6 mg/L) for all of the wines, from 0.6 to 23.5 mg/L (mean = 11.2 mg/L) for the white wines, and from 2.3 to 53.5 mg/L (mean = 30.5 mg/L) for the red wines (Table 3). These levels may reflect the effects of the different methods of wine-making in red and white wines.

This study demonstrates for the first time that *trans*-astringin is present in significant amounts in wines (Figure 3). Its level is highest in red wines: 53% of them are in the class 9−35 mg/L, whereas only 26% of the white wines are in the class 9−15.6 mg/L. ^{1}H NMR data for **1** are reported in Table 4. By comparison with the literature (Qiu et al., 1996; Waffo-Teguo et al., 1996b) and with results on the pure compound, the stilbene was confirmed to be *trans*-astringin. Moreover, a much higher *trans*-astringin concentration (80 mg/L) was found in a press young wine after a conventional red wine vinification of the Syrah variety. The influence of enological practices on the evolution of this compound in wine is under study.

The highest levels of *trans*-piceid were in red wine. Figure 3 shows that only 29% of the red wines were in the class 0−5 mg/L, unlike 82% of the white wines. Most (59%) of the red wines were between 5 and 25 mg/L, and 12% were >25 mg/L.

cis-Piceid, an isomer of *trans*-piceid, is typically found at lower concentrations than *trans*-piceid in wines (Figure 3). Indeed, 73% of the white wines were between 0 and 2 mg/L, and 23% were in the group 2−4 mg/L. In the red wines, only 48 and 9% were in the classes 0−2 and 2−4 mg/L, respectively. Around 28% of the red wines were between 4 and 10 mg/L, and nearly 15% of the reds were >10 mg/L.

trans- and *cis*-resveratrol levels are also shown in Figure 3. Close to 100% of white wines were between 0 and 2 mg/L for both compounds. For the red wines, around 20 and 45% were between 2 and 10 mg/L, respectively, for *trans*- and *cis*-resveratrol. However, 80 and 55% of the red wines were in the group 0−2 mg/L. This result corroborates other studies which show that red wines from various countries and regions have a low mean concentration of *trans*- and *cis*-resveratrol, <5

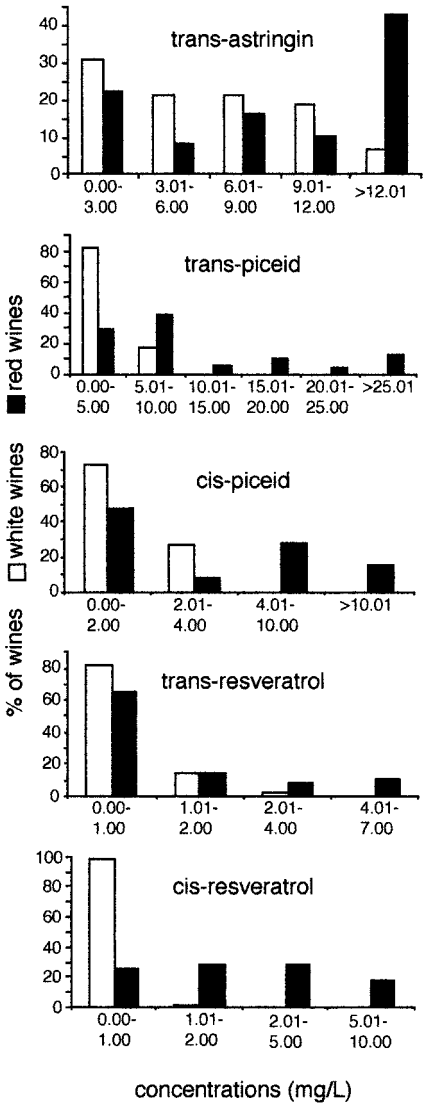

Figure 3. Frequency distribution of stilbene concentrations of white and red wines from Portugal and France.

mg/L (Goldberg et al., 1995b; Lamuela-Raventos et al., 1995). Concerning *trans*- and *cis*-piceid, its level exceeded that of the free isomers and reached high values, 50 and 18 mg/L, respectively, which are superior to those found in the previous studies.

Estimation of Stilbene Intake from Wine. It is important to monitor food and beverages for phenolic antioxidants because wine phenolics appear to have properties that could reduce the heart disease mortality rates observed in moderate wine drinkers. All samples analyzed showed significant stilbene levels, and the compounds are recognized as potent antioxidants. For

Table 4. ^{1}H NMR Data of 1 Extracted from Red Wine

proton	δ	δ^a
$H_{2'}$	6.97 d (2.05)	7.02 d (2.00)
H_8	6.93 d (16.02)	6.97 d (16.2)
$H_{6'}$	6.84 dd (2.2; 8.1)	6.88 dd (2.00; 8.1)
H_7	6.78 d (16.02)	6.82 d (16.2)
H_2	6.76 d (2.00)	6.80 d (2.00)
$H_{5'}$	6.73 d (8.1)	6.77 d (8.1)
H_6	6.59 Br	6.63 Br
H_4	6.44 t (2.00)	6.48 t (2.00)
glucose H_1	4.87 d (7.1)	4.92 d (7.1)

[a] The assignment reported by Waffo-Téguo et al. (1996).

example in vitro, *trans*- and *cis*-resveratrol, *trans*- and *cis*-piceid, and *trans*-astringin are known to be significantly active molecules against low-density lipoprotein oxidation (Waffo-Teguo et al., 1998). For this reason, we estimated stilbene intake from wine of Portuguese origin. On the basis of a consumption of 160 mL/day/individual, the current daily intake of stilbenes from wine (red and white) for the Portuguese population can be estimated as 3.0 mg/day/individual. For people drinking only red wine, the current daily intake of stilbenes can be estimated at 4.9 mg/day/individual and at 1.8 mg/day/individual for people drinking only white wines. We therefore assume that wine contributes to total daily stilbene intake, which is considerably greater than previously thought. Moreover, other unidentified stilbenes are perhaps present in wine such as *cis*- and *trans*-resveratrolosides, which we have characterized in *V. vinifera* cell cultures (Waffo-Teguo et al., 1998). Further investigations are in progress to identify these compounds in different types of wine. Moreover, we are developing an HPLC method for the determination of stilbenes in wine using sensitive fluorescence detection.

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LITERATURE CITED

Clifford, A. J.; Ebeler, S. E.; Ebeler, J. D.; Bills, N. D.; Hinrichs, S. H.; Teissedre, P.-L.; Waterhouse, A. L. Delayed tumor onset in transgenic mice fed an amino acid-based diet supplemented with red wine solids. *Am. J. Clin. Nutr.* **1996**, *64*, 748−756.

Fauconneau, B.; Waffo Teguo, P.; Huguet, F.; Barrier, L.; Decendit, A.; Mérillon, J. M. Comparative study of radical scavenger and antioxidant properties of phenolic compounds from *Vitis vinifera* cell cultures using in vitro tests. *Life Sci.* **1997**, *61*, 2103−2110.

Frankel, E.; Kanner, J.; German, J.; Parks, E.; Kinsella, J. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993a**, *341*, 454−457.

Frankel, E.; Waterhouse, A.; Kinsella, J. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993b**, *341*, 1103−1104.

Geahlen, R. L.; McLaughlin, J. L. Piceatannol (3,4,3′,5′-tetrahydroxy-trans-stilbene) is a naturally occurring protein-tyrosine kinase inhibitor. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 241−245.

Goldberg, D. M. Does wine work? *Clin. Chem.* **1995**, *41*, 14−16.

Goldberg, D. M.; Ng, E.; Karumanchiri, A.; Yan, J.; Diamandis, E. P.; Soleas, G. J. Assay of resveratrol glucosides and isomers in wine by direct-injection high-performance liquid chromatography. *J. Chromatogr. A* **1995a**, *708*, 89−98.

Goldberg, D. M.; Karumanchiri, A.; Ng, E.; Yan, J.; Diamandis, E. P.; Soleas, G. J. Direct Gas Chromatographic-Mass Spectrometric Method to Assay *cis*-Resveratrol in Wines: Preliminary Survey of Its Concentration in Commercial Wines. *J. Agric. Food Chem.* **1995b**, *43*, 1245−1250.

Gronbaek, M.; Deis, A.; Sorensen, T.; Becker, U.; Schnohr, P.; Jensen, G. Mortality associated with moderate intakes of wine, beer, or spirits. *Br. Med. J.* **1995**, *310*, 1165−1169.

Hart, J. H. Role of phytostilbenes in decay and disease resistance. *Annu. Rev. Phytopathol.* **1981**, *19*, 437−458.

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.

Jeandet, P.; Bessis, R.; Gautheron, B. The production of resveratrol (3,5,4′-trihydroxystilbene) by grape berries in different developmental stages. *Am. J. Enol. Vitic.* **1991**, *42*, 41−46.

Jeandet, P.; Bessis, R.; Maume, B. F.; Meunier, P.; Peyron, D.; Trollat, P. Effect of enological practices on the resveratrol isomer content of wine. *J. Agric. Food Chem.* **1995**, *43*, 316−319.

Lamikanra, O.; Grimm, C. C.; Ben Rodin, J.; Inyang, I. D. Hydroxylated stilbenes in selected American wines. *J. Agric. Food Chem.* **1996**, *44*, 1111−1115.

Lamuela-Raventós, R. M.; Romero-Pérez, A. I.; Waterhouse, A. L.; De la Torre-Boronat, M. C. Direct HPLC analysis of *cis*- and *trans*-resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.* **1995**, *43*, 281−283.

Mannila, E.; Talvitie, A. Stilbenes from *Picea abies* bark. *Phytochemistry* **1992**, *31*, 3228−3229.

Mannila, E.; Talvitie, A.; Kolehmainen, E. Anti-leukaemic compounds derived from stilbenes in *Picea abies* bark. *Phytochemistry* **1993**, *33*, 813−816.

Mattivi, F.; Reniero, F.; Korhammer, S. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.* **1995**, *43*, 1820−1823.

Oliver, J.; Burg, D.; Wilson, B.; McLaughlin, J.; Geahlen, R. Inhibition of mast cell Fc epsilon R1-mediated signaling and effector function by the Syk-selective inhibitor, piceatannol. *J. Biol. Chem.* **1994**, *269*, 29697−703.

Orsini, F.; Pelizzoni, F.; Verotta, L.; Aburjai, T. Isolakion, synthesis, and antiplatelet aggregation activity of resveratrol 3-*O*-β-D-glucopyranoside and related compounds. *J. Nat. Prod.* **1997**, *60*, 1082−1087.

Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. *Clin. Chim. Acta* **1995**, *235*, 207−219.

Pezet, R.; Pont, V.; Cuenat, P. Method to determine resveratrol and pterostilbene in grape berries and wines using high-performance liquid chromatography and highly sensitive fluorimetric detection. *J. Chromatogr. A* **1994**, *663*, 191−197.

Qiu, F.; Komatsu, K.; Kawasaki, K.; Saito, K.; Yao, X.; Kano, Y. A novel stilbene glucoside, oxyresveratrol 3′-O-β-glucopyranoside, from the root bark of *Morus alba*. *Planta Med.* **1996**, *62*, 559−561.

Renaud, S.; de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

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

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

Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of *in vitro* human LDL oxidation

by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

Varache-Lembège, M.; Waffo-Teguo, P.; Decendit, A.; Devaux, G.; Deffieux, G.; Mérillon, J. M. Polyhydroxystilbenes from *Vitis Vinifera* L. cells: inhibitory effect on human platelet aggregation and molecular modeling. *18th International Conference on Polyphenols*; 1996.

Waffo-Teguo, P.; Decendit, A.; Vercauteren, J.; Deffieux, G.; Mérillon, J.-M. Trans-resveratrol-3-O-*β*-glucoside in cell suspension cultures of *Vitis vinifera*. *Phytochemistry* **1996a**, *42*, 1591−1593.

Waffo-Teguo, P.; Decendit, A.; Krisa, S.; Deffieux, G.; Vercauteren, J.; Mérillon, J.-M. The accumulation of stilbene glycosides in *Vitis vinifera* cell suspension cultures. *J. Nat. Prod.* **1996b**, *59*, 1189−1191.

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

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