Phenolic Composition of Grape Stems

Jean-Marc Souquet,* Benoît Labarbe, Christine Le Guernevé, Véronique Cheynier, and Michel Moutounet

ISVV-INRA, Institut des Produits de la Vigne, Unité de Recherche Biopolymères et Arômes, 2 place Viala, 34060 Montpellier Cedex 2, France

Grape stems contain significant amounts of polyphenolic compounds, especially phenolic acids, flavonols, and flavanonols such as astilbin. The tannin content was characterized after the depolymerization reaction thiolysis. Tannins consisted of polymeric proanthocyanidins (up to 27 units) mainly consisting of (−)-epicatechin units along with smaller amounts of (+)-catechin, (−)-epicatechin gallate, and (−)-epigallocatechin. Flavanonols (astilbin) have been identified for the first time in stem and characterized by LC/MS and NMR. All phenolic compounds in grape stems were quantified by HPLC: quercetin 3-glucuronide was the most important, followed by catechin, caffeoyltartaric acid, and dihydroquercetin 3-rhamnoside (astilbin). Comparison was made of proanthocyanidin characteristics in different white and red grape varieties and also among parts of the cluster (skin, seed, and stem). Stem-condensed tannins were qualitatively intermediate between seed and skin but could not be differentiated between red and white varieties.

Keywords: *Grape; stems; proanthocyanidins; flavanols; flavonols; flavanonols; phenolic acids*

INTRODUCTION

Polyphenolic compounds have been well-studied and elucidated in different grape or vine parts such as skins (Ribéreau-Gayon, 1964, 1965; Hrazdina and Franzese, 1974; Singleton et al., 1978; Trousdale and Singleton, 1983; Cheynier and Rigaud, 1986; Roggero et al., 1988; Souquet et al., 1996), seeds (Ricardo Da Silva et al., 1991; Thorngate and Singleton, 1994; Prieur et al., 1994), and leaves and shoots (Bourzeix et al., 1986; Boukharta et al., 1988). This term includes phenolic acids, flavanols, flavonols, flavanonols, proanthocyanidins, and anthocyanins, which are responsible for many properties of fruit, juices, and fermented beverages such as color, browning, bitterness, and astringency (Haslam, 1980; Cheynier et al., 1990). All these native phenolic compounds are more or less reactive and, depending on their relative amounts, lead to various products formed by enzymatic or chemical reactions during food processing (Fulcrand et al., 1996, 1998). The new constituents formed may have different properties from those of the precursors. A better understanding of all the phenolic compounds present in grape materials during winemaking may help with elucidation of new compounds. It is well-known that the influence of destemming on the composition and quality of wines is very important. Wines made from a non-destemmed crop generally contain higher levels of phenolic compounds. The quantity of stems varies between 14 g/kg for Negrette and 70 g/kg for Tannat, and practice shows that up to 8% are still present after destemming.

The purpose of this work was to identify the major phenolic compounds of grape stems which may influence the quality of wine. Moreover, with regard to the stem, a better knowledge of its composition can make it possible to find industrial uses to add value to this waste.

* Corresponding author (tel, 33 4 99 61 25 24; fax, 33 4 99 61 26 83; e-mail, souquet@ensam.inra.fr).

The investigation was made by different techniques including reversed-phase chromatography that allows separation of polyphenolic constituents and depolymerization followed by HPLC analysis to determine the nature of the constitutive units of polymeric proanthocyanidins. Characterization of some compounds was performed by coupling HPLC with electrospray ionization mass spectrometry (LC/ESI-MS) and by 1D and 2D NMR spectroscopy. The results on tannins allow us to compare them qualitatively and quantitatively with skin and seed tannins and with those from stems of other varieties.

MATERIALS AND METHODS

Standards. (+)-Catechin and (−)-epicatechin were provided by Sigma (St. Louis, MO). Epicatechin 3-gallate and thiol derivatives were obtained and characterized as already described (Prieur et al., 1994; Souquet et al., 1996). Cyanidin, delphinidin, and quercetin 3-glucoside were purchased from Extrasynthese (Genay, France). *trans*-Caffeoyltartaric acid and *p*-coumaroyltartaric acid were isolated from must as described by Cheynier and Van Hulst (1988). Astilbin was isolated from the first fraction eluted from the TSK gel (see below), by semipreparative C18 HPLC.

Extraction and Isolation of Stem Polyphenols. Grape stems from *Vitis vinifera* vars. Merlot, Negrette, Pinot, Tannat, Chardonnay, and Clairette were harvested at commercial maturity from the INRA collection at Domaine du Chapitre (Villeneuve les Maguelone, France). Stems were ground under liquid nitrogen with a Dangoumau mill (Prolabo, Fontenay-sous-bois, France), and 1 g was extracted three times with 20 mL of acetone/water (60:40, v/v), filtered, taken to dryness by rotary evaporation, and dissolved in 2 mL of ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v). This extract was then chromatographed on TSK HW-50F (Tosohaas, Japan) (12 × 1 cm i.d., 1 mL/min). Phenolic acids, flavonols, flavanonols, and flavanol monomers were eluted with 50 mL of ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v), and the tannin fraction was eluted with 50 mL of acetone/water (60:40, v/v). Tannins were taken to dryness under vacuum, dissolved in methanol, and fractionated by chromatography on normal-phase HPLC

10.1021/jf991171u CCC: $19.00
Published on Web 03/18/2000

as described earlier (Rigaud et al., 1993; Prieur et al., 1994; Souquet et al., 1996).

Thiolysis. A 100-μL portion of the tannin fraction in MeOH was added to an equal volume of thiolytic reagent (5% toluene-α-thiol in 0.2 N HCl in MeOH). The sealed vial was kept for 2 min at 90 °C, and the mixture was then analyzed in triplicate by HPLC under the conditions described by Souquet et al. (1996).

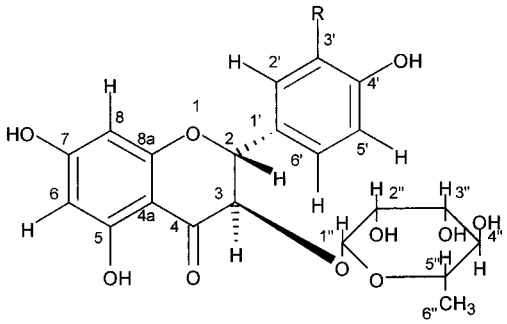
Analytical HPLC/DAD Analyses. These analyses were performed on a Waters 2690 HPLC system including an autosampler, a Waters 996 photodiode array detector, and Millenium 32 chromatography manager software (Milford, MA). The column was a reversed-phase Lichrospher 100-RP 18 (Merck, Darmstadt, Germany) (5 μm packing, 250 × 4 mm i.d.) protected with a guard column of the same material. Elution conditions were as follows: 1 mL/min flow rate at 30 °C; solvent A, water/formic acid (95/5, v/v); solvent B, acetonitrile/water/formic acid (80/15/5, v/v/v); isocratic for 7 min with 3% B, from 3 to 20% B in 15 min, from 20 to 40% B in 18 min, and from 40 to 50% B in 10 min, followed by washing and reequilibrating the column. The different fractions were analyzed in triplicate, and calibration curves were established from standards.

LC/ESI-MS. Negative-ion mode ESI-MS spectra were recorded on a Sciex API I plus (Sciex, Thornhill, Ontario, Canada) simple quadrupole mass spectrometer with a nominal mass range up to *m/z* 2400, equipped with an ion spray source. LC/ESI-MS was performed using the following conditions: −4 kV was applied to the electrospray needle and −60 V to the orifice under normal operating conditions and −150 V in collision-induced dissociation mode (CID). The mass was scanned from *m/z* 280 to 1180, in steps of 0.3 u and with a dwell time of 0.9 ms. Chromatographic separation was done on C18 Nucleosil (3 μm, 250 × 2 mm; Macherey-Nagel, Hoerdt, Germany). The chromatograph consisted of an Applied Biosystem 140B pump (Foster City, CA) and a 785A UV detector set at 280 nm. The elution conditions were as follows: flow rate 280 μL/min, temperature 30 °C. The solvent system used was a gradient of solvent A (HCOOH, 2% v/v in H_2O) and solvent B (CH_3CN, solvent A, 80/20, v/v), isocratic with 10% B for 4 min, linear gradient from 10 to 15% B in 11 min and from 15 to 40% B in 25 min, followed by washing and reconditioning the column. Split was done with 70 μL/min going through the mass detector and 210 μL/min into the diode array detector. Continuous flow injection (CFI-MS) was performed with a medical syringe infusion pump (Harvard Apparatus, model 22, South Natick, MA) with a constant flow rate of 10 μL/min and scanned from *m/z* 250 to 2400 with steps of 0.2 u and a dwell time of 1 ms.

NMR. Spectra were recorded on a Varian Unity Inova 500 (Palo Alto, CA) equipped with a 3-mm reverse probe, and chemical shifts (δ) were referenced to solvent signal (2.5 ppm for ^{1}H and 39.5 ppm for ^{13}C): ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C); ^{13}C NMR (125.7 MHz, DMSO-d_6, 25 °C).

RESULTS AND DISCUSSION

Low-pressure chromatography of a grape stem extract (*V. vinifera* var. Merlot) on Toyopearl TSK-HW 50F allowed us to separate phenolic acids, flavonols, and flavanol monomers, in fraction A, from condensed tannins in fraction B (Souquet et al., 1996). Fraction A was then studied by LC/DAD/ESI-MS, in the negative ion mode, that permitted identification and quantitation of major phenolic compounds. This analysis showed the presence of *trans*-caffeoyltartaric acid (caftaric acid) with a pseudomolecular ion $[M - H]^-$ at *m/z* 311 and a λ_{max} at 328 nm (298 sh) and *trans*-coumaroyltartaric acid (coutaric acid) with a signal at *m/z* 295 and a λ_{max} at 313 nm (298 sh). These two products are the main phenolic acids already identified in skins and pulp from grapes of *Vitis* varieties (Singleton et al., 1978). The major compound present in this fraction showed the UV



Dihydroquercetin 3-rhamnoside (Astilbin) : R= OH

Dihydrokaempferol 3-rhamnoside (Engeletin) : R= H

Figure 1. Structure of dihydroquercetin 3-rhamnoside (astilbin) and dihydrokaempferol 3-rhamnoside (engeletin).

spectum typical of a flavonol (252, 260 sh, 300 sh, 353) and in LC/MS a pseudomolecular ion $[M - H]^-$ at *m/z* 477. With a higher orifice voltage (150 V), ions are in CID conditions and a characteristic fragmentation $[(M - C_6H_8O_6) - H]^-$ at *m/z* 301 that corresponds to the cleavage of the glucuronic acid moiety from the glycosylated flavonol was observed. These results suggested that this compound is quercetin 3-glucuronide known to occur in grapes. LC/ESI-MS analysis also indicated the presence of quercetin 3-glucoside close to the previous component, detected at *m/z* 463 and yielding in the CID mode a fragment ion $[(M - C_6H_{10}O_5) - H]^-$ at *m/z* 301. Mass analysis indicated that this fraction contained other additional flavonols in lower quantities such as kaempferol 3-glucoside (molecular ion at *m/z* 447, fragment ion at *m/z* 285). Two compounds more polar than quercetin derivatives were detected with mass spectral characteristics of flavonols. The first one, yielding an ion at *m/z* 479 in the negative ion mode and an additional ion at *m/z* 317 in the CID mode, was identified as myricetin 3-glucoside. The second compound showing ions at *m/z* 493 and 317 corresponded to myricetin 3-glucuronide. All these flavonols have already been described in grape skins (Ribéreau-Gayon, 1964; Moskowitz and Hrazdina, 1981; Cheynier and Rigaud, 1986). Flavanol monomers and some dimers were also present in fraction A. Catechin was the major flavanol monomer in grape stem, and epicatechin was also present in trace amounts.

An additional group of compounds was also present in fraction A. LC/ESI-MS analysis showed a compound coeluting with quercetin 3-glucuronide, with a molecular ion $[M - H]^-$ at *m/z* 449 and a trifluoroacetic acid (TFA) adduct at *m/z* 563 in normal conditions. In more drastic conditions (CID mode) it yielded a fragment ion at *m/z* 303 which corresponds to the loss of a rhamnose moiety $[(M - C_6H_{10}O_4) - H]^-$. From these results and the UV characteristics (λ_{max} 290, 327 sh), this compound was tentatively identified as a flavanonol, astilbin (dihydroquercetin 3-rhamnoside) (Figure 1), reported earlier in the skin of white grape varieties, in white wine (Trousdale and Singleton, 1983), and in grape pomace of Chardonnay (Lu and Foo, 1999). This product was purified by semipreparative chromatography in larger amounts to allow NMR analyses and HPLC external calibration. ^{1}H NMR analysis showed signals for 15 protons including the methyl group of rhamnose, with a typical shift at 1.07 ppm. The anomeric proton of the sugar was at 4.04 ppm with a large singlet that indicated an α configuration. Protons of the C ring have

Table 1. 500-MHz ^{1}H and 125.7-MHz ^{13}C Chemical Shifts and Multiplicity (*J*, Hz) of Astilbin (Dihydroquercetin 3-Rhamnoside) in Acetone-d_6 (Referenced to the Solvent Signal)

	δ ^{1}H (ppm)	δ ^{13}C (ppm)
	Aglycone	
2	5.16 (d, 9.85)	81.08
3	4.55 (d, 9.85)	75.42
4		194.20
4a		100.28
5		163.20
6	5.76 (d, 2)	95.86
7		170.14
8	5.78 (d, 2)	96.70
8a		162.14
1′		126.90
2′	6.88 (br s)	114.67
3′		145.50
4′		144.84
5′	6.72 (br s)	115.3
6′	6.72 (br s)	118.62
	Rhamnose	
1″	4.04 (s)	99.70
2″	3.41 (m)	69.93
3″	3.35 (m)	70.27
4″	3.12 (m)	71.48
5″	3.91 (m)	68.68
6″	1.07 (d, 5.85)	17.5

chemical shifts at 4.55 and 5.16 ppm with a large coupling constant ($J_{2,3}$ = 9.85 Hz), and the C-2 chemical shift was above 80 ppm, which is characteristic of a 2,3-*trans* configuration (Foo and Karchesy, 1989; Lu and Foo, 1999). The HMBC 2D NMR sequence allowed us to distinguish a correlation between the proton C-3 and the carbon of the anomeric position, indicating that the linkage between the rhamnose and dihydroquercetin moieties was C-3−O−C-1″. Other ^{1}H and ^{13}C NMR data (Table 1) were in agreement with the literature (Trousdale and Singleton, 1983; Lu and Foo, 1999). All these analyses proved, for the first time, the presence of astilbin in grape stems. Finally, LC/ESI-MS analysis of the polyphenolic stem fraction A showed the presence of another compound with a mass signal at $[M - H]^-$ *m*/*z* 433, less polar than astilbin and in very low amount, that could be engeletin (dihydrokaempferol 3-rhamnoside) (Figure 1) according to the results on the skin and grape pomace (Trousdale and Singleton, 1983; Lu and Foo, 1999).

Stem-condensed tannins (fraction B) were characterized by two depolymerization reactions performed on the total extract and on eight fractions obtained by normal-phase chromatography (Rigaud et al., 1993; Prieur et al., 1994). Finally, the total extract was analyzed by LC/ESI-MS to detect oligomers and polymers. HPLC analysis after depolymerization in strong acidic medium (Bate−Smith reaction) (Porter et al., 1986) showed the presence of cyanidin and delphinidin and their butyl ether derivatives which also absorb in the visible. These results indicate that stem-condensed tannins consisted of both (epi)gallocatechin units and (epi)catechin units. In the presence of weaker acid and nucleophilic reagents such as toluene-α-thiol, we detected terminal units of polymers in the free form and extension units as benzyl thioether derivatives by HPLC. LC/ESI-MS of the units released during this degradation allowed detection in the negative ion mode of (epi)catechin (*m*/*z* 289) and (−)-epicatechin gallate (*m*/*z* 441) as terminal units and the corresponding derivatives as extension units. The (−)-epigallocatechin benzyl thioether (*m*/*z* 427), also detected, confirmed the presence of prodelphinidins in grape stems, as already found in grape skins (Souquet et al., 1996). The total extract was then fractionated by normal-phase chromatography (Prieur et al., 1994; Souquet et al., 1996).

The thiolysis reaction in the presence of toluene-α-thiol was carried out on the eight fractions thus obtained. HPLC analysis of the various degradation products formed gave us information on the structure of polymers, including the mean degree of polymerization (mDP) and the proportion of the quantifiable monomeric flavanols from which they are constituted. mDP is estimated by calculating the ratio between the total number of released units and the number of terminal units. Quantitative data obtained on the total extract and on each fraction are presented in Table 2. All fractions contained the different flavanol monomers found in the total extract. (−)-Epicatechin was the major unit (~60%) of grape stem tannins although (+)-catechin predominated as free monomer in the first fraction. (−)-Epicatechin represented 73% of the extension units and only 16.5% of the terminal units, in which (+)-catechin reached 78%. The percentage of (−)-epigallocatechin units (2.44%) was low. This trihydroxylated unit was present in all fractions but never detected in the terminal position. mDP calculated from the results of thiolysis increased up to 27.6 in fraction V (9.17 in the total extract) and decreased in the subsequent ones. This result has already been observed on seed tannins, which did not contain trihydroxylated units, and might be due to the lower solubility of these fractions (Labarbe et al., 1999). The proportion of gallates was independent from mDP and close to 15% in all fractions. On the basis of peak areas obtained from normal-phase HPLC at 280 nm of fraction B, the

Table 2. Composition and Characteristics of Grape Stem Proanthocyanidin Fractions and Total Extract Determined by HPLC following Thiolysis Degradation[a]

		fractions								
		I	II	III	IV	V	VI	VII	VIII	total
terminal units	Cat	15.44	10.85	6.33	7.03	2.80	3.75	4.27	14.22	8.55
	Ec	2.10	0.90	2.41	0.87	0.28	0.31	0.50	2.18	1.82
	EcG	2.85	1.87	0.93	1.68	0.53	1.04	0.56	3.66	0.60
extension units	EGC	5.26	4.52	5.75	2.19	4.90	4.69	10.08	2.73	2.44
	Cat	7.78	8.65	7.35	6.88	6.21	6.11	6.38	7.13	5.80
	Ec	56.77	60.59	64.55	65.40	69.27	66.89	63.79	57.10	65.84
	EcG	9.76	12.59	12.65	15.91	15.98	17.18	14.40	12.94	14.95
galloylation (%)		12.62	14.46	13.58	17.59	16.52	18.23	14.96	16.60	15.55
mDP		4.90	7.33	10.32	10.41	27.63	19.56	18.71	4.97	9.17
trihydroxylated units (%)		5.26	4.52	5.75	2.19	4.90	4.69	10.08	2.73	2.44

[a] Cat, Ec, EcG, EGC: catechin, epicatechin, epicatechin gallate, and epigallocatechin units. All amounts represent relative concentrations (in mol).

Table 3. Comparison of the Proanthocyanidins Characteristic of Different Red (R) and White (W) Varieties

	mDP	%Cat	%Ec	%EcG	%EGC	mg tannins/ kg crop
Merlot (R)	9.2	14.4	67.7	15.5	2.9	221.3
Negrette (R)	10.2	11.7	61.7	21.1	5.4	388.1
Pinot (R)	8.2	15.3	65.1	18.1	1.5	228.8
Tannat (R)	8.7	13.0	65.5	19.8	1.7	302.0
Chardonnay (W)	9.1	14.0	69.4	15.7	0.8	27.9
Clairette (W)	7.7	17.3	68.4	13.4	0.9	217.8

Table 4. Comparison with the Different Parts of the Grape Cluster in Merlot[a]

	mDP	%gallate	%EGC	%Ec	%Cat	mg tannins/ kg crop
skins	28.18	5.16	22.69	67.20	4.96	1605.0
seeds	10.35	32.45		54.36	13.19	2323.5
stems	9.17	15.55	2.44	67.66	14.35	221.3

[a] Tannin sample weight was obtained by summing the weights of all units released by the thiolysis reaction.

oligomers (DPm < 10) account for 18.62%, polymers 81.30%, and monomers less than 1%. The amount of tannins in the stem of Merlot represented about 5 g/kg of fresh material and 220 mg/kg of crop.

All of the characteristics calculated from thiolysis were determined in five other *V. vinifera* varieties, namely three red grapes: Negrette, Pinot Noir, and Tannat, and two white cultivars: Chardonnay and Clairette. Results (Table 3) showed that the presence and proportion of trihydroxylated units cannot be used as a factor to differentiate white and red grapes. All of the values were close to those found in Merlot except for tannin amounts (per kg of crop). Chromatographic analysis of the first fraction eluted from Toyopearl showed the presence of astilbin in all white and red varieties.

Investigations were carried out in LC/ESI-MS, to determine the presence or absence of mixed tannins (containing both dihydroxylated and trihydroxylated units). CFI is generally used in the case of large molecules (beyond DP5) which cannot be properly separated in HPLC, but the presence of multicharged species of all the possible combinations of units renders interpretation difficult. Nevertheless, the analysis in CFI-MS made it possible to detect signals at *m*/*z* 577 and 593 corresponding to dimers, consisting of two (epi)catechin units and one (epi)catechin unit with one (epi)gallocatechin unit, respectively, as well as their monogalloylated derivatives (*m*/*z* 729 and 745). Signals at *m*/*z* 865, 881, and 1033 can be similarly interpreted as trimers with three (epi)catechin units, two (epi)-catechin units plus one (epi)gallocatechin unit, and one (epi)catechin unit plus one epicatechin gallate unit and one (epi)gallocatechin unit. Their trifluoroacetate adducts were also well-detected. Unlike what was observed in wine tannins (Fulcrand et al., 1999), no pure (epi)gallocatechin oligomers were detected in grape stems, probably due to the lower proportion of epigallocatechin units. This suggests that constitutive units are incorporated at random along the tannin chain, as postulated earlier (Haslam, 1980).

Finally, we can compare tannin composition in different parts of the Merlot grape that are of great interest in wine-making (skin, seed, and stem) (Table 4). Stem tannins may have less importance due to the low proportion of this tissue in the cluster; nevertheless their qualitative composition is intermediate between those of skin and seed. Stem tannins contain prodelphinidins, but their level is lower than in the skin (31%). mDP and percentage of galloylation are close to values found in the seeds which are characterized by the absence of prodelphinidins. Skin tannins differ from the other parts by a higher mDP (33) and a lower amount of gallates (~4%). (−)-Epicatechin is the unit most represented in the cluster. Stems may be a source of phenolic acids (caftaric and coutaric acids) in lower quantities than pulp and skin and also of flavonols, especially quercetin 3-glucuronide. Astilbin is the main flavanonol present in the stem of white and red varieties although its presence was only noticed in white skin and pomace (Trousdale and Singleton, 1983; Lu and Foo, 1999). Finally, the concentration of the different phenolic compounds present in the stems of Merlot is shown in Table 5.

Table 5. Concentration of the Different Phenolic Compounds Present in the Stems of Merlot

compounds	mg/kg stem
caftaric	40
coutaric	4.5
quercetin 3-glucuronide	200
quercetin 3-glucoside	18
kaempferol 3-glucoside	traces
myricetin 3-glucoside	traces
myricetin 3-glucuronide	traces
catechin	60
epicatechin	traces
astilbin	35
engeletin	traces

Extraction and diffusion of the phenolic compounds from the different parts of the cluster, during wine-making, should be the next step of our investigation.

LITERATURE CITED

Boukharta, M.; Girardin, M.; Metche, M. Procyanidines galloylées du sarment de vigne (*Vitis vinifera*). *J. Chromatogr.* **1988**, *45*, 406−409.

Bourzeix, M.; Weyland, D.; Heredia, N. Etudes des catéchines et des procyanidols de la grappe de raisin, du vin et d'autres dérivés de la vigne. *Bull. OIV* **1986**, *669*−*670*, 1171−1254.

Cheynier, V.; Rigaud, J. HPLC separation and characterization of flavonols in the skins of *Vitis vinifera* var. Cinsault. *Am. J. Enol. Vitic.* **1986**, *37*, 248−252.

Cheynier, V.; Van Hulst, M. Oxidation of trans-caftaric acid and 2-S-glutathionylcaftaric acid in model solutions. *J. Agric. Food Chem.* **1988**, *36*, 10−15.

Cheynier, V.; Rigaud, J.; Souquet, J.-M.; Duprat, F.; Moutounet, M. Must browning in relation to the behaviour of phenolic compounds during oxidation. *Am. J. Enol. Vitic.* **1990**, *41*, 346−349.

Foo, L. Y.; Karchesy, J. J. Procyanidin dimer and trimer from Douglas fir inner bark. *Phytochemistry.* **1989**, *28*, 1743−1747.

Fulcrand, H.; Cameira Dos Santos, P.-J.; Sarni Manchado, P.; Cheynier, V.; Fabre Bonvin, J. Structure of new anthocyanin-derived wine pigments. *J. Chem. Soc., Perkin Trans.* **1996**, *1*, 735−739.

Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* **1998**, *47*, 1401−1407.

Fulcrand, H.; Remy, S.; Souquet, J.-M.; Cheynier, V.; Moutounet, M. Study of wine tannin oligomers by on line liquid chromatography electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **1999**, *47*, 1023−1028.

Haslam, E. In vino veritas: Oligomeric procyanidins and the aging of red wines. *Phytochemistry* **1980**, *19*, 2577−2582.

Hrazdina, G.; Franzese, A. J. Structure and properties of the acylated anthocyanins from *Vitis* species. *Phytochemistry* **1974**, *13*, 225−229.

Labarbe, B.; Cheynier, V.; Brossaud, F.; Souquet, J.-M.; Moutounet, M. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem*. **1999**, *47*, 2719−2723.

Lu, Y.; Foo, L. Y. The polyphenol constituents of grape pomace. *Food Chem*. **1999**, *65*, 1−8.

Moskowitz, A. H.; Hrazdina, G. Vacuolar contents of fruit subepidermal cells from *Vitis* species. *Plant Physiol*. **1981**, *68*, 686−692.

Porter, L. J.; Hrstich, L. N.; Chan, B. G. the conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25*, 223−230.

Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.

Ribéreau-Gayon, P. Les composés phénoliques du raisin et du vin II. Les flavonosides et les anthocyanosides. *Ann. Physiol. Vég*. **1964**, *6*, 211−242.

Ribéreau-Gayon, P. Identification d′esters des acides cinnamiques et de l′acide tartrique dans les limbes et les baies de *Vitis vinifera. C. R. Acad. Sci., Paris* **1965,** *260*, 341−343.

Ricardo Da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidin dimers and trimers from grape seeds. *Phytochemistry* **1991**, *30*, 1259−1264.

Rigaud, J.; Escribano-Bailon, M. T.; Prieur, C.; Souquet, J. M.; Cheynier, V. Normal phase high-performance liquid chromatographic separation of procyanidins from cacao beans and grape seeds. *J. Chromatogr.* **1993**, *654*, 255−260.

Roggero, J. P.; Larice, J. L.; Rocheville Divorne, C.; Archier. P.; Coen, S. Composition anthocyanique des cépages. I: Essai de classification par analyse en composantes principales et par analyse factorielle discriminante. *Rev. Fr. d′Œnol*. **1988**, *112*, 41−48.

Singleton, V. L.; Timberlake, C. F.; Lea, A. G. H. The phenolic cinnamates of white grapes and wine. *J. Sci. Food Agric.* **1978**, *29*, 403−410.

Souquet, J.-M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509−512.

Thorngate, J. H.; Singleton, V. L. Localization of procyanidins in grape seeds. *Am. J. Enol. Vitic.* **1994**, *45*, 259−262.

Trousdale, E. K.; Singleton, V. L. Astilbin and engeletin in grapes and wine. *Phytochemistry* **1983**, *22*, 619−620.

Received for review October 28, 1999. Revised manuscript received January 25, 2000. Accepted January 26, 2000.

JF991171U