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Isolation, Identification, and Antioxidant Activity of Three Stilbene Glucosides Newly Extracted from *Vitis vinifera* Cell Cultures

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Suspension cultures of *Vitis vinifera* L. (Vitaceae) produce many hydroxylated stilbene glucosides found in red wine. From these cells, we isolated and characterized glycosylated stilbenes, (*Z*)-piceatannol (3,5,3′,4′-tetrahydroxystilbene) -3-*O*-β-D-glucopyranoside (**6**) and (*E*)- and (*Z*)-resveratrol (3,5,4′-trihydroxystilbene)-4′-*O*-β-D-glucopyranoside (**2** and **7**, respectively), which have not previously reported to be constituents of *Vitis vinifera* or wine. The ability of these compounds to act as radical scavengers was investigated using 1,1 diphenyl-2-picryl-hydrazyl, a stable free radical. Antioxidant activities were assessed by their capacity to prevent Cu^{2+}-induced lipid peroxidation in human low-density lipoprotein.

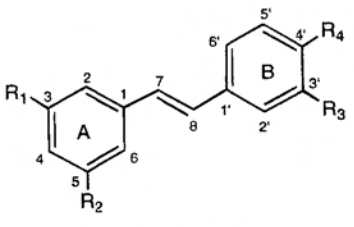
Numerous epidemiological studies in France have shown a negative correlation between moderate red wine consumption and the incidence of cardiovascular diseases.[1–3] This is the so-called French paradox. Wine contains natural plant phenolic compounds that may protect circulating lipoproteins from oxidative damage.[4] Stilbene has attracted a great deal of interest because relatively high quantities are found in grapes and wine, which are considered the most important dietary sources of these substances.[2,5] On the other hand, stilbene derivatives seem to have a variety of biological activities.[6,7] We reported previously that (*E*)- and (*Z*)-piceid and (*E*)-astringin, stilbene glucosides isolated from cell cultures of *Vitis vinifera* L. (Vitaceae),[8,9] inhibit the lipid peroxidation induced by Cu^{2+}.[10]

In this study we isolated and characterized stilbene glucosides, (*E*)- and (*Z*)-resveratroloside and (*Z*)-astringin, not previously reported to be constituents of *Vitis vinifera* or of wine. We assessed the relative activities of a range of stilbene glucosides as scavengers of radicals and their properties to prevent Cu^{2+}-induced lipid peroxidation in low-density lipoprotein (LDL). We further analyzed the structure–antioxidant activity relationship of these compounds *in vitro*.

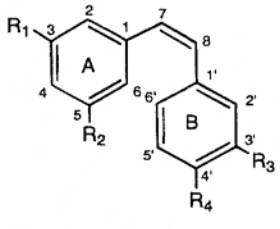
Stilbene compounds **1**, **2**, **3**, and **6**–**8** were purified from the EtOAc extract of cell suspension of *Vitis vinifera* by a combination of chromatographic techniques. Compound **1** was identified as (*E*)-astringin.[8] Compounds **3** and **8** were identified as (*E*)- and (*Z*)-piceid.[8,9]

The structures of **2**, **6**, and **7**, three stilbene glucosides newly isolated from cell suspension of *Vitis vinifera*, were deduced by spectrometric methods. Assignments of proton and carbon resonances were deduced from analysis of $^1H-^1H$ COSY,[11] heteronuclear HMQC,[12] and HMBC[13] 2D chemical shift correlations. Compounds **2** and **7** were characterized as (*E*)- and (*Z*)-3,5,4′-trihydroxystilbene-4′-*O*-β-D-glucopyranoside, respectively, by comparison with literature data.[14] These compounds have been found in the roots of *Polygonum cuspidatum*[14] and are also called (*E*)- and (*Z*)-resveratroloside. For **6**, our results are similar to those previously reported by Strack et al.[15] and indicate that this compound is (*Z*)-piceatannol-3-*O*-β-D-glucopyranoside or (*Z*)-astringin.

The antioxidant activities of compounds **1**–**9** were studied (Table 1). Coexistence of an antioxidant A and a free radical R° (such as reactive oxygen species generated by an oxidative stress, or 1,1 diphenyl-2-picryl-hydrazyl (DPPH) leads to the disappearance of this free radical and to the appearance of the free radical A° according to the reaction: A + R° → A° + R. On the stilbenes studied, the conjugation between rings A and B via a planar C2 unsaturated structure allows an electron delocalization across the molecules for stabilization of the radical, which explains the relative antioxidant properties of all these compounds.



	R_1	R_2	R_3	R_4
1	GlcO	OH	OH	OH
2	OH	OH	H	GlcO
3	GlcO	OH	H	OH
4	OH	OH	OH	OH
5	OH	OH	H	OH



	R_1	R_2	R_3	R_4
6	GlcO	OH	OH	OH
7	OH	OH	H	GlcO
8	GlcO	OH	H	OH
9	OH	OH	H	OH

On Cu^{2+}-induced lipid peroxidation on the LDL, the results (Table 1) show no important difference between

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Table 1. IC_{50} Values[a] for the Antioxidant Activities of Stilbenes Extracted from *Vitis Vinifera* Cells and of Trolox

compound	IC_{50} values (μM) DPPH	LDL
1	30.2 ± 1.8	3.0 ± 0.2
2	1000 ± 95	100 ± 11.7
3	198 ± 16.8	19.1 ± 3.0
4	28 ± 1.2	1.8 ± 0.2
5	72 ± 4.5	2.4 ± 0.2
6	29 ± 1.9	2.5 ± 0.3
7	>1000	150 ± 16.1
8	142 ± 7.9	16.8 ± 1.6
9	95 ± 3.8	18 ± 2.0
trolox	10.1 ± 0.5	4.7 ± 0.4

[a] Each value is the mean of at least three independent experiments ± SD. Statistical analysis was performed by using Student's *t*- test.

(*E*) and (*Z*) structures of each molecule, except for (*E*)- and (*Z*)-resveratrol, with a better activity for (*E*)-resveratrol.

The glycosylation of (*E*)-stilbenes reduces their activity when compared to the corresponding aglycons (respectively, seven times for (*E*)-piceid vs. (*E*)-resveratrol, $p < 0.001$; 35 times for (*E*)-resveratroloside vs. (*E*)-resveratrol, $p < 0.001$; 1.6 times for astringin vs. piceatannol, $p < 0.05$). This difference is less important in the (*Z*) structure. Blocking the 4′-hydroxyl group in the B ring by a glycosyl moiety decreases dramatically the antioxidant activity [(*E*)-resveratroloside vs. (*E*)-resveratrol] compared to glycosylation in the 3-position on A ring [(*E*)-piceid vs. (*E*)-resveratrol].

Considering the antioxidant activities of these molecules, it is worth noting the importance of the two hydroxyl groups in the *ortho*-diphenolic arrangement in the B ring. Actually, astringin, which possesses this catechol structure and consequently a supplementary OH on the B ring as compared to piceid, has an activity six times higher for the (*E*) and (*Z*) structure ($p < 0.001$). Astringin, despite the presence of glycoside in the 3-position on ring A, has an activity close to that observed with (*E*)-resveratrol.

Among these molecules, the most potent antioxidant is piceatannol, which possesses four hydroxyl groups, including the catechol structure in the B ring. Furthermore, piceatannol is two times more efficient than trolox, the water-soluble vitamin E analogue ($p < 0.01$).

On DPPH, Table 1 shows no important difference between (*E*) and (*Z*) structures of each molecule. The glycosylation of (*E*)- and (*Z*)-stilbenes reduces their activity when compared to the corresponding aglycons [(*E*)- and (*Z*)-piceid vs. (*E*)- and (*Z*)-resveratrol; (*E*)- and (*Z*)-resveratroloside vs. (*E*)- and (*Z*)-resveratrol], but the difference of activity between (*E*)-astringin and piceatannol is less than that observed on the LDL test.

The glycosylation of resveratrol in the 3-position in the A ring leads to piceid, which has an activity about two times lower for (*E*) and (*Z*) structures ($p < 0.01$). When glycosylation is performed in the 4′-position in B ring, the antioxidant activities of the molecules obtained, that is, (*E*)- and (*Z*)-resveratroloside, decrease dramatically, as compared to those of (*E*)- and (*Z*)-resveratrol.

These results show that the catechol structure is essential for the antioxidant activities of stilbenes, as reported by Rice-Evans et al.[16] for flavonoids. But (*E*)- and (*Z*)-resveratrolosides may be hydrolyzed by glycosidases in the human gastrointestinal tract.[2] Further work is being undertaken to characterize theses substances (**1**, **2**, **4**, **6**, **7**) in wine.

Experimental Section

General Experimental Procedures. UV spectra were measured in MeOH using a Hitachi U-2000 spectrophotometer. IR spectra were obtained on a KBr disk using a Shimadzu IR-470 spectrophotometer. NMR spectra were performed with a Bruker AMX-500 spectrometer. FABMS were recorded using glycerol as matrix, in positive-ion mode. (*E*)-Piceatannol (**4**, 6 mg) and (*Z*)-resveratrol (**9**, 5 mg) were obtained by enzymatic hydrolysis of (*Z*)-piceid (10 mg) and (*E*)-astringin (10 mg), respectively.

Cell Culture. Cell suspension cultures of *Vitis vinifera* L. cv Gamay Fréaux var. Teinturier were maintained as previously described.[17] The maintenance medium (MM) contained B5 macroelements,[18] microelements,[19] and vitamins,[20] and was supplemented with 58 mM sucrose, 250 mg/L casein hydrolysate, 0.54 μM 1-naphthaleneacetic acid, and 0.93 μM kinetin. Experiments were carried out by inoculating a 7-day-old cell suspension into an induction medium (IM_1) at a 1:8 (v/v) ratio, for one transfer.[17] IM_1 was the same as MM, but contained 2 mM $(NH_4)_2SO_4$, 2.2 mM NaH_2PO_4, 2 mM $MgSO_4$, and 175 mM sucrose. Harvesting was made on day 12 corresponding to the optimal period for the production of stilbenes by grape cells in this medium.[8] Cells were collected through filtration under partial vacuum (nylon cloth, 30 μm), rapidly washed with cold distilled H_2O, and then extracted.

Test on Low-Density Lipoproteins (LDL). This assay was previously described.[10] Briefly, human LDL were oxidized by cupric ions. Lipid peroxidation was assessed by TBARS (thiobarbituric acid reactive substances) measurement, using thiobarbituric acid (TBA) colorimetric method.[21] The absorbance is read at 532 nm. The stilbenes added in this mixture inhibited this peroxidation, and the efficient concentration (IC_{50}) was the concentration that inhibited 50% of coloration.

Test on DPPH. DPPH is a dyed free radical. The trapping effect of the molecules tested was assessed by measuring the absorbance change at 515 nm of a DPPH solution[22] (100 μM) in the presence of different concentrations of the stilbenes. Measurements were performed at least in triplicate. The efficient concentration (IC_{50}) is the concentration that inhibited 50% of coloration.

Extraction, Isolation, and Identification of the Stilbene Compounds. Frozen cells (900 g) were homogenized with Me_2CO-H_2O as previously described.[8,9] The extract was concentrated *in vacuo*, and the aqueous mixture was extracted with EtOAc. The EtOAc extract was chromatographed over a cation-exchange resin column (1.5 × 60 cm) and eluted by H_2O–MeOH gradient. The stilbenes were eluted by 50% MeOH. For the further fractionation, the crude stilbenes were divided into fractions on a Sephadex LH-20 column (1.5 × 60 cm). Two main fractions were obtained. The mixture of (*Z*)-stilbenes was eluted by 20% MeOH and the mixture of (*E*)-stilbenes by 30% MeOH. Extracts were constantly protected from light to avoid (*E*–*Z*) isomerization.

Compounds **1** (*E*)-astringin (12 mg), **2** (*E*)-resveratroloside (8 mg), and **3** (*E*)-piceid (67 mg), **6** (*Z*)-astringin (7 mg), **7** (*Z*)-resveratroloside (7 mg), and **8** (*Z*)-piceid (52 mg), were obtained as pure compounds by semi-prep. HPLC on an Ultrasep RP18 (6 μm) reversed-phase C18 column (8 mm i.d. × 250 mm) with column guard eluted by gradient system solvent: A, H_2O adjusted to pH 2.4 with TFA; B, 20% A with 80% MeCN. The elution program at 3 mL min^{-1} was as follows: 18% B (0−10 min); 18−23% B (10−17 min); 23−24.5% B (17−21 min); 24.5−31.5% B (21−27 min); 31.5−50% B (27−30 min); 50−60% B (30−35 min); 60−100% B (35−40 min). The chromatogram was monitored at dual mode 286−306 nm using an UV detector.

Compound **2**: UV (MeOH) λ_{max} (log ϵ) 241 (4.52), 261 (4.52), 304 (4.89) nm; IR (KBr) ν_{max} 3400, 1600 cm^{-1}.

Compound **6:** UV (MeOH) λ_{max} (log ϵ) 302 (4.08) nm; IR (KBr) ν_{max} 3400, 1600 cm^{-1}.

Compound **7:** UV (MeOH) λ_{max} (log ϵ) 283 (4.02) nm; IR (KBr) ν_{max} 3450, 1600 cm^{-1}.

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References and Notes

(1) Renaud, S.; De Lorgeril, M. *Lancet* **1992**, *339*, 1523−1526.
(2) Goldberg, D. M. *Clinic. Chem.* **1995**, *41*, 14−16.
(3) Waterhouse, A. L. *Chem. Ind.* **1995**, 338−341.
(4) Fuhrman, B.; Lavy, A.; Aviram, M. *Am. J. Clin. Nutr.* **1996**, *42*, 1591−1593.
(5) Mattivi, F.; Reniero, F.; Korhammer, S. *J. Agric. Food Chem.* **1995**, *43*, 1820−1823.
(6) Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. *Lancet* **1993**, *341*, 1103−1104.
(7) 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. *Science* **1997**, *275*, 218−220.
(8) Waffo Teguo, P.; Decendit, A.; Krisa, S.; Vercauteren, J.; Deffieux, G.; Mérillon, J.-M. *J. Nat. Prod.* **1996**, *59*, 1189−1191.
(9) Waffo Teguo, P.; Decendit, A.; Vercauteren, J.; Deffieux, G.; Mérillon, J.-M. *Phytochemistry* **1996**, *42*, 1591−1593.
(10) Mérillon, J.-M.; Fauconneau, B.; Waffo Teguo, P.; Barrier, L.; Vercauteren, J.; Huguet, F. *Clinic. Chem.* **1997**, *43*, 1092−1093.
(11) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229−2246.
(12) Bax, A.; Subramanian, S. *J. Magn. Res.* **1986**, *67*, 565−569.
(13) Bax, A.; Summer, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(14) Jayatilake, G. S.; Jayasuriya, H.; Lee, E. S.; Koonchanok, N. M.; Geahlen, R. L.; Ashendel, C. L.; McLaughlin, J. L.; Chang C. J. *J. Nat. Prod.* **1993**, *56*, 1805−1810.
(15) Strack, D.; Heilemann, J.; Wray, V.; Dirks, H. *Phytochemistry* **1989**, *28*, 2071−2078.
(16) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. *Free Rad. Res.* **1995**, *22*, 375−383.
(17) Decendit, A.; Mérillon, J.-M. *Plant Cell Rep.* **1996**, *15*, 762−765.
(18) Gamborg, O. L.; Miller, R. A.; Ojima K. *Exp. Cell. Res.* **1968**, *50*, 151−156.
(19) Murashige, T.; Skoog, F. *Physiol. Plant.* **1962**, *15*, 473−497.
(20) Morel, G. *Physiol. Vég.* **1970**, *8*, 189−191.
(21) Buege, J. A.; Aust, S. D. *Methods Enzymol.* **1978**, *52*, 302−310.
(22) Tamura, A.; Sato, T.; Fuhu, T. *Chem. Pharm. Bull.* **1990**, *38*, 255−257.

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