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Extraction, Detection, and Quantification of Flavano-Ellagitannins and Ethylvescalagin in a Bordeaux Red Wine Aged in Oak Barrels

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An extraction procedure and an analytical method have been developed to detect and quantify for the first time a series of ellagitannin derivatives formed in wine during aging in oak barrels. The method involves a preliminary purification step on XAD7 HP resin followed by a second purification step on TSK 40 HW gel. The resulting extract is analyzed for compound identification and quantitative determination by high-performance liquid chromatography−electrospray ionization−mass spectrometry in single ion recording mode. Reference compounds, which are accessible through hemisynthesis from the oak *C*-glycosidic ellagitannin vescalagin, were used to build calibration curves, and chlorogenic acid was selected as an internal standard. This method enabled us to estimate the content of four flavano-ellagitannins and that of another newly identified wine polyphenol, β-1-*O*-ethylvescalagin, in a Bordeaux red wine aged for 18 months in oak barrels. All five ellagitannin derivatives are derived from the nucleophilic substitution reaction of vescalagin with the grape flavan-3-ols catechin and epicatechin or ethanol.

KEYWORDS: Wine; polyphenols; tannins; ellagitannins; catechin; epicatechin; acutissimin; epiacutissimin; ethylvescalagin

INTRODUCTION

The tannins contained in red wines play a major role for their taste and color properties (*1*−*4*). The primary source of wine tannin is of course grape berry seeds and skins (*5*, *6*) that contain proanthocyanidins or condensed tannins in their vacuoles and cell walls (*7*, *8*). These molecules are polymers of flavanols such as (+)-catechin (**1a**) or (−)-epicatechin (**1b**; **Figure 1**). Once in wine, these tannins may react with anthocyanins to form new pigments that have different colors and chemical properties than the native anthocyanins (*9*−*15*). They are also important for the taste attributes of wine such as bitterness and astringency because of their property to interact strongly with protein in general and salivary proteins in particular (*16*−*19*).

Another source of tannins for wine is the oak heartwood used in barrel aging (*20*, *21*). These tannins are ellagitannins or hydrolyzable tannins that are derived from galloyl units esterified to a sugar core (*22*, *23*). The monomeric (−)-vescalagin (**2a**) and (−)-castalagin (**2b**) found in oak species used to make barrels are depicted in **Figure 1** (*24*). Dimers and lyxose/xylose derivatives have also been identified in oak woods (*25*−*27*). The level of their occurrence in the cooperage wood depends in part on the species used and also on the type and length of drying and toasting (*28*, *29*). Their quantification both in wood and in wine is based on high-performance liquid chromatography (HPLC) methods, which quantify either each known

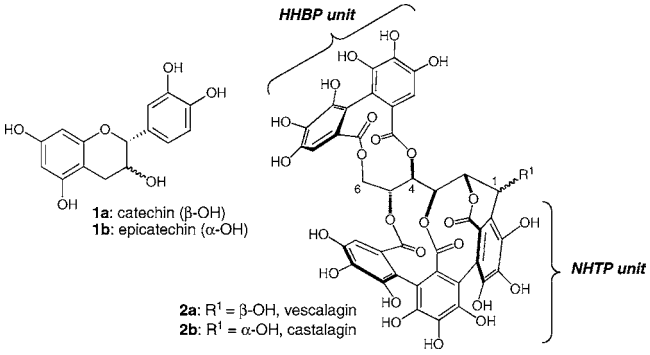

Figure 1. Structures of grape flavan-3-ols **1a/b** and oak *C*-glycosidic ellagitannins **2a/b**.

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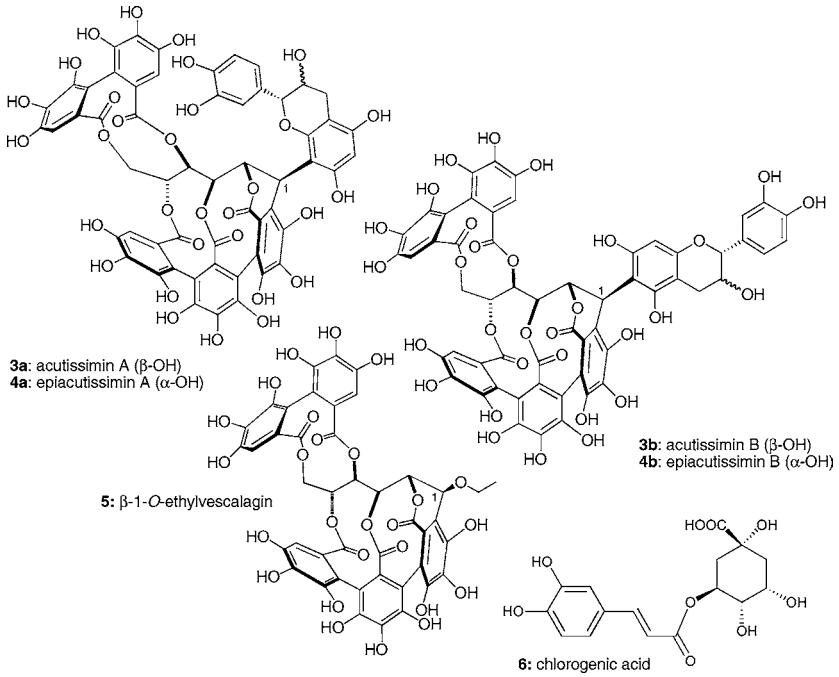

Figure 2. Structures of the acutissimins **3** and **4**, ethylvescalagin **5**, and chlorogenic acid **6** (internal standard).

monomers, dimers, and derivatives or the ellagic acid released during acid hydrolysis (*30*). The results found by these two methods are generally different because higher oligomeric forms and other possible derivatives are not taken into account when integrating only the known oak-derived native entities. These molecules are gradually released in wine during oak barrel aging. However, only small amounts have been found in wine implying that these molecules undergo chemical transformations via, e.g., oxidation or reactions with other wine species. Among the numerous possible reactions, we decided to focus on some acid-catalyzed reactions that have not been previously investigated for the ellagitannins in enology. In particular, the reaction of oak ellagitannins with grape flavanols was the subject of our recent studies in this field (*31*, *32*). Four flavano-ellagitannins, including two novel molecules, were hemisynthesized. These molecules are acutissimin A (**3a**) and B (**3b**) and epiacutissimin A (**4a**) and B (**4b**), which result from a nucleophilic substitution reaction between (−)-vescalagin (**2a**) and (+)-catechin (**1a**) or (−)-epicatechin (**1b**). The same chemical reaction leads to the formation of the *β*-1-*O*-ethylated vescalagin derivative **5** from **2a** and ethanol. The goal of the work described herein was to develop a method to detect and to quantify these ellagitannin derivatives in an oak-aged red wine sample.

MATERIALS AND METHODS

General. (−)-Vescalagin (**2a**) and (−)-castalagin (**2b**) were extracted from the heartwood of *Quercus robur* and purified as previously described (*33*, *34*). This heartwood material does not contain any UV-detectable amounts of the flavano-ellagitannins of interest. Acutissimin A (**3a**) and B (**3b**), epiacutissimin A (**4a**) and B (**4b**), and *β*-1-*O*-ethylvescalagin (**5**) were obtained by hemisynthesis as previously described (*31*, *32*). Chlorogenic acid (**6**) was used as an internal standard and was purchased from Aldrich. XAD7 HP resin was purchased from Supelco, and the TSK gel HW 40F was purchased from VWR International. Methanol was of HPLC quality, and Milli-Q (Millipore) water was used for HPLC analyses. HPLC-MS analyses were carried out on a Hewlett-Packard 1100 series system coupled to a Micromass Platform II for electrospray ionization mass spectrometry (ESI-MS) analysis.

HPLC-ESI-MS Conditions. The mobile phase was composed of solvent A [H_2O−HCOOH (990:10, v/v)] and solvent B [MeOH−HCOOH (990:10)]. For the identification of the compounds of interest, a gradient elution (0−20 min, 0−30% solvent B; 20−35 min, 30−100% solvent B; and 35−40 min, 100% solvent B) was applied at a flow rate of 1 mL min^{-1} with detection at 280 nm. For the quantitative determination of the same compounds, the gradient elution was modified (0−35 min, 0−25% solvent B; 35−45 min, 25−100% solvent B; and 45−50 min, 100% solvent B) because of the coelution of the internal standard with one of the compounds of interest when using the previous gradient elution. The ESI-MS analyses were performed in negative ion mode with the following optimized parameters: source temperature, 120 °C; nebulizer gas flow, 9 L/h; desolvation gas flow, 220 L/h; and capillary voltage, 3.5 kV. For the identification of the compounds in the wine sample (vide infra), the cone voltage was set at −90 eV. For their quantitative determination, as well as the ESI-MS analysis of their corresponding reference compounds, the cone voltage was set at −60 eV.

Red Wine Sample Preparation. A sample (100 mL) of a red wine (Bordeaux 2001, aged for 18 months in oak barrels) was evaporated under reduced pressure, and the resulting dark red viscous residue (3.83 g) was dissolved in water (20 mL). This solution was loaded on a column (150 mm × 40 mm) that has been packed with Amberlite XAD7 HP resin, previously swelled in methanol overnight, and equilibrated with H_2O−HCOOH (996:4, 250 mL). The same acidic aqueous solvent (250 mL) was first used to wash out tartaric acid and sugars (2.82 g), and an acidic hydromethanolic solvent [H_2O−MeOH−HCOOH (796/200/4, 250 mL)] was then used to elute the ellagitannin fraction. This fraction (250 mL) was evaporated under reduced pressure to furnish a dark pinkish residue (372 mg), which was dissolved in water (10 mL) and further purified by TSK HW 40F gel chromatography using a 120 mm × 18 mm column. The acidic aqueous solvent (50 mL) and a different acidic hydromethanolic solvent [H_2O−MeOH−HCOOH (296/700/4), 50 mL] were first used successively to separate further the sample mixture. These two solvents yielded a colorless fraction (154 mg) and a light red fraction (155 mg), respectively. The ellagitannins and flavano-ellegitannins of interest were then eluted using H_2O−acetone−HCOOH (296/700/4, 50 mL). This fraction was evaporated under reduced pressure to furnish a reddish light brown residue (52 mg), which was dissolved in water (400 *μ*L) for HPLC-ESI-MS analysis (50 *μ*L injection). For the quantification of **2a**/**b**, **3a**/**b**, **4a**/**b**, and **5**, sample preparation was realized on two different aliquots from the same wine and each prepared sample was injected in HPLC-ESI-MS in duplicate for deviation determination. Before injection, the reddish light brown residue was dissolved in water (400 *μ*L) containing

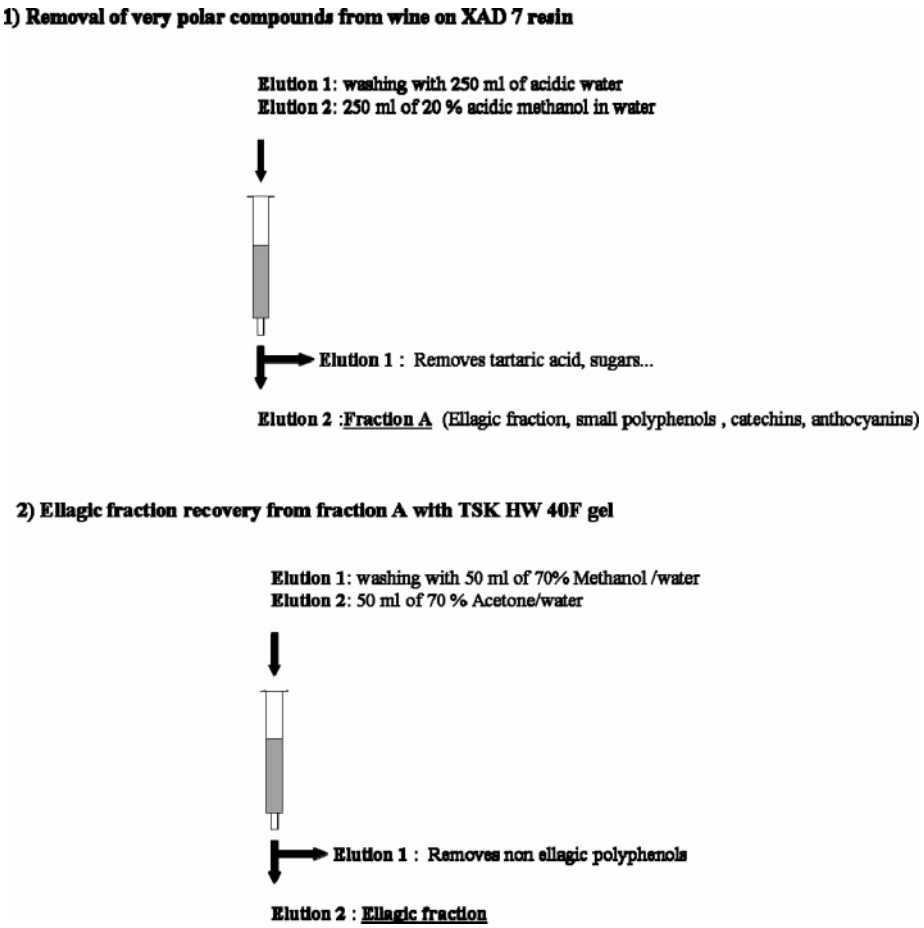

Figure 3. Extraction and purification procedures followed for the separation of the ellagitannin/flavano-ellagitannin fraction from red wine.

20 mg/L of chlorogenic acid (**6**) used as the internal standard and then an aliquot (50 μL) was injected.

Calibration Curves and Quantification in Red Wine by HPLC-ESI-MS. The calibration curves were established from injection of aqueous solutions of the pure vescalagin (**2a**), castalagin (**2b**), and hemisynthetic acutissimins **3a**/**b**, epiacutissimins **4a**/**b**, and β-1-*O*-ethylvescalagin (**5**) with increasing concentrations ranging from 25 to 200 mg/L using 20 mg/L of chlorogenic acid (**6**) as the internal standard. This concentration range turned out to be appropriate for calibrating the quantification of the compounds of interest in the red wine sample after its partial purification, during which the sample volume is decreased 250-fold (i.e., from 100 to 0.4 mL, vide supra). The response coefficients of each compound submitted through the HPLC-ESI-MS protocol were then determined by comparing their ESI-MS response in single ion recording (SIR) mode to the same signal of the internal standard at a fixed concentration. Calibrations curves were then obtained to have this response factor based on the following equation:

$$K_{X/IS} = \frac{[X]A_{IS}}{[IS]A_X}$$

where [X] is the concentration of product X in mg/L, [IS] is the concentration of the internal standard in mg/L, A_X is the peak area of compound X in SIR mode, and A_{IS} is the peak area of the internal standard in SIR mode.

The peaks corresponding to the substances to quantify are then integrated, and the concentrations in the wine are calculated as follows:

$$[X]_W = [IS] \times K_{X/IS} \times \frac{A_X}{A_{IS}} \times \frac{0.4}{V_W}$$

where $[X]_W$ is the concentration of compound X in mg/L in wine, [IS] is the concentration of the internal standard in mg/L in the injected sample (20 mg/L), A_X is the peak area of compound X in SIR mode, A_{IS} is the peak area of the internal standard in SIR mode, and V_W is the volume of wine (mL) used before the extraction procedures.

RESULTS AND DISCUSSION

In order to separate and concentrate the ellagitannins and flavano-ellagitannins of interest from red wine, we set up a specific sample preparation (**Figure 3**). This procedure first involves an Amberlite XAD7 HP resin purification step to remove the more polar compounds (*35*−*37*). The red wine is evaporated under reduced pressure, and the resulting dark red viscous residue is dissolved in water for elution through the XAD7 HP resin-filled column. An elution with some acidified water is initially used to remove highly polar compounds such as tartaric acid and glycerol. A fraction containing the ellagitannins and the flavano-ellagitannins together with almost all of the other phenolic compounds, such as phenolic acids, flavanols, oligomeric proanthocyanidins, and anthocyanins, is then obtained by eluting with an acidified aqueous methanol solvent mixture (see Materials and Methods).

The second step of the procedure involves a purification using a Toyopearl TSK HW40F gel to separate all tannins from other phenolic compounds (*6*). Nonellagic polyphenols were thus removed by eluting with an acidified aqueous methanol solvent mixture, and the desired ellagic polyphenols were then eluted with an acidified aqueous acetone solvent mixture (see Materials and Methods). The resulting fraction was dissolved in water and analyzed by HPLC-ESI-MS analysis. The complexity of the resulting UV-detected HPLC profile precluded any direct identification of the compounds of interest. We thus relied on the SIR MS detection mode to get around this difficulty, and unambiguous identification was based on the comparison of

Table 1. HPLC Retention Times, Mass Fragmentation Patterns, and Response Coefficients of the Reference Compounds **2**–**5** and the Internal Standard **6**

compounds	retention time (min)	*m*/*z* (negative ion scan mode)	response coefficients (K_X/IS)
vescalagin (**2a**)	16.1	935, 915, 613, 301	1.1
castalagin (**2b**)	18.2	935, 915, 613, 301	0.9
acutissimin A (**3a**)	25.7	1205, 915, 613, 602, 301	2.6
acutissimin B (**3b**)	32.3	1205, 915, 613, 602, 301	2.3
epiacutissimin A (**4a**)	36.8	1205, 1053, 915, 613, 602, 301	2.6
epiacutissimin B (**4b**)	22.1	1205, 1053, 915, 613, 602, 301	2.4
ethylvescalagin (**5**)	24.8	961, 915, 480, 301	1.2
chlorogenic acid (**6**)	35.1	353	1

HPLC retention times and MS fragmentation patterns with those of our five hemisynthetic reference compounds **3a**/**b**, **4a**/**b**, and **5** (**Table 1**).

This analysis of the 2001 Bordeaux red wine sample revealed the presence of all five compounds, as well as that of oak native *C*-glycosidic ellagitannins, such as vescalagin (**2a**) and castalagin (**2b**). Calibration curves were thus also established for the quantification of these two compounds. Hence, it allowed us to determine the amount of vescalagin **2a** relative to that of its epimer **2b** and to those of **3a**/**b**, **4a**/**b**, and **5**, which are all derived from **2a** and not from **2b** (*32*). The detection of these five new wine components is shown on the negative mode ESI (ESI^-) ion trace mass chromatograms displayed in **Figure 4**. An early eluting compound (i.e., **7**) was also detected together with the ethylvescalagin **5**, whose molecular ion $[M - H]^-$ peak is observed at *m*/*z* 901 (**Figure 4b**). This compound could not be identified here, but it is probably not a *C*-glycosidic ellagic structure, for its mass fragmentation pattern does not show any of the characteristic fragments observed in the ESI^- mass spectra of all of the other compounds analyzed in this study. These characteristic fragments are observed at *m*/*z* 915 (loss of the substitutent at C-1 of the vescalagin-derived core structure), 613 (loss of the 4,6-hexahydroxybiphenoyl unit from the latter fragment), and 301 (ellagic acid) (see **Figures 1** and **2**). The results of the quantitative determination of the five newly identified wine compounds, as well as **2a**/**b**, are given in **Table 2**. Chlorogenic acid (**6**) was chosen as the internal standard because of its absence in wine made from *Vitis vinifera* berries (*38*−*41*) and its relatively short retention time. It has previously been used for quantitative analyses of ellagitannins (*42*).

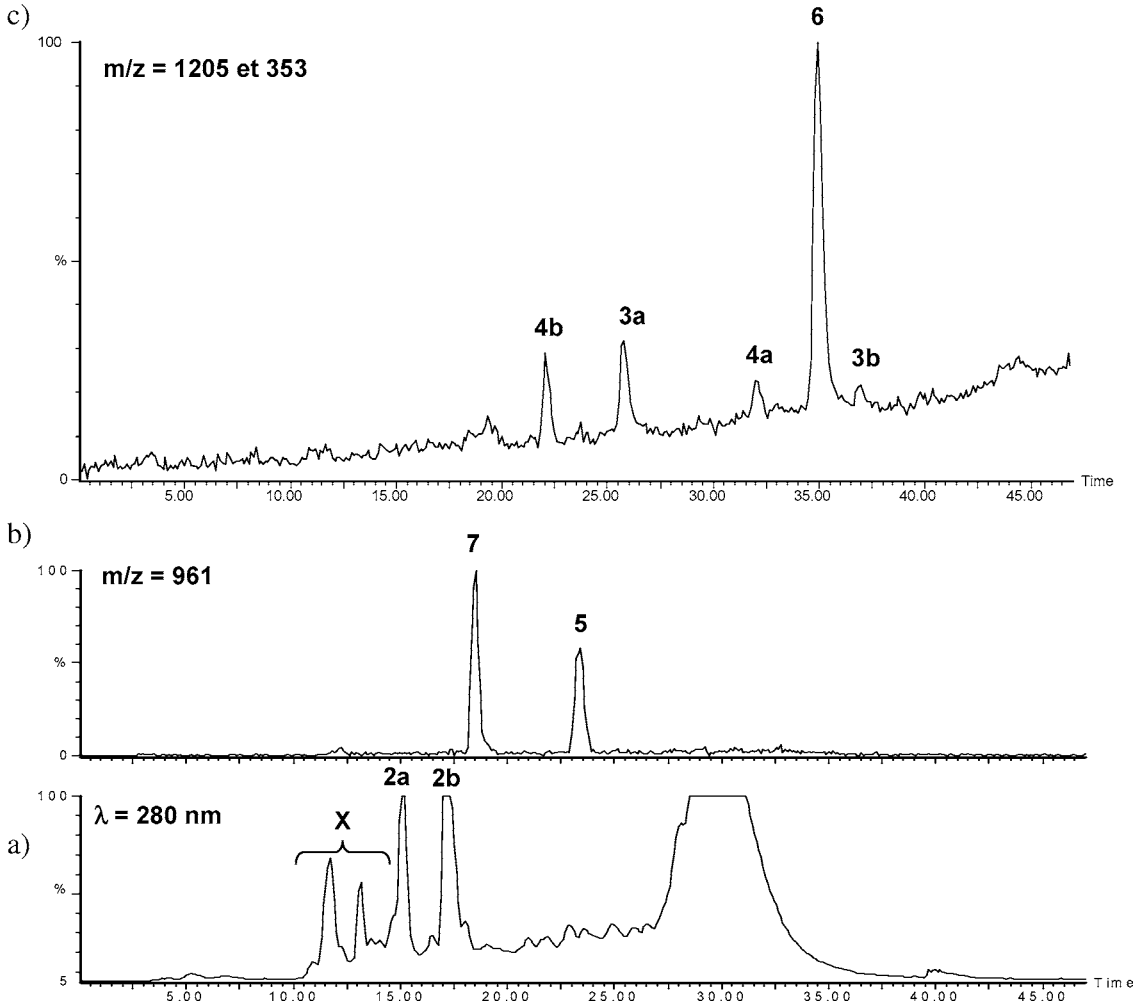

Figure 4. HPLC/MS profiles of the ellagitannin/flavano-ellagitannin fraction separated from an aged-in-oak red wine sample. (**a**) UV detection at 280 nm; X, roburins A-C + grandinin; **2a**, vescalagin; and **2b**, castalagin. (**b**) Negative-mode ESI SIR trace chromatogram (*m*/*z* = 901); **5**, β-1-*O*-ethylvescalagin; and **7**, unknown compound (see text). (**c**) Negative-mode ESI SIR trace chromatogram (*m*/*z* = 1205 and 353); **3a**/**b**, acutissimins A/B; **4a**/**b**, epiacutissimins A/B; and **6**, chlorogenic acid (internal standard).

Table 2. Quantitative Estimation of a Selection of Ellagitannins and Flavano-ellagitannins in an 18 Month Oak-Aged 2001 Bordeaux Red Wine

compd	vescalagin (**2a**)	castalagin (**2b**)	ethylvescalagin (**5**)	acutissimin A (**3a**)	acutissimin B (**3b**)	epiacutissimin A (**4a**)	epiacutissimin B (**4b**)
concn (mg/L)	2.20 ± 0.15	8.10 ± 0.31	0.85 ± 0.05	0.40 ± 0.03	0.28 ± 0.02	0.30 ± 0.02	0.35 ± 0.02

This first estimation of the amounts of each of these five ellagitannin derivatives in aged-in-oak red wine indicates that they individually constitute only minor components in wine, with a total amount reaching only about 2 mg/L (**Table 2**). However, of most importance at this stage of our investigation is the fact that their occurrence in wine unambiguously proves that oak *C*-glycosidic ellagitannins do participate in bond-forming events with grape-derived nucleophilic species during aging in oak barrels. Indeed, the acutissimins **3a**/**b** and **4a**/**b** and the ethylvescalgin **5** investigated herein represent only just a few examples of a large repertoire of compounds that can conceivably be derived from vescalagin (**2a**), as well as from other oak *C*-glycosidic ellagitannins presenting the same structural features, such as the dimeric roburin A (vide infra) (*25*, *26*). Among the other wine nucleophiles susceptible to react with such ellagitannins, one can first cite the flavan-3-ol-derived proanthocyanidins but also the anthocyanin pigments and some sulfur-based nucleophiles such as glutathione. An evidence of the natural occurrence of procyanidino-ellagitannins can be found in a report by Nishioka et al. (*43*), who isolated, from the bark of *Quercus mongolica* var. *grosseserrata*, a compound referred to as mongolicanin and similarly derived from a condensation reaction between **2a** and procyanidin B3 (not shown). On our side, we have recently proven the feasibility of the formation of the latter two types of ellagic hybrids from **2a** in a wine model system (*32*). In fact, **2a** can be considered as a nucleophile trapping agent in the slightly acidic aqueous wine solution, for it can condense with all kinds of phenols, alcohols, amines, carboxylic acids, enolizable carbonyl compounds, and thiols present in such a beverage (*44*). Furthermore, it should be kept in mind that wine is a complex multicomponent reaction system that slowly but continuously evolves under mildly acidic and oxidative conditions. For example, the acutissimins that we have analyzed herein are certainly further transformed in wine via phenolic oxidation process, but they continue to form as long as the flavan-3-ols **1a**/**b** and **2a** are present in the system. A final observation worth noting from the results of this quantitative analysis is the lower content of **2a** as compared to that of its epimer **2b** (i.e., 2 vs 8 mg/L; see **Table 2**). Other analyses have indicated amounts comprised in the same range between 0 and 7 mg/L for **2a** and between 5 and 21 mg/L for **2b** (*45*, *46*). This difference in the amounts of these two epimeric compounds is likely due to their difference in chemical reactivity. The chemical inertness of **2b** relatively to **2a** has been previously observed by others in several instances and rationalized by us at the molecular level as far as the reactivity of their C-1 OH group is concerned (*32*, *34*). This OH group has to be in a β-orientation such as in **2a** to enable acid-catalyzed condensation reactions at this site. Hence, as alluded to above, the oak dimeric roburin A can also engage itself in similar chemistry but probably not its epimer roburin D (*25*, *26*), in which the C-1 OH group is α-oriented like in **2b**.

In summary, we have developed a method to extract, detect, and estimate for the first time the amounts of four flavano-ellagitannins (**3a**/**b** and **4a**/**b**) and β-1-*O*-ethylvescalagin (**5**) formed in wine during aging in oak barrels. Using this new method, the concentrations of **3a**/**b**, **4a**/**b**, and **5** need to be estimated on several wine samples from different origins and with different aging times in oak barrels to examine the kinetic evolution of those compounds during wine aging and hence to evaluate further their impact on the chemical profile of wines. Our findings open up new molecular level insights about the influence of the use of oak-made barrels in wine aging. The take-home message of this investigation is that a major oak ellagitannin like vescalagin (**2a**) is capable of forming novel entities through covalent chemistry by reacting with nucleophilic species present in wine. Future studies should address the role played by such oak-derived ellagic hybrids and derivatives in the elaboration of the organoleptic properties of fine wines.

Supporting Information Available: HPLC-ESI-MS graphs, ion trace chormatograms, and calibration curves of various compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Ribéreau-Gayon, P. Interprétation chimique de la couleur des vins rouges. *Vitis* **1973**, *12*, 119−142.

(2) Glories, Y. La couleur des vins rouges. *Connaiss. Vigne Vin.* **1984**, *18*, 195−217.

(3) Glories, Y. La couleur des vins rouges. II. Mesure, origine et interprétation. *Connaiss. Vigne Vin.* **1984**, *18*, 253−271.

(4) Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: A critical rewiew. *Am. J. Enol. Vitic.* **2001**, *52*, 67−87.

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

(6) Souquet, J. M.; Cheynier, V.; Broussaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509−512.

(7) Amrani Joutei, K.; Glories, Y.; Mercier, M. Localisation des tannins dans les pellicules de raisin. *Vitis* **1994**, *33*, 133−138.

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

(9) Somers, T. The polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175−2186.

(10) Timberlake, C. F.; Bridle, P. Interaction between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97−105.

(11) Ribéreau-Gayon, P. Interprétation chimique de la couleur des vins rouges. *Vitis* **1973**, *12*, 119−142.

(12) Ribéreau-Gayon, P.; Pontallier, P.; Glories, Y. Some interpretation of colour changes in young red wines during their conservation. *J. Sci. Food Agric.* **1983**, *34*, 505−516.

(13) Remy, S.; Fulcrand, H.; Labarde, B.; Cheynier, V.; Moutounet, M. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. *J. Sci. Food Agric.* **2000**, *80*, 745−751.

(14) Salas, E.; Atanasova, V.; Poncet-Legrand, C.; Meudec, E.; Mazauric, J. P.; Cheynier, V. Demonstration of the occurrence of flavanol−anthocyanin adducts in wine and in model solutions. *Anal. Chim. Acta* **2004**, *513*, 325−332.

(15) Salas, E.; Le Guernevé, C.; Fulcrand, H.; Poncet-Legrand, C.; Cheynier, V. Structure determination and colour properties of a new directly linked flavanol−anthocyanin dimer. *Tetrahedron Lett.* **2004**, *45*, 8725−8729.

(16) Bate-Smith, E. C. Astringency in foods. *Food* **1954**, *23*, 419−429.

(17) Haslam, E. Polyphenol-protein interaction. *Biochem. J.* **1974**, *139*, 285−288.

(18) Haslam, E. The association of proteins with polyphenols. *J. Chem. Soc. Chem. Commun.* **1981**, 309−311.

(19) McManus, J. P.; Davis, K. G.; Beart, J. E.; Gaffney, S.; Lilley, T. H.; Haslam, E. Polyphenol interactions. Part 1. Introduction: Some observations on the reversible complexation of polyphenols with proteins and polysaccharides. *J. Chem. Soc. Perkin Trans. II* **1985**, 1429−1438.

(20) Moutounet, M.; Rabier, P.; Puech, J. L.; Verette, E.; Barillère, J. M. Analysis by HPLC of extractible substances in oak wood. Application to a Chardonnay wine. *Sci. Aliments* **1989**, *9*, 35−51.

(21) Chatonnet, P.; Dubourdieu, D. Comparative study of the characteristic of American white oak (*Quercus alba*) and European oak (*Quercus petraea* and *Q. robur*) for production of barrels used in barrel aging of wines. *Am. J. Enol. Vitic.* **1998**, *49*, 79−85.

(22) Quideau, S.; Feldman, K. S. Ellagitannin chemistry. *Chem. Rev.* **1996**, *96*, 475−503.

(23) Haslam, E.; Cai, Y. Plant polyphenols (vegetable tannins): Gallic acid metabolism. *Nat. Prod. Rep.* **1994**, 41−66.

(24) Nonaka, G.-I.; Sakai, T.; Tanaka, T.; Mihashi, K.; Nishioka, I. Tannins and related compounds. XCVII. Structure revision of *C*-glycosidic ellagitannins, castalagin, vescalagin, casuarinin and stachyurin, and related hydrolyzable tannins. *Chem. Pharm. Bull.* **1990**, *38*, 2151−2156.

(25) Hervé Du Penhoat, C. L. M.; Michon, V. M. F.; Ohassan, A.; Peng, S.; Scalbert, A.; Gage, D. Roburine A, a dimeric ellagitannin from heartwood of *Quercus robur*. *Phytochemistry* **1991**, *30*, 329−332.

(26) Hervé Du Penhoat, C. L. M.; Michon, V. M. F.; Peng, S.; Viriot, C.; Scalbert, A.; Gage, D. Structural elucidation of new dimeric ellagitannins from *Quercus robur* L. Roburins A-E. *J. Chem. Soc. Perkin Trans. 1* **1991**, 1653−1660.

(27) Nonaka, G.-I.; Ishimaru, K.; Azuma, R.; Ishimatsu, M.; Nishioka, I. Tannins and related compounds. LXXXV. Structures of novel *C*-glycosidic ellagitannins, grandinin and pterocarinins A and B. *Chem. Pharm. Bull.* **1989**, *37*, 2071−2077.

(28) Masson, G.; Moutounet, M.; Puech, J. L. Ellagitannins content of oak wood as a function of species and of sampling position in the tree. *Am. J. Enol. Vitic.* **1995**, *46*, 262−268.

(29) Doussot, F.; De Jéso, B.; Quideau, S.; Pardon, P. Extractives content in cooperage oak wood during natural seasoning and toasting: Influence of tree species, geographic location, and single-tree effects. *J. Agric. Food Chem.* **2002**, *50*, 5955−5961.

(30) Scalbert, A.; Monties, B.; Janin, G. Tannins in wood: Comparaison of different estimation methods. *J. Agric. Food Chem.* **1989**, *37*, 1324−1329.

(31) Quideau, S.; Jourdes, M.; Saucier, C.; Glories, Y.; Pardon, P.; Baudry, C. DNA. topoisomerase II inhibitor acutissimin A and other flavano-ellagitannins in red wine. *Angew. Chem. Int. Ed.* **2003**, *42*, 6012−6014.

(32) Quideau, S.; Jourdes, M.; Lefeuvre, D.; Montaudon, D.; Saucier, C.; Glories, Y.; Pardon, P.; Pourquier, P. The chemistry of wine polyphenolic C-glycosidic ellagitannins targeting human topoisomerase II. *Chem. Eur. J.* **2005**, *11*, 6503−6513 and references cited within.

(33) Scalbert, A.; Duval, L.; Peng, S.; Monties, B.; Hervé Du Penhoat, C. Polyphenols of *Quercus robur* L. II. Preparative isolation by low-pressure and high-pressure liquid chromatography of heartwood ellagitannins. *J. Chromatogr.* **1990**, *502*, 107−119.

(34) Quideau, S.; Varadinova, T.; Karagiozova, D.; Jourdes, M.; Pardon, P.; Baudry, C.; Genova, P.; Diakov, T.; Petrova, R. Main structural and stereochemical aspects of the antiherpetic activity of nonahydroxyterphenoyl-containing *C*-glycosidic ellagitannins. *Chem. Biodiversity* **2004**, *1*, 247−258 and references cited within.

(35) Degenhart, A.; Knapp, H.; Winterhalter, P. Rapid isolation of malvidin3-glucoside from red wine by hight speed countercurrent chromatography (HSCCC). *Vitis* **2000**, *39*, 43−44.

(36) Degenhart, A.; Knapp, H.; Winterhalter, P. Separation and purification of anthocyanins by high-speed coutercurrent chromatography and screening for antioxidant activity. *J. Agric. Food Chem.* **2000**, *48*, 338−343.

(37) Di Stefano, R.; Guidoni, S. XAD. 2 Resin in separation of phenolics. *Vitis* **1990**, *29*, 71−78.

(38) 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**, *280* (D), 341−343.

(39) Singleton, V. L.; Zaya, J.; Trousdale, E.; Salgues, M. Caftaric acid in grapes and conversion to a reaction product during processing. *Vitis* **1984**, *23*, 113−120.

(40) Somers, T.; Vérette, C.; Pocock, E. Hydroxycinnamates esters of *Vitis vinifera*: changes during white vinification and effects of exogenous enzymic hydrolysis. *J. Sci. Food Agric.* **1987**, *40*, 67−68.

(41) Makris, D. P.; Psarra, E.; Kallithraka, S.; Kefalas, P. The effect of polyphenolic composition as related to antioxidant capacity in white wines. *Food Res. Int.* **2003**, *36*, 805−814.

(42) Salminen, J. P.; Ossipov, V.; Haukioja, E.; Pihlaja, K. Seasonal variation in the content of hydolysable tannins in leaves of *Betula pubescens*. *Phytochemistry* **2001**, *57*, 15−22.

(43) Ishimaru, K.; Ishimatsu, M.; Nonaka, G. I.; Mihashi, K.; Iwase, Y.; Nishioka, I. Tannins and related compounds. LXXII. Isolation and characterisation of mongolicanin (procyanidino-ellagitannin), mongolinin A, acutissimin C and vescalagin carboxylic acid, novel tannins from *Quercus mongolica* var. *grosserrata*. *Chem. Pharm. Bull.* **1988**, *36*, 3319−3327.

(44) Cabanis, J. C.; Canabis, M. T.; Cheynier, V.; Teissedre, P. L. Tables de composition. In *Œnologie—Fondements Scientifiques et Technologiques*; Flanzy, C., Ed.; TEC & DOC Lavoisier: Paris, 1998; Chapter 8, pp 315−336.

(45) Moutounet, M.; Rabier, P.; Sarni, F.; Scalbert, A. Les tanins du bois de chêne. Les conditions de leur présence dans les vins. In *Le bois et la Qualité des Vins et Eaux de Vie*; *J. Int. Sci. Vigne Vin* **1992**, *Hors série*, 75−79.

(46) Moutounet, M.; Rabier, P.; Puech, J. L.; Barillere, J. M. Analysis by HPLC of extractable substances in oak wood. Application to a Chardonnay wine. *Sci. Aliments* **1989**, *9*, 35−51.

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