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Comparative Analyses of Stilbenoids in Canes of Major *Vitis vinifera* L. Cultivars

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ABSTRACT: Grapevine canes are rich in resveratrol and its complex derivatives. These compounds have many biological activities and are needed mainly for health purposes. Canes, which are often wasted, can be used to produce these high-value compounds at low cost. We studied sixteen *Vitis vinifera* L. cultivars among the most widely cultivated ones worldwide. Polyphenols were extracted from their canes and identified by liquid chromatography−nuclear magnetic resonance spectroscopy. We accurately determined the content of *E*-ε-viniferin, *E*-resveratrol, *E*-piceatannol, and vitisin B and, for the first time, that of hopeaphenol and miyabenol C. The canes did not contain these major stilbene compounds in similar proportions, and their abundance and order of abundance varied according to the cultivar. For instance, Pinot noir has very high levels of *E*-resveratrol and *E*-ε-viniferin; Gewurztraminer has very high levels of vitisin B, and Carignan and Riesling have very high levels of hopeaphenol. These findings suggest that the right cultivar should be used to obtain the highest yield of a polyphenol of interest.

KEYWORDS: *grapevine, polyphenol, stilbene, viniferins, LC−NMR, LC−MS*

INTRODUCTION

Stilbenoids are phenolic compounds that are derived from the phenylpropanoid pathway. They are particularly abundant in Vitaceae such as grapevines, in which they have considerable physiological importance.[1] For instance, they play a major role in defense reactions by acting as phytoalexins (low-molecular-weight molecules accumulating upon pathogen infection and displaying antimicrobial activities).[2,3] For example, the level of resveratrol and viniferins, which are resveratrol oligomers, is indicative of downy mildew,[4] powdery mildew,[5] and gray mold resistance.[6] The antimicrobial activity of stilbenes has been demonstrated by the action of resveratrol toward *Botrytis cinerea* (the agent of gray mold)[7,8] and *Plasmopara viticola* (the downy mildew agent)[4] by that of ε-viniferin toward *B. cinerea*[9] and by that of vitisin B and hopeaphenol toward *P. viticola*.[9] Our group has recently reported that stilbene oligomers like miyabenol C, isohopeaphenol, and vitisins A and B greatly reduce the growth of some major wood decay fungi.[10] Additionally, resveratrol and its derivatives have attracted much attention owing to their various benefits in human health. It is now widely accepted that resveratrol possesses a large number of pharmacological properties including cardioprotective, antioxidant, and anticancer effects.[11−13] Piceatannol, a resveratrol analogue with an additional hydroxyl group in position 3, also displays a wide spectrum of bioactive activities.[14] Stilbenoid oligomers are also potent pharmacological biomolecules. For example, viniferins can inhibit human cytochrome P450 enzymes, improve the function of vascular endothelial cells and the heart, inhibit β-amyloid peptide aggregation, inhibit growth of human colon tumorigenic cells, and protect cells in models of Huntington disease.[15−19] Hopeaphenol is known for its antitumoral and anti-inflammatory activities and its strong growth-inhibiting activity in various cancer cell lines.[20−23] Miyabenol C exhibits strong DPPH scavenging activity and lipid peroxidation inhibitory activities,[24] high antiproliferative effects against colon cancer cell lines,[16] and is a potent antitumoral agent.[25] Vitisins display antioxidant and cardio-protection properties[26] and neuroprotective ones. Indeed, a recent study conducted in our group with twenty-five stilbenoids showed that vitisins A and B were the most effective molecules against nitric oxide production in lipopolysaccharide-activated microglia cells.[27] Since these compounds have many biological properties, the demand for resveratrol and its derivatives is ever-increasing. They now need to be produced for nutraceutical, cosmetic, and putatively pharmaceutical uses. Furthermore, owing to their antifungal properties, stilbenoid compounds could be a "green" method to replace the use of pesticides in agronomical practice, especially in the vineyard.[9] Currently, commercialized *E*-resveratrol is mainly produced from the roots of Japanese knotweeds (*Fallopia japonica*) that are cultivated in fields. Various studies have been conducted to develop efficient alternative methods for their large-scale cost-effective production. Chemical synthesis is a potential approach, such as the Wittig and Heck reaction, but this often requires relatively long synthetic sequences and the use of expensive catalysts and reagents. Biotechnology has also been used to produce resveratrol and stilbene derivatives from plant cell cultures and microorganisms.[28] However, these in vitro technologies still require optimization and do not allow the production of large amounts of stilbenoid oligomers. Furthermore, large quantities of plant material rich in complex stilbenoids are wasted each year.

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Indeed, the grape industry does not optimize cane pruning as canes are often composted or burned for disposal. This represents a huge source of plant material as vineyards worldwide comprised a total surface area of 7.5 million hectares in 2012 (http://www.oiv.int). Consequently, extracting stilbenoids from canes would be a good way to utilize canes by obtaining high-value phytochemicals at low cost. Aaviksaar et al.,[29] Pussa et al.,[30] and Rayne et al.[31] rank among the first authors to propose this idea. To optimize a stilbenoid of biological interest in canes, it is essential to know which polyphenols are present in harvested canes and in which quantity. Some authors have identified stilbenes and/or their content in grapevine canes, but this was only in one *Vitis vinifera* L. cultivar: Pinot Noir.[32,33] Others measured stilbene concentrations in several *Vitis* such as *V. vinifera* hybrids and cultivars grown in Estonia,[29,30] Greek *V. vinifera* cultivars, and cultivars commonly cultivated in Chile[33,34] and in wild grapevine species.[35] However, in many of these publications, only the content of the two major stilbenes was evaluated (i.e., *E*-resveratrol and *E*-ε-viniferin) and the results were expressed as *E*-resveratrol equivalents.[30−32,34] Moreover, among the thousands of *V. vinifera* cultivars, the most widespread, at least for wine production, are Chardonnay, Cabernet Sauvignon, Shiraz (Syrah), Merlot, and Sauvignon blanc, sometimes called the 'Big Five'.[36,37] To the best of our knowledge, stilbene contents in Big Five canes have never been compared in the same experiment. We therefore decided to fill this gap by analyzing a *V. vinifera* germplasm collection located in a single geographic place. In this way, we were free of the influence of growth environment and agronomic factors, which could impact polyphenolic composition. To complement the study, we analyzed other commonly cultivated varieties (Carignan, Chenin, Cinsault, Gamay, Gewurztraminer, Grenache, Melon, Pinot noir, Riesling, Semillon, and Ugni blanc) (i.e., a total of 16 different cultivars). We first conducted hydroxystilbenoid identification from the *V. vinifera* L. cv. canes by liquid chromatography−nuclear magnetic resonance (LC−NMR) spectroscopy. Then, we accurately determined the contents of the main compounds identified, namely *E*-resveratrol, *E*-piceatannol, *E*-ε-viniferin, miyabenol C, vitisin B, and hopeaphenol by comparison with the corresponding pure standards.

MATERIALS AND METHODS

Plant Material. The sixteen *V. vinifera* L. cultivars (eight red cultivars: Cabernet Sauvignon, Carignan, Cinsault, Gamay, Grenache, Merlot, Pinot noir, Shiraz; seven white cultivars: Chardonnay, Chenin, Melon, Riesling, Sauvignon blanc, Semillon, Ugni blanc; and a pink cultivar: Gewurztraminer) of interest were cultivated at the field station in Villenave d'Ornon (France) and belong to the INRA germplasm collection. They were planted in 2001 and similarly grafted. The grapevine plants were managed by conventional methods. For each cultivar, two one-year-old canes from three plants were collected in January 2011. Each sample was cut into pieces and dried at 40 °C for 15 days. Then the samples were ground in powder on a 0.75 μm sieve and stored at −20 °C.

Chemicals. *E*-Resveratrol and *E*-piceatannol were purchased from Sigma Chemical Company (St Louis, MO). We extracted and purified hopeaphenol, *E*-ε-viniferin, miyabenol C, vitisin B from *V. vinifera* roots and woody stems of cv. Merlot.[38] Acetone was provided by Xilab (Bruges, France). High-performance liquid chromatography (HPLC)-grade methanol was purchased from Carlo Erba (Rodano, Italy). Water for HPLC−MS was purified using an Elga water purification system (Bucks, U.K.). LC−MS-grade acetonitrile (ACN, Scharlau), formic acid (Fischer Scientific, Loughborough, U.K.), and trifluoroacetic acid (TFA, Sigma−Aldrich, St Louis, MO, USA) were used for the LC−MS analyses.

Conditions for LC−MS Analysis. The chromatography apparatus, an Agilent 1200 from Agilent Technologies (Santa Clara, CA), consisted of an autosampler module, a degasser, a binary pump, a column heater/selector, and a UV−visible-diode array detector (DAD) from the same provider. A Prontosil C18 5 μm, 150 × 4.6 mm column was used for all LC applications (Bischoff, Leonberg, Germany). Samples were dissolved in 1:1 MeOH:water (4 mg/mL), filtered, and injected using 10 μL injection volumes. The fractions were eluted with a gradient consisting of water acidified with 0.1% formic acid (solvent A) and acetonitrile acidified with 0.1% formic acid (solvent B) at 0.8 mL min^{-1}. A 60 min elution was performed at 25 °C with a gradient of 0 min (95% A, 5% B) to 50 min (65% A, 35% B), followed by a 10 min wash (100% B) and a 10 min re-equilibration. The HPLC was coupled to an Esquire 3000 Plus ion trap mass spectrometer using an ESI source (Bruker-Daltonics, Billerica, MA). The chromatographic conditions were as above, and the HPLC output was split 1:10 in the MS detector. Total ion chromatograms were obtained using alternating positive and negative modes with a range of m/z 110−1500. Nitrogen was used as the drying gas at 5 L/min with nebulizer pressure of 15 psi at 325 °C. For negative ion mode, capillary voltage was 3100 V, capillary end voltage at −127.7 V, skimmer voltage at −40 V, and trap drive at 71.0. For positive ion mode, capillary voltage was −3700 V, capillary end voltage 127.7 V, skimmer voltage 40 V, and trap drive 68.7. Data analysis was performed with Bruker Data Analysis 3.2.

Conditions for LC−NMR and NMR Analysis. The chromatography apparatus for LC−NMR was identical to that for LC−MS analyses: an Agilent 1200 (Agilent Technologies, Santa Clara, CA). During LC−NMR experiments, samples were eluted with a mixture of three solvents, deuterated water (solvent A), acetonitrile acidified with 0.02% TFA (solvent B), and water acidified with 0.02% TFA (solvent C). The flow rate was 0.8 mL/min using the following gradient: 0 min (30% A, 5% B, 65% C) to 50 min (30% A, 35% B, 35% C), followed by a 10 min wash (100% B) and a 10 min re-equilibration. The LC−NMR experiments were performed with the BPSU HP interface coupled to a Bruker Avance III 600 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany). The NMR was equipped with a $^{1}H-^{13}C$ inverse-detection LC probe, with an active volume of 60 μL. The ^{1}H NMR spectra were acquired in a stopped-flow mode. Solvent suppression of the water and acetonitrile peaks was achieved by a NOESY-type presaturation pulse sequence. The acetonitrile peak was used as the chemical shift reference and was offset to $\delta = 1.96$ ppm. The number of scans varied according to sample concentration, ranging from 512 to 2048. For 2D-NMR experiments, compounds were directly collected after on-flow ^{1}H-LC−NMR analysis onto a FOXY collector from Teledyne ISCO (Lincoln, NE), lyophilized, and analyzed by using classical NMR experiments.

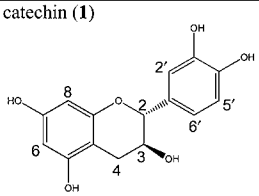
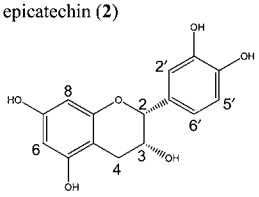
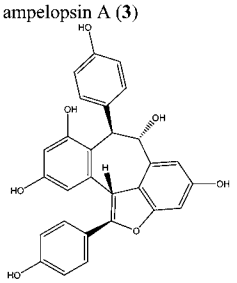
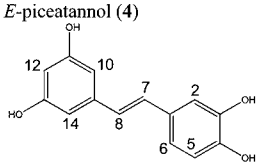
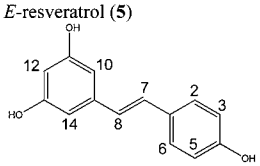
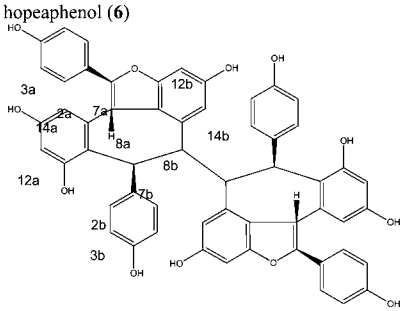
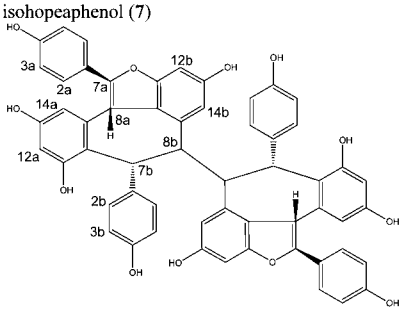
Quantification of Stilbenoids. Two-hundred milligrams of cane powder were extracted in 10 mL of acetone/H_2O mixture (6:4) overnight at room temperature. After centrifugation (2500 rpm, 5 min), 5 mL of supernatant were evaporated and the dry extract was suspended in 2 mL of methanol/H_2O (1:1). The solution was filtered through PTFE membrane filters (0.45 μm). Analysis of stilbenes was performed by HPLC-DAD−MS in the same conditions as those used for identification in the positive mode. Separation of 10 μL of cane extract was performed at a flow rate of 1 mL/min with a mobile phase composed of (A) distilled water 0.1% formic acid and (B) acetonitrile 0.1% formic acid. The run was as follows: 0 to 5 min, 17% B; 5 to 25 min from 17% B to 30% B; 25 to 35 min, from 30% B to 38% B; 35 to 45 min 38% B to 100% B; 45 to 55 min, 100% B; 55 to 56 min, from 100% B to 17% B; 56 to 70 min 17% of B. UV detection was performed at 280 and 325 nm. Stilbene contents were determined from calibration curves of pure standards (injected concentrations ranging from 2 to 500 μg/mL). The linearity of the response of the standard molecules was checked by plotting the peak area versus the concentration of the compounds.

Statistical Analysis. All analyses were done by using XLstat (Addinsoft) with $p < 0.05$ as significant. One-way ANOVA and the Kruskall−Wallis test investigated the difference between red and white cultivars. Principal component analysis (PCA) was performed with Spearman correlations to investigate stilbenoid profile and cultivar relationship.

RESULTS AND DISCUSSION

Identification of Hydroxystilbenoids from Grapevine Canes. Eight stilbenoids (ampelopsin A, *E*-piceatannol, *E*-resveratrol, *E*-ε-viniferin, hopeaphenol, isohopeaphenol,

Table 1. Name, Peak Number, Retention Time, MS (Molecular Ion, Positive Mode) and ^{1}H-LC−NMR Data of Compounds Identified by LC−MS and Stop-Flow LC−NMR

Compound	t_R (min)	*m/z*	δ_H
catechin (**1**) 	7.8	291	6.80 (1H, brs, H-2′), 6.79 (1H, d, *J* = 8.1 Hz, H-5′), 6.73 (1H, dd, *J* = 8.1, 1.6 Hz, H-6′), 5.96 (1H, brs, H-8), 5.88 (1H, brs, H-6), 4.67 (1H, d, *J* = 7.6 Hz, H-2), 6.80 (1H, m, H-3), 2.74 (1H, dd, *J* = 16.1, 5.3 Hz, H-4b), 2.42 (1H, dd, *J* = 16.1, 7.9 Hz, H-4a)
epicatechin (**2**) 	10.8	291	6.90 (1H, brs, H-2′), 6.80 (1H, d, *J* = 8.1 Hz, H-5′), 6.79 (1H, brd, *J* = 8.1, H-6′), 5.96 (1H, brs, H-6), 5.94 (1H, brs, H-8), 4.85 (1H, s, H-2), 4.19 (1H, brs, H-3), 2.79 (1H, dd, *J* = 17.0, 4.5 Hz, H-4b), 2.61 (1H, dd, *J* = 17.0, 1.9 Hz, H-4a)
ampelopsin A (**3**) 	16.1	470	identified by classical NMR after isolation
E-piceatannol (**4**) 	16.5	245	7.00 (1H, d, *J* = 2.0 Hz, H-2), 6.93 (1H, d, *J* = 16.4 Hz, H-7), 6.88 (1H, *J* = 2.0; 8.4 Hz, H-6) 6.80 (1H, d, *J* = 16.4 Hz, H-8), 6.77 (1H, d, *J* = 8.4, H-5), 6.45 (2H, d, J = 2.1 Hz, H-10,14), 6.14 (1H, t, *J* = 2.1 Hz, H-12)
E-resveratrol (**5**) 	22.9	229	7.36 (2H, d, *J* = 8.5 Hz, H-2,6), 6.99 (1H, d, *J* = 16.4 Hz, H-7), 6.82 (1H, d, *J* = 16.4 Hz, H-8), 6.76 (2H, d, *J* = 8.5, H-3,5), 6.44 (2H, d, J = 2.1 Hz, H-10,14), 6.13 (1H, t, *J* = 2.1 Hz, H-12)
hopeaphenol (**6**) 	27.2	907	7.07 (2H, d, *J* = 8.5 Hz, H-2b,6b), 6.79 (2H, d, *J* = 8.5 Hz, H-2a,6a), 6.76 (2H, d, *J* = 8.5 Hz, H-3b,5b), 6.56 (2H, d, *J* = 8.5 Hz, H-3a,5a), 6.39 (1H, brs, H-12b), 6.19 (1H, brs, H-14b), 5.73 (1H, d, *J* = 12.2 Hz, H-7b), 5.72 (1H, brs, H-12a), 5.42 (1H, d, brs, H-14a), 4.85 (1H, brs, H-7a), 4.08 (1H, d, *J* = 12.2 Hz, H-8b), 3.76 (1H, brs, H-8a)
isohopeaphenol (**7**) 	27.9	907	7.46 (2H, d, *J* = 8.4 Hz, H-2a,6a), 6.95 (2H, d, *J* = 8.4 Hz, H-3a,5a), 6.30 (2H, d, *J* = 8.4 Hz, H-2b,6b), 6.23 (2H, d, J = 8.4, H-3b,5b), 6.22 (1H, brs, H-12a), 6.01 (1H, brs, H-14a), 5.80 (1H, d, brs, H-12b), 5.51 (1H, d, *J* = 10.8 Hz, H-7a), 5.31 (1H, d, brs, H-14b), 5.27 (1H, d, *J* = 10.8 Hz, H-8a), 4.77 (1H, brs, H-7b), 3.23 (1H, brs, H-8b)

miyabenol C, and vitisin B) and two flavonoids (catechin and epicatechin) were found in the cane extracts of the sixteen cultivars. Successful application of liquid chromatography in combination with MS (LC−MS) and NMR (LC−NMR) was

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recently reported for the direct chemical characterization of stilbenoids in wine.[35] LC−MS provides useful structural data with high sensitivity. However, MS data do not provide detailed and conclusive structural information, especially when isomeric compounds are studied like hopeaphenol (**6**) and isohopeaphenol (**7**). In such cases, the structural discriminating abilities of NMR are necessary for unequivocal identification of the individual stilbenoids. LC−NMR analysis enables the identification of individual compounds without the need for their isolation.

Quantitative differences were observed in the HPLC profiles. Thus, Merlot and Cabernet Sauvignon extracts were used to identify each compound. Eleven compounds were identified: two flavanols (**1**−**2**) and nine stilbenoids (**3**−**11**). Table 1 summarizes the results for the individual compounds, including the peak number, retention time, molecular ions (positive mode), and ^{1}H-LC−NMR data that were obtained from LC−MS and LC−NMR analysis.

The ^{1}H−LC−NMR spectra of compounds **1** and **2** are shown in Figure 1A. The molecular ion $[M + H]^+$ of m/z 291 was detected and is typical of flavanol. Compounds **1** and **2** were identified by comparing their ^{1}H-LC−NMR spectra (Table 1 and Figure 1A). Close examination of the ^{1}H NMR data of compound **1** established it to be catechin with the 2,3-trans configuration, as indicated by the specific coupling constants of H-2 (J = 7.6 Hz) and H-4.[39] The NMR spectra of compound **2** were almost identical to those of **1**, except for the coupling constants of H-2 (broad singlet) and H-4, indicating a 2,3-cis configuration.[39] Thus, compound **2** was epicatechin.

The ^{1}H−LC−NMR spectra of compounds **4** and **5** are shown in Figure 1B. The molecular ions $[M + H]^+$ of m/z 245 (compound **4**) and 229 (compound **5**) were typical of stilbene monomers. Compounds **4** and **5** were characterized as *E*-piceatannol and *E*-resveratrol, respectively, by analyzing ^{1}H−LC−NMR (Table 1) and by comparison with the literature data.[40,41]

The MS and LC−NMR analysis of compounds **3**, **8**, and **10** (Table 1) revealed the presence of three stilbene dimers. The MS analysis of compound **3** with [M + H]+ of m/z 470 in the positive ion mode showed it to be ampelopsin A.[42] Owing to its low concentration in the extracts, compound **3** was collected and accumulated after LC−NMR analysis in a fraction collector, lyophilized, and subsequently analyzed with a classical NMR probe as previously described.[43] Identification as ampelopsin A was confirmed by comparison of the ^{1}H NMR shifts and coupling constants with the literature data.[42] The MS analysis of compounds **8** and **10** showed a molecular ion $[M + H]^+$ of m/z 455, which is typical of viniferin derivatives. Comparison of their ^{1}H-LC−NMR data with our previous data[35] revealed *E*-*ε*-viniferin (**8**) and *E*-*ω*-viniferin (**10**).

The MS analysis of compound **9** showed compounds with $[M + H]^+$ of m/z 681 in the positive ion mode, which are typical of stilbene trimers. As for the identification of ampelopsin A (**3**), compound **9** was collected, accumulated, and subsequently analyzed with a classical NMR probe owing to its low concentration in the analyzed extracts. Identification as miyabenol C was confirmed by comparison of the ^{1}H NMR data with the literature data.[44]

The MS analysis of compounds **6**, **7**, and **11** showed the molecular ion $[M + H]^+$ of m/z 907, which is typical of stilbene tetramers. ^{1}H-LC−NMR analysis (Figure 1C and Table 1) allowed the identification of these compounds. The ^{1}H-LC−NMR of compound **7** matched with our previously reported data for isohopeaphenol.[35] The ^{1}H-LC−NMR data of compound **6**, which contained only two para-disubstituted phenyl rings, were similar to that of compound **7**. Upon careful examination, the LC−NMR data matched the literature for hopeaphenol, a symmetrical molecule.[42] The ^{1}H NMR of compound **11** contained three sets of para-disubstituted phenyl rings, two sets of meta-coupled aromatic hydrogens, two sets of AX2-type meta-coupled aromatic hydrogens, a 1-oxy-2,4-disubstituted benzene ring, and an *E* double bond, in addition to six aliphatic signals. The ^{1}H NMR of compound **11** matched the literature values for vitisin B.[45] Online LC−MS and LC−NMR combination techniques provide structurally rich information with very small quantities of material for characterizing complex plant extracts. These techniques provide efficient structural identification without preliminary isolation or purification steps.

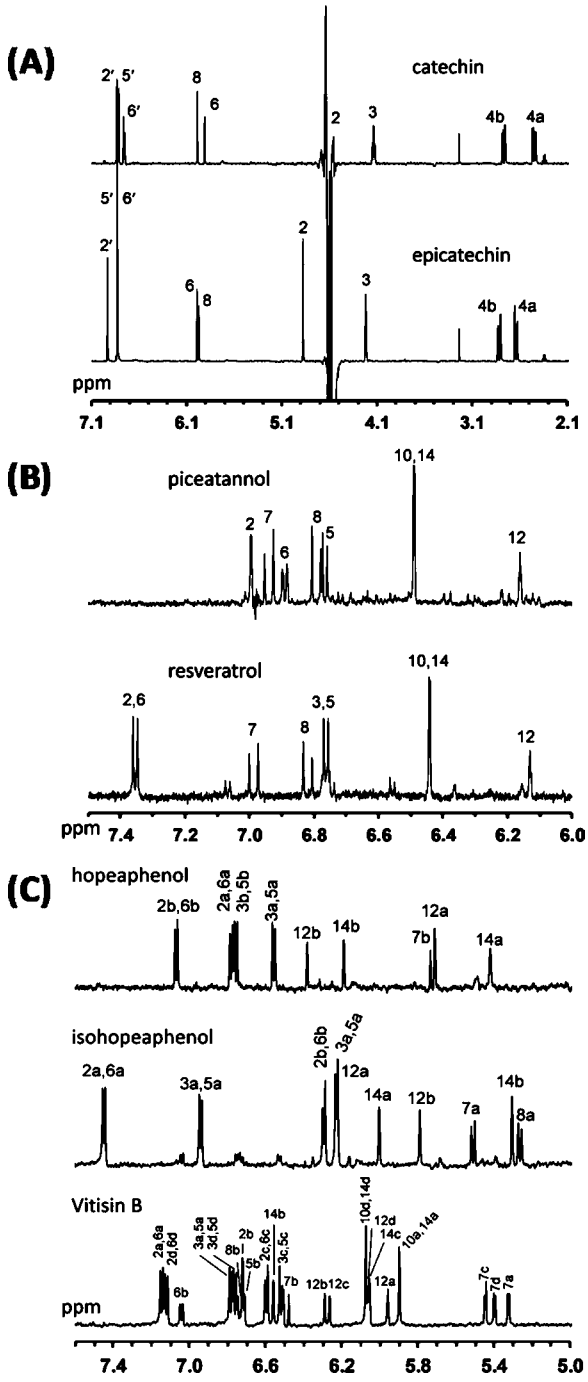

Figure 1. ^{1}H-LC−NMR spectra of phenolics identified in woody canes.

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Table 2. Concentrations of Stilbenoids in Woody Canes of 16 *Vitis vinifera* L. Cultivars[a]

	stilbene concentration (mg kg^{-1} DW)						
cultivar	*E*-resveratrol	*E*-piceatannol	*E*-ε-viniferin	miyabenol C	vitisin B	hopeaphenol	total
Cabernet Sauvignon	871 (202)	735 (142)	2379 (1123)	30 (12)	420 (109)	1346 (294)	5781
Carignan	880 (304)	519 (80)	967 (71)	87 (21)	NQ	1439 (214)	3892
Chardonnay	190 (87)	190 (67)	2089 (334)	NQ	NQ	766 (149)	3235
Chenin	794 (161)	1227 (267)	2218 (274)	35 (23)	NQ	623 (175)	4897
Cinsault	486 (226)	298 (268)	1629 (100)	106 (12)	NQ	339 (96)	2858
Gamay	980 (201)	843 (138)	1828 (157)	NQ	102 (53)	1085 (182)	4838
Gewurztraminer	649 (290)	490 (150)	2199 (379)	NQ	1116 (380)	1118 (357)	5572
Grenache	752 (392)	372 (195)	1792 (110)	NQ	88 (22)	465 (123)	3469
Melon	963 (189)	561 (359)	1970 (193)	NQ	126 (44)	645 (188)	4265
Merlot	1181 (189)	947 (353)	2263 (220)	22 (14)	146 (48)	642 (163)	5201
Pinot noir	1526 (293)	1710 (224)	3737 (421)	73 (22)	313 (156)	1126 (294)	8485
Riesling	605 (258)	270 (101)	1716 (441)	174 (12)	88 (54)	1468 (601)	4321
Sauvignon blanc	730 (34)	607 (294)	2697 (167)	36 (17)	369(212)	841 (263)	5280
Semillon	872 (263)	471 (208)	2448 (186)	NQ	252 (106)	287 (124)	4330
Shiraz	481 (373)	460 (253)	2507 (462)	38 (14)	182 (244)	586 (456)	4254
Ugni blanc	689 (119)	1056 (295)	2292 (259)	39 (12)	138 (21)	818 (202)	5032
Means	791	672	2171	40	209	850	4732

[a]Two sets of extractions were conducted on separate days on three samples collected on three different vinestocks. Results are shown in milligrams per kilogram of dry weight. Standard deviation (SD) is given in parentheses. NQ means detected but not quantified because of low levels.

Nevertheless, stopped-flow LC−NMR has potential drawbacks.[35] In such cases, LC−NMR analysis through peak collection in a fraction collector allows structural identification via classical NMR experiments.

Accurate Quantification of Hydroxystilbenoids from Grapevine Canes. Only the six major stilbenoids (*E*-piceatannol, *E*-resveratrol, hopeaphenol, *E*-ε-viniferin, miyabenol C, and vitisin B) were quantified as the other molecules were not present in sufficient quantities. To determine with accuracy the stilbene concentration of the cane extracts, we performed calibration curves with pure hydroxystilbenoid standards. These compounds were extracted and purified from *V. vinifera* roots or woody stems of cv. Merlot, and we characterized them by NMR and LC-ESI-MS, as previously described.[38] We first checked the linearity of response of the standard molecules (peak area versus the concentration of the compounds) at 325 nm, except for hopeaphenol at 280 nm. As regards the profile of these curves, the slope decreased as the degree of polymerization increased. Indeed, the molar extinction coefficient is lower for oligomers than monomers.[46] Since all the molecules present in the canes except *E*-resveratrol and *E*-piceatannol are not commercially available, many authors have quantified these other compounds as *trans*-resveratrol equivalents.[30,31,34] For instance, this means that the determination of *E*-ε-viniferin in *E*-resveratrol equivalent leads to the underestimation of the dimer by about 2-fold. In this study, we quantified the 6 major hydroxystilbenoids from calibration curves of the corresponding standard molecules (Table 2). The most abundant stilbenoid in canes was *E*-ε-viniferin (mean of 2171 mg kg^{-1} DW), followed by hopeaphenol, *E*-resveratrol, and *E*-piceatannol (850, 791, and 672 mg kg^{-1} DW, respectively), vitisin B (209 mg kg^{-1} DW), and miyabenol C (40 mg kg^{-1} DW), and a total of stilbenes of 4732 mg kg^{-1} DW. However, this order of abundance varied according to the cultivar. For example, Carignan was richer in hopeaphenol (1439 ± 214 mg kg^{-1} DW) than in *E*-ε-viniferin (967 ± 71 mg kg^{-1} DW), *E*-resveratrol (880 ± 304 mg kg^{-1} DW), *E*-piceatannol (519 ± 80 mg kg^{-1} DW), or miyabenol C (87 ± 21 mg kg^{-1} DW), while vitisin B could not be quantified. In Semillon, the main compound was *E*-ε-viniferin (2448 ± 186 mg kg^{-1} DW), followed by *E*-resveratrol (872 ± 263 mg kg^{-1} DW), *E*-piceatannol (471 ± 208 mg kg^{-1} DW), hopeaphenol (287 ± 124 mg kg^{-1} DW), and vitisin B (252 ± 106 mg kg^{-1} DW) but there was no miyabenol C. Therefore, the content of a specific stilbenoid may vary greatly depending on the cultivar, so it is important to select cultivars on the basis of the compound to be isolated.

The highest concentrations of *E*-resveratrol in our samples was obtained with Pinot noir (1526 ± 293 mg kg^{-1} DW) and Merlot (1181 ± 189 mg kg^{-1} DW), while the lowest one was with Chardonnay (190 ± 87 mg kg^{-1} DW), with an average level for all the cultivars of 791 mg kg^{-1} DW. With regard to *E*-resveratrol content, Aaviksaar et al.[29] and Püssa et al.[30] found values in Estonian grape cultivars between 100 and 4700 mg kg^{-1} DW and between 1100 and 3200 mg kg^{-1} DW (average 1727 and 2133 mg kg^{-1} DW, respectively). In Pinot noir, Rayne et al.[31] and Karacabey and Mazza[32] found 3450 and between 1290 and 4060 mg kg^{-1} DW (average 3068 mg kg^{-1} DW), respectively. Anastasiadi et al.[33] reported values between 74 and 266 mg kg^{-1} DW (average 131 mg kg^{-1} DW) for native Greek *V. vinifera* cultivars, while Vergara et al.[34] found between 383 and 6533 mg kg^{-1} DW (average 3471 mg kg^{-1} DW) for various cultivars in Chile. Our findings are in general agreement with the literature despite significant variability, owing perhaps to different extraction systems and/or different plant physiological states of the cane when harvesting was done. On the latter point, Aaviksaar et al.[29] observed that *E*-resveratrol content in canes increased throughout the growing season. A similar expression profile was noted for *E*-ε-viniferin, whose concentrations in our hands ranged from 3737 ± 421 mg kg^{-1} DW for Pinot noir to 967 ± 71 mg kg^{-1} DW for Carignan, with an average level for all cultivars studied of 2171 mg kg^{-1} DW. Caution is required when comparing the *E*-ε-viniferin contents that we obtained with those by other authors because the latter often expressed their results as resveratrol equivalents.[29−32,34] Nevertheless, at least two recent papers report *E*-ε-viniferin content by using a purified standard. Anastasiadi et al.[33] found concentrations between 167 and 499 mg kg^{-1} DW for native Greek cultivars, and Pawlus et al.[35] reported values between 728 and 5739 mg kg^{-1} DW for grapevine wild-type species and 2584 for Cabernet Sauvignon. Our results are therefore in agreement with others. In our

investigation of *E*-piceatannol content, we found the highest level of 1710 ± 224 mg kg^{-1} DW in Pinot noir and the lowest of 190 ± 67 mg kg^{-1} DW in Chardonnay, with an average of 672 mg kg^{-1} DW for all cultivars studied. As with *E*-ε-viniferin, our values cannot easily be compared to those in the literature, as most authors did not use the corresponding standard to express their results. Nevertheless, absolute *E*-piceatannol content was assessed by Pawlus et al.[35] who found levels from 194 to 1962 mg kg^{-1} DW in *Vitis* wild species and 573 in Cabernet Sauvignon, which is in agreement with our present findings. In our samples, the highest quantities of hopeaphenol were 1468 ± 601 mg kg^{-1} DW in Riesling and 1439 ± 214 mg kg^{-1} DW in Carignan, while the lowest was 287 ± 124 mg kg^{-1} DW in Semillon. The average level of 850 mg kg^{-1} DW was found for all cultivars together. To our knowledge, this is the first time that hopeaphenol content has been precisely assayed. Vitisin B could also easily be quantified with results ranging from 1116 ± 380 mg kg^{-1} DW for Gewurztraminer to no detectable levels in Carignan, Chardonnay, Chenin, and Cinsault. Pawlus et al.[35] also found that this molecule could not be detected in some grapevine species, whereas others contained up to 7019 mg kg^{-1} DW. Miyabenol C, which was found in minor quantities in our extracts, represented 174 ± 12 mg kg^{-1} DW of Riesling canes and was absent in Chardonnay, Gamay, Gewurztraminer, Grenache, Melon, and Semillon. As in the case of hopeaphenol, the content of this molecule cannot be compared to values reported in the literature.

The mean total amounts of stilbenoids that we obtained were about 4700 mg kg^{-1} DW, with the highest value of 8485 mg kg^{-1} DW for Pinot noir and the lowest of 2858 mg kg^{-1} DW for Cinsault. These values are in accordance to those of Cabernet Sauvignon in the study by Pawlus et al.[35] who also quantified many stilbenoid compounds. In the latter paper, the authors found that canes of wild *Vitis* species contained about 10000 mg kg^{-1} DW and could reach 19500 mg kg^{-1} for *V. rupestris*. These species are very rich in stilbenoids but are not a commercial prospect since they are not cultivated as extensively as *V. vinifera* around the world. Principal component analysis (PCA) comparing the mean content of each of the 6 quantified stilbenoids in the 16 cultivars (Figure 2) found that about 62% of the total variance was explained by F1 and F2. Pinot noir (PN) differed considerably from the other cultivars by its high overall amounts of all hydroxystilbenoids. Indeed, Pinot noir is known to contain high stilbene levels in berries[47] and stems.[31,33] Cinsault (Ci) can be also considered as a cultivar apart as its stilbene content was low except for miyabenol C. Riesling (Ri) and Carignan (Ca) formed a group characterized by higher quantities in hopeaphenol and miyabenol C and rather low quantities of *E*-ε-viniferin and vitisin B. Chardonnay (Cha), Grenache (Gr), and Sh (Shiraz) had low concentrations in hopeaphenol, resveratrol, and piceatannol. Semillon (Sem), Sauvignon (Sau), and Gewurztraminer (Gew) were found to be rich in *E*-ε-viniferin and vitisin B. Gamay (Ga), Chenin (Che), Ugni Blanc (UB), Cabernet Sauvignon (CS), Merlot (Mer) and Melon (Mel) form a group as they were found to contain average levels of each phenolic compound quantified. Significant differences in stilbenoid content between cultivars were also reported in grape berry by Gatto et al.[47] who underlined the importance of genotype on the control of stilbene accumulation. While PCA showed that red and white varieties may share a common chemical profile, we observed that red cultivars had on average a higher level than white and pink ones. A one-way ANOVA revealed that this difference was significant with a p value of 0.014, as confirmed by a Kruskall-Wallis test (p value = 0.003).

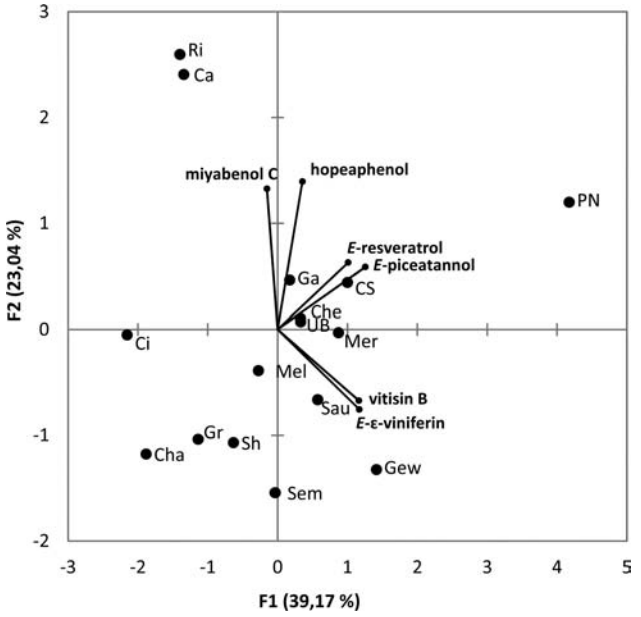

Figure 2. Distribution of the 16 grape cultivars in the two-dimensional space F1 F2. CS: Cabernet Sauvignon, Ca: Carignan, Cha: Chardonnay, Che: Chenin, Ci: Cinsault, Ga: Gamay, Gew: Gewurztraminer, Gr: Grenache, Mel: Melon, Mer: Merlot, PN: Pinot noir, Ri: Riesling, Sau: Sauvignon, Sem: Semillon, Sh: Shiraz, and UB: Ugni Blanc.

Similarly, Gatto et al.[47] noted that the *E*-resveratrol and piceid levels allowed the discrimination of red and white varieties in grape berry.

The present study shows that the canes of the major cultivated *V. vinifera* cultivars contain a variety of stilbenoids from monomers to oligomers and that these compounds may be found in considerable quantities, particularly *E*-ε-viniferin, hopeaphenol, *E*-resveratrol, and *E*-piceatannol. This is the first report of the accurate determination of a number of stilbenoids in the five major cultivated grapevines in the world and 11 other French *V. vinifera* cultivars. Moreover, we demonstrate that the content of a specific stilbenoid differs from one cultivar to another. For instance, Pinot noir has very high levels of *E*-resveratrol and *E*-ε-viniferin, compared to the others, while Gewurztraminer has high levels of vitisin B and Carignan and Riesling are high in hopeaphenol. This underlines the importance of determining the presence and concentration of a stilbenoid of interest in harvested *V. vinifera* canes before performing extraction, in order to obtain the maximal amount of the molecule. The findings also demonstrate the potential for using grapevine canes to obtain high-value phytochemicals of biological importance for human and plant health.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Rivière, C.; Pawlus, A. D.; Mérillon, J. M. Natural stilbenoids: Distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. *Nat. Prod. Rep.* **2012**, *29*, 1317−1333.

(2) Jeandet, P.; Douillet-Breuil, A. C.; Bessis, R.; Debord, S.; Sbaghi, M.; Adrian, M. Phytoalexins from the Vitaceae: Biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem.* **2002**, *50*, 2731−2741.
(3) Jeandet, P.; Delaunois, B.; Conreux, A.; Donnez, D.; Nuzzo, V.; Cordelier, S.; Clément, C.; Courot, E. Biosynthesis, metabolism, molecular engineering and biological functions of stilbene phytoalexins in plants. *BioFactors* **2010**, *36*, 331−341.
(4) Pezet, R.; Gindro, K.; Viret, O.; Richter, H. Effect of resveratrol, viniferins and pterostilbene on *Plasmopara viticola* zoospore mobility and disease development. *Vitis* **2004**, *43*, 145−148.
(5) Schnee, S.; Viret, O.; Gindro, K. Role of stilbenes in the resistance of grapevine to powdery mildew. *Physiol. Mol. Plant Pathol.* **2008**, *72*, 128−133.
(6) Bavaresco, L.; Petegolli, D.; Cantù, E.; Fregoni, M.; Chiusa, G.; Trevisan, M. Elicitation and accumulation of stilbene phytoalexines in grapevine berries infected by *Botrytis cinerea*. *Vitis* **1997**, *36*, 77−83.
(7) Adrian, M.; Jeandet, P.; Veneau, J.; Weston, L. A.; Bessis, R. Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. *J. Chem. Ecol.* **1997**, *23*, 1689−1702.
(8) Adrian, M.; Jeandet, P. Effects of resveratrol on the ultrastructure of *Botrytis cinerea* conidia and biological significance in plant/pathogen interactions. *Fitoterapia* **2012**, *83*, 1345−1350.
(9) Schnee, S.; Queiroz, E. F.; Voinesco, F.; Marcourt, L.; Dubuis, P. H.; Wolfender, J. L.; Gindro, K. *Vitis vinifera* canes, a new source of antifungal compounds against *Plasmopara viticola*, *Erysiphe necator*, and *Botrytis cinerea*. *J. Agric. Food Chem.* **2013**, *61*, 5459−5467.
(10) Lambert, C.; Bisson, J.; Waffo-Téguo, P.; Papastamoulis, Y.; Richard, T.; Corio-Costet, M. F.; Mérillon, J. M.; Cluzet, S. Phenolics and their antifungal role in grapevine wood decay: Focus on the Botryosphaeriaceae family. *J. Agric. Food Chem.* **2012**, *60*, 11859−11868.
(11) Baur, J. A.; Sinclair, D. A. Therapeutic potential of resveratrol: The *in vivo* evidence. *Nat. Rev. Drug Discovery* **2006**, *5*, 493−506.
(12) Saiko, P.; Szakmary, A.; Jaeger, W.; Szekeres, T. Resveratrol and its analogs: Defense against cancer, coronary disease and neurodegenerative maladies or just a fad? *Mutat. Res.* **2008**, *658*, 68−94.
(13) Smoliga, J. M.; Baur, J. A.; Hausenblas, H. A. Resveratrol and health: A comprehensive review of human clinical trials. *Mol. Nutr. Food Res.* **2011**, *55*, 1129−1141.
(14) Piotrowska, H.; Kucinska, M.; Murias, M. Biological activity of piceatannol: Leaving the shadow of resveratrol. *Mutat. Res.* **2012**, *750*, 60−82.
(15) Piver, B.; Berthou, F.; Dreano, Y.; Lucas, D. Differential inhibition of human cytochrome P450 enzymes by ε-viniferin, the dimmer of resveratrol: Comparison with resveratrol and polyphenols from alcoholized beverages. *Life Sci.* **2003**, *73*, 1199−1213.
(16) Gonzalez-Sarrías, A.; Gromek, S.; Niesen, D.; Seeram, N. P.; Henry, G. E. Resveratrol oligomers isolated from *Carex* species inhibit growth of human colon tumorigenic cells mediated by cell cycle arrest. *J. Agric. Food Chem.* **2011**, *59*, 8632−8638.
(17) Richard, T.; Poupard, P.; Nassra, M.; Papastamoulis, Y.; Iglésias, M. L.; Krisa, S.; Waffo-Teguo, P.; Mérillon, J. M.; Monti, J. P. Protective effect of ε-viniferin on β-amyloid peptide aggregation investigated by electrospray ionization mass spectrometry. *Bioorg. Med. Chem.* **2011**, *19*, 3152−3155.
(18) Fu, J.; Jin, J.; Cichewicz, R. H.; Hageman, S. A.; Ellis, T. K.; Xiang, L.; Peng, Q.; Jiang, M.; Arbez, N.; Hotaling, K.; Ross, C. A.; Duan, W. *Trans*-(−)-ε-viniferin increases mitochondrial sirtuin 3 (SIRT3), activates AMP-activated Protein Kinase (AMPK), and protects cells in models of Huntington disease. *J. Biol. Chem.* **2012**, *287*, 24460−24472.
(19) Zghonda, N.; Yoshida, S.; Ezaki, S.; Otake, Y.; Murakami, C.; Mliki, A.; Ghorbel, A.; Miyazaki, H. ε-Viniferin is more effective than its monomer resveratrol in improving the functions of vascular endothelial cells and the heart. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 954−960.
(20) Sahidin; Hakim, E. H.; Juliawaty, L. D.; Syah, Y. M.; Bin Din, L.; Ghisalberti, E. L.; Latip, J.; Said, I. M.; Achmad, S. A. Cytotoxic properties of oligostilbenoids from the tree barks of *Hopea dryobalanoides*. *Z. Naturforsch.* **2005**, *60*, 723−727.
(21) Huang, K. S.; Lin, M.; Cheng, G. F. Anti-inflammatory tetramers of resveratrol from the roots of *Vitis amurensis* and the conformations of the seven-membered ring in some oligostilbenes. *Phytochemistry* **2001**, *58*, 357−362.
(22) Muhtadi; Hakim, E. H.; Juliawaty, L. D.; Syah, Y. M.; Achmad, S. A.; Latip, J.; Ghisalberti, E. L. Cytotoxic resveratrol oligomers from the tree bark of *Dipterocarpus hasseltii*. *Fitoterapia* **2006**, *77*, 550−555.
(23) Rohaiza, S.; Yaacob, W. A.; Din, L. B.; Nazlina, I. Cytotoxic oligostilbenes from *Shorea hopeifolia*. *Afr. J. Pharm. Pharmacol.* **2011**, *5*, 1272−1277.
(24) Jin, Q.; Han, X. H.; Hong, S. S.; Lee, C.; Choe, S.; Lee, D.; Kim, Y.; Hong, J. T.; Lee, M. K.; Hwang, B. Y. Antioxidative oligostilbenes from *Caragana sinica*. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 973−976.
(25) Barjot, C.; Tournaire, M.; Castagnino, C.; Vigor, C.; Vercauteren, J.; Rossi, J. F. Evaluation of antitumor effects of two vine stalk oligomers of resveratrol on a panel of lymphoid and myeloid cell lines: Comparison with resveratrol. *Life Sci.* **2007**, *81*, 1565−1574.
(26) Huang, Y. L.; Tsai, W. J.; Shen, C. C.; Chen, C. C. Resveratrol derivates from roots of *Vitis thunbergii*. *J. Nat. Prod.* **2005**, *68*, 217−220.
(27) Nassra, M.; Krisa, S.; Papastamoulis, Y.; Kapche, G. D.; Bisson, J.; André, C.; Konsman, J. P.; Schmitter, J. M.; Mérillon, J. M.; Waffo-Téguo, P. Inhibitory activity of plant stilbenoids against nitric oxide production by lipopolysaccharide-activated microglia. *Planta Med.* **2013**, *79*, 966−970.
(28) Donnez, D.; Jeandet, P.; Clément, C.; Courot, E. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. *Trends Biotechnol.* **2009**, *27*, 706−713.
(29) Aaviksaar, A.; Haga, M.; Püssa, T.; Roasto, M.; Tsoupras, G. Purification of resveratrol from vine stems. *Proc. Est. Acad. Sci.* **2003**, *52*, 155−164.
(30) Püssa, T.; Floren, J.; Kuldkepp, P.; Raal, A. Survey of grapevine *Vitis vinifera* stem polyphenols by liquid chromatography-diode array detection-tandem mass spectrometry. *J. Agric. Food Chem.* **2006**, *54*, 7488−7494.
(31) Rayne, S.; Karacabey, E.; Mazza, G. Grape cane waste as a source of *trans*-resveratrol and *trans*-viniferin: High-value phytochemicals with medicinal and anti-phytopathogenic applications. *Ind. Crops Prod.* **2008**, *27*, 335−340.
(32) Karacabey, E.; Mazza, G. Optimization of solid-liquid extraction of resveratrol and other phenolic compounds from milled grape canes (*Vitis vinifera*). *J. Agric. Food Chem.* **2008**, *56*, 6318−6325.
(33) Anastasiadi, M.; Pratsinis, H.; Kletsas, D.; Skaltsounis, A. L.; Haroutounian, S. A. Grape stem extracts: Polyphenolic content and assessment of their *in vitro* antioxidant properties. *LWT−Food Sci. Technol.* **2012**, *48*, 316−322.
(34) Vergara, C.; von Baer, D.; Mardones, C.; Wilkens, A.; Wernekinck, K.; Damm, A.; Macke, S.; Gorena, T.; Winterhalter, P. Stilbene levels in grape cane of different cultivars in southern Chile: Determination by HPLC-DAD-MS/MS method. *J. Agric. Food Chem.* **2012**, *60*, 929−933.
(35) Pawlus, A. D.; Sahli, R.; Bisson, J.; Rivière, C.; Delaunay, J. C.; Richard, T.; Gomès, E.; Bordenave, L.; Waffo-Téguo, P.; Mérillon, J. M. Stilbenoid profiles of canes from *Vitis* and *Muscadinia* species. *J. Agric. Food Chem.* **2013**, *61*, 501−511.
(36) Vivier, M. A.; Pretorius, I. S. Genetically tailored grapevines for the wine industry. *Trends Biotechnol.* **2002**, *20*, 472−478.
(37) Vezzulli, S.; Troggio, M.; Coppola, G.; Jermakow, A.; Cartwright, D.; Zharkikh, A.; Stefanini, M.; Grando, M. S.; Viola, R.; Adam-Blondon, A. F.; Thomas, M.; This, P.; Velasco, R. A reference integrated map for cultivated grapevine (*Vitis vinifera* L.) from three crosses, based on 283 SSR and 501 SNP-based markers. *Theor. Appl. Genet.* **2008**, *117*, 499−511.
(38) Zga, N.; Papastamoulis, Y.; Toribio, A.; Richard, T.; Delaunay, J. C.; Jeandet, P.; Renault, J. H.; Monti, J. P.; Mérillon, J. M.; Waffo-Téguo, P. Preparative purification of antiamyloidogenic stilbenoids from *Vitis vinifera* (Chardonnay) stems by centrifugal partition chromatography. *J. Chromatogr., B* **2009**, *877*, 1000−1004.

(39) Martin, T. S.; Kikuzaki, H.; Hisamoto, M.; Nakatani, N. Constituents of *Amomum tsao-ko* and their radical scavenging and antioxidant activities. *J. Am. Oil Chem. Soc.* **2000**, *77*, 667−673.
(40) Bavaresco, L.; Fregoni, M.; Trevisan, M.; Mattivi, F.; Vrhovsek, U.; Falchetti, R. The occurrence of the stilbene piceatannol in grapes. *Vitis* **2002**, *41*, 133−136.
(41) Mattivi, F.; Reniero, F.; Korhammer, S. Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *J. Agri. Food Chem.* **1995**, *43*, 1820−1823.
(42) Reniero, F.; Rudolph, M.; Angioni, A.; Bernreuther, A.; Cabras, P.; Mattivi, F. Identification of two stilbenoids from *Vitis* roots. *Vitis* **1996**, *35*, 125−127.
(43) Acevedo De la Cruz, A.; Hilbert, G.; Rivière, C.; Mengin, V.; Ollat, N.; Bordenave, L.; Decroocq, S.; Delaunay, J. C.; Delrot, S.; Mérillon, J. M.; Monti, J. P.; Gomès, E.; Richard, T. Anthocyanin identification and composition of wild *Vitis* spp. accessions by using LC-MS and LC−NMR. *Anal. Chim. Acta* **2012**, *732*, 145−152.
(44) Mattivi, F.; Vrhovsek, U.; Malacarne, G.; Masuero, D.; Zulini, L.; Stefanini, M.; Moser, C.; Velasco, R.; Guella, G. Profiling of resveratrol oligomers, important stress metabolites, accumulating in the leaves of hybrid *Vitis vinifera* (Merzling × Teroldego) genotypes infected with *Plasmopara viticola*. *J. Agric. Food Chem.* **2011**, *59*, 5364−5375.
(45) Oshima, Y.; Kamijou, A.; Ohizumi, Y.; Niwa, M.; Ito, J.; Hisamichi, K.; Takeshita, M. Novel oligostilbenes from *Vitis coignetiae*. *Tetrahedron* **1995**, *51*, 11979−11986.
(46) Mattivi, F.; Reniero, F. Relationship between UV spectra and molecular structure of resveratrol oligomers. *Polyphenols Communications* **1996**, 125−126.
(47) Gatto, P.; Vrhovsek, U.; Muth, J.; Segala, C.; Romualdi, C.; Fontana, P.; Prueffer, D.; Stefanini, M.; Moser, C.; Mattivi, F.; Velasco, R. Ripening and genotype control stilbene accumulation in healthy grapes. *J. Agric. Food Chem.* **2008**, *56*, 11773−11785.