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Hemisynthesis and Structural and Chromatic Characterization of Delphinidin 3-*O*-Glucoside−Vescalagin Hybrid Pigments

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ABSTRACT: During red wine maturation in the presence of oak wood, reactions involving anthocyanins and ellagitannins might affect wine organoleptic properties such as color and astringency. In this work, the condensation reaction between myrtillin (delphinidin 3-*O*-glucoside) and vescalagin has been performed to determine the behavior of this anthocyanin in this kind of reaction and to assess the possible impact of such a reaction in wine color modulation. Two different hybrid pigments have been hemisynthetized and characterized by HPLC-DAD-MS and NMR spectroscopy. These pigments have been identified as 1-deoxyvescalagin-(1β→8)-myrtillin (major) and 1-deoxyvescalagin-(1β→6)-myrtillin (minor). The minor pigment could be formed both by the condensation reaction and by a regioisomerization process from the major pigment. Moreover, the chromatic properties of these pigments have been studied and compared to those of myrtillin. The hybrid pigments showed an important bathochromic shift (ca. 20 nm) in the maximum absorbance wavelength and lower molar absorption coefficients.

KEYWORDS: *C-glucosidic ellagitannin, oak wood, red wine, myrtillin (delphinidin 3-O-glucoside), vescalagin, anthocyanin reactivity*

INTRODUCTION

During wine maturation, anthocyanins from red grape progressively disappear as a result of various chemical reactions that lead to new derivatives resulting in changes of wine color. These reactions, which usually involve different other compounds from grape or from the oak wood made barrels in which wine is commonly aged, might produce both qualitative and quantitative changes in wine, affecting its organoleptic properties such as color and astringency.[1−5]

Most of the reactions involving wine anthocyanins result in the formation of more stable derived pigments. Different kinds of derived pigments such as pyranoanthocyanins and flavanol−anthocyanin condensation products have been detected in wines. The first ones result from the cycloaddition of wine nucleophiles at C4 and O5 of the anthocyanin flavylium nucleus (followed by aromatization through autoxidation), which lead to an additional pyrane ring in the pigment structure. Some nucleophilic compounds present in wines, such as the enol forms of pyruvic acid or acetaldehyde and vinylphenols among others, can thus react with anthocyanins to generate these kinds of pigments.[6−11] Anthocyanins have also been shown to react with flavanols, involving acetaldehyde or not, to generate either acetaldehyde-derived or direct flavanol−anthocyanin condensation products.[4,12−17] The anthocyanin acts as a nucleophile in the formation of acetaldehyde-derived flavanol−anthocyanin products,[4,13] whereas two different mechanisms have been postulated to explain direct reactions between anthocyanins and flavanols during which the anthocyanin could act either as an electrophile or as a nucleophile to form either anthocyanin−flavanol (A-F) or flavanol−anthocyanin (F-A$^+$) adducts.[15]

Moreover, among the different substances released from oak wood into the wine solution during aging, the *C*-glucosidic ellagitannins can take part in some of the anthocyanin reactions, hence affecting the wine properties.[4,5] The most representative structures of found-in-wine *C*-glucosidic ellagitannins are the monomeric forms vescalagin and castalagin, which are the most abundant ellagitannins in oak wood with contents ranging from 39 to 73% of the total ellagitannin content.[18−21] Lyxose/xylose derivatives (grandinin and roburin E, respectively) and dimeric forms (roburins A, B, C, and D) have also been described.[20,22−25] In oak heartwood, the *C*-glucosidic ellagitannins may represent up to 10% of the dry wood weight.[18] These highly hydrophilic compounds are easily solubilized by wine, in which they can be involved in different chemical reactions.

However, there are important differences in the reactivity of the different *C*-glucosidic ellagitannins. For example, it has been proven that castalagin expresses a much lower reactivity than vescalagin in direct condensation reactions with other wine components.[24,26] In fact, only vescalagin reacts with flavanols or anthocyanins to form adducts such as flavano-ellagitannins[27,28] or anthocyano-ellagitannins.[28,29] The reactions of vescalagin with (epi)catechins yields (epi)acutissimins. These flavano-ellagitannins feature a C−C linkage between carbon-1 of the vescalagin moiety and either carbon-8 or carbon-6 of the A ring of the (epi)catechin unit.[26−28] The catechin variants of these compounds were first isolated and characterized by Ishimaru and co-workers from the bark of *Quercus acutissima*,[30] and some studies have also reported their formation during the aging of wine in contact with oak wood.[27,31,32]

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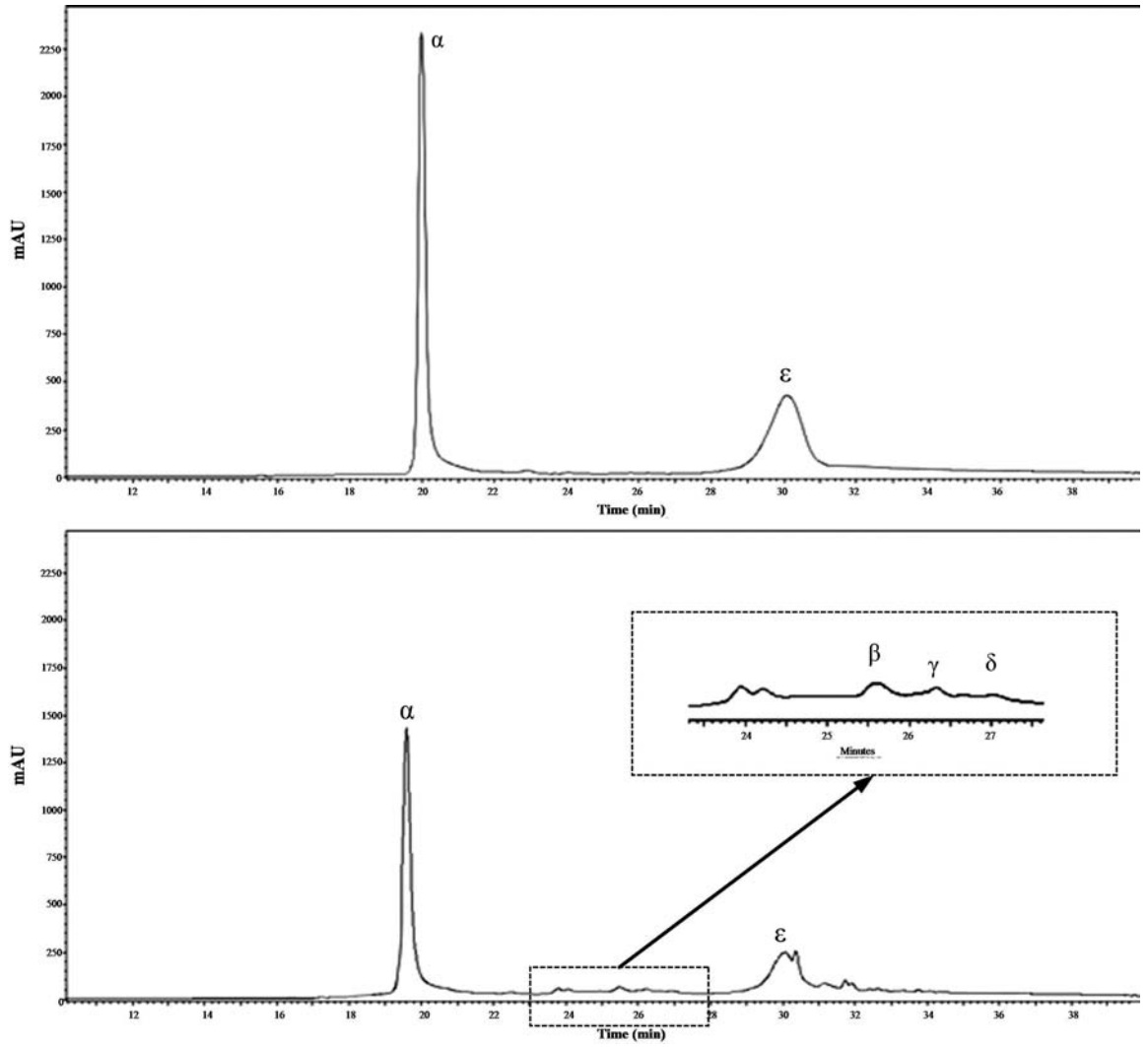

Figure 1. HPLC-DAD chromatogram (λ = 520 nm) of the reaction between vescalagin and myrtillin: (a) t = 0; (b) t = 24 h. α, vescalagin; β, product **1**; γ, product **3**; δ, product **2**; ε, myrtillin.

The formation of anthocyano-ellagitannin hybrid pigments has been demonstrated by Quideau and co-workers,[28,29] who were able to achieve the first hemisynthesis of 1-deoxyvescalagin-(1β→8)-malvidin and 1-deoxyvescalagin-(1β→8)-oenin.[28] These authors have also demonstrated the formation of these hybrid pigments in a standard wine model solution, confirming the feasibility of these condensation reactions in such a medium.[28] These anthocyano-ellagitannin hybrid pigments show purple hues in accordance with the bathochromic shift observed in the absorption band of their visible spectra. Moreover, differences in their kinetic and thermodynamic parameters as compared to those of the native anthocyani(di)ns have been reported.[29] Therefore, ellagitannins might also be directly involved in color-modulating reactions in wine via the formation of this kind of hybrid pigment.

Although the major anthocyanins in grapes and wines are usually malvidin derivatives, other anthocyanins such as delphinidin or petunidin derivatives may be, depending on the grape variety, as quantitatively relevant as malvidin derivatives.[33,34] Moreover, there have been reported differences in the reactivity of the anthocyanidins upon oxidation depending on the different substituents of the B-ring,[35,36] which may indicate the importance of the substitution pattern of the B-ring in the reactivity of the different anthocyanins. For these reasons, it appears interesting to study the behavior of anthocyanins other than the oenin in this kind of reaction. In the work described herein, the hemisynthesis of myrtillin (delphinidin 3-*O*-glucoside)−vescalagin hybrid pigments has been carried out, and their chromatic properties have been examined with the aim of assessing the role of the formation of such anthocyano-ellagitannins in the modulation of wine color.

MATERIALS AND METHODS

Chemicals. Myrtillin (delphinidin 3-*O*-glucoside) was extracted from the skins of *Vitis vinifera* L. cv. Tempranillo grapes. Extraction was carried out three times using MeOH/12 M HCl 999:1 (v/v), and the extracts were gathered and evaporated under reduced pressure to remove the methanol. The residue was redissolved in aqueous HCl (0.1 M, pH 1) and then loaded on a Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA) column (30 × 300 mm), which was previously conditioned using 1 L of aqueous HCl (0.1 M, pH 1). Elution was carried out using the same aqueous HCl solution, and fractions (25 mL each) were collected. Myrtillin eluted in two fractions after the elution of the other anthocyanidin-3-*O*-glucosides. These two fractions were gathered and then freeze-dried to furnish a reddish purple powder. Myrtillin purity (>95%) was determined by HPLC-DAD-MS analysis. (−)-Vescalagin (Vg) was extracted from *Quercus robur*

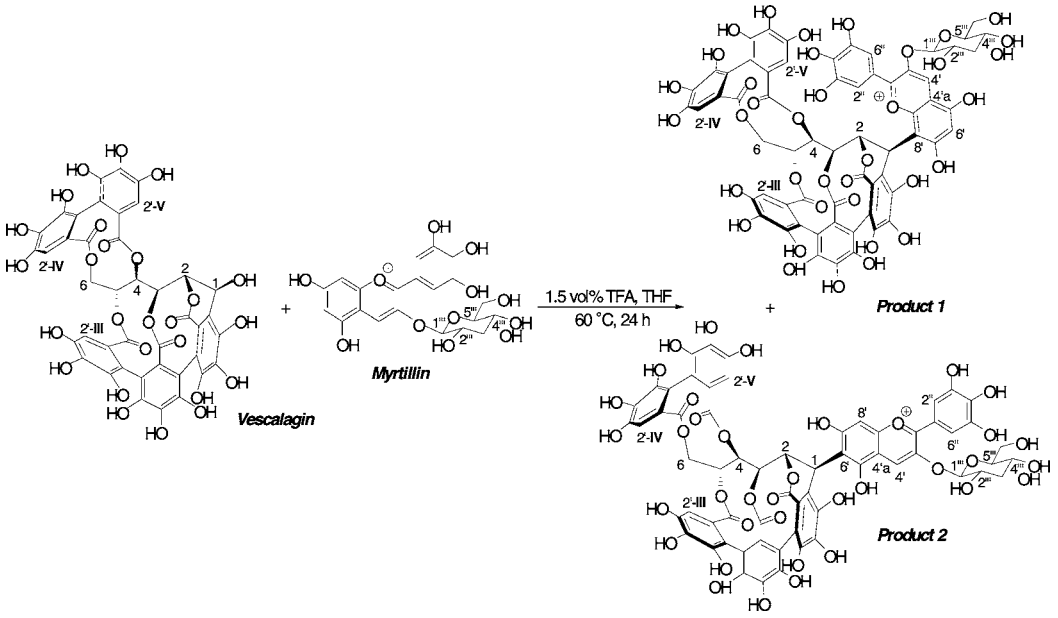

Figure 2. Reaction between vescalagin and myrtillin in an acidic organic solution.

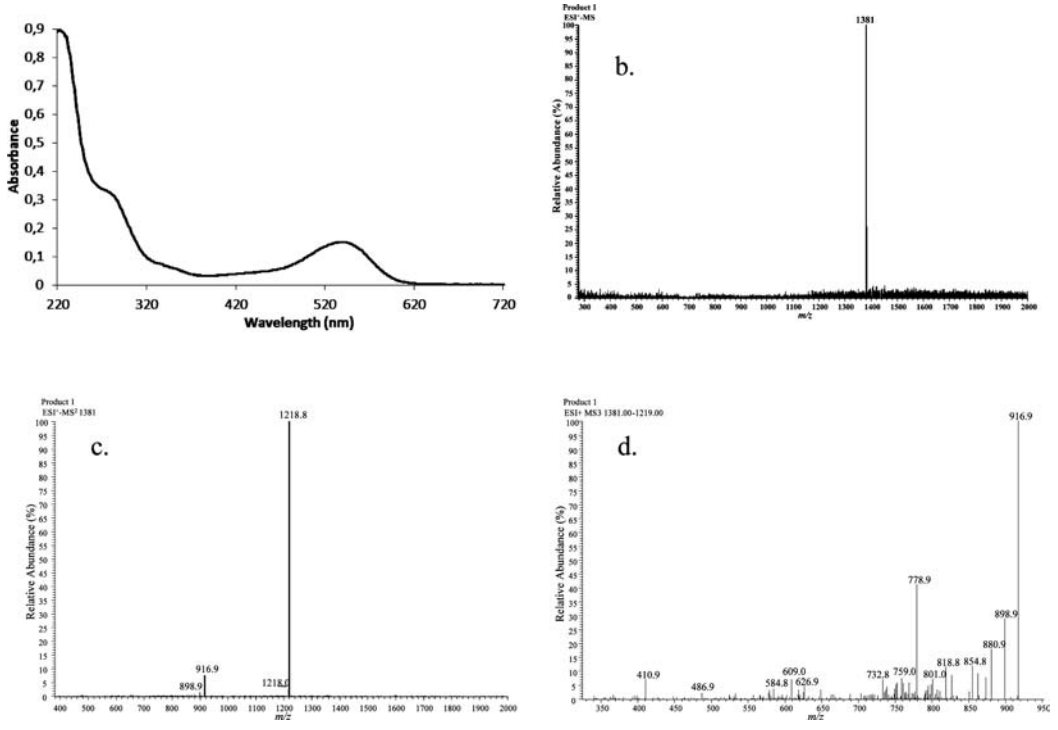

Figure 3. (a) UV−visible spectrum of the major product of the reaction between vescalagin and myrtillin (product **1**). (b) Full mass spectrum of product **1**. (c) MS^2 fragmentation pattern of the molecular ion of product **1** (m/z 1381). (d) MS^3 fragmentation pattern of the main fragment (m/z 1219) obtained in the MS^2 analysis.

heartwood and purified as described previously.[37] All of the solvents used were of analytical HPLC grade and were purchased from Sigma-Aldrich.

Procedures. *Reaction.* The reaction between vescalagin and myrtillin was performed according to the procedure described by Quideau and co-workers for the hemisynthesis of oenin− and malvidin−vescalagin hybrid pigments.[28] Vescalagin (20 mg, 0.021 mmol) and myrtillin chloride (12 mg, 0.024 mmol) were dissolved in an anhydrous tetrahydrofuran solution containing 1.5 vol % of trifluoroacetic acid (12 mL). The reaction mixture was stirred at 60 °C for 24 h, and then the reaction was stopped by evaporation under reduced pressure. The residue was dissolved in acidic water (H_2O/HCOOH 999:1) and purified by semipreparative HPLC.

Purification and Structural Characterization. Semipreparative HPLC purification of the products was performed on a Varian ProStar system using a Dynamax C-18, 5 μm, 21.4 × 250 mm column (Agilent Technologies, Waldbronn, Germany). This liquid chromatographic purification method used H_2O/HCOOH 999:1 as solvent A and MeOH/HCOOH 999:1 as solvent B in a gradient elution (0−20 min, 0−20% solvent B; 20−35 min, 20−100% solvent B; 35−40 min, 100% solvent B) with a flow rate of 6 mL/min. Column effluent was monitored by UV detection at 280 nm using a Varian ProStar 320 UV−visible detector controlled by Star Chromatography Workstation

6.41 software (Agilent Technologies, Waldbronn, Germany). The obtained fractions were analyzed by HPLC-DAD-ESI$^+$-MS in a Thermo Finnigan Spectra Systems P1000XR equipped with a Spectra System UV6000 LP detector (controlled by ChromQuest 4.2 software Thermoquest, San Jose, CA, USA). The mass analyses were performed with a Finnigan LCQ ion trap instrument (Thermoquest) equipped with an electrospray ionization (ESI) interface. HPLC-DAD-MS analyses were performed using a previously developed method for the analysis of oenin−vescalagin hybrid pigments.[28,29] The fractions containing each compound of interest were gathered, concentrated under reduced pressure, and repurified using the same semipreparative HPLC procedure.

^{1}H NMR analyses were carried out in a Bruker DPX 800NB NMR spectrometer equipped with a 5 mm direct QNP probed with gradient capabilities, using MeOH-d_4/TFA (99:1) as solvent. Data processing was performed with Topspin software (Bruker, Billerica, MA, USA) using a sine-bell multiplication in both dimensions.

Chromatic Characterization. The absorption spectra were recorded on a Hewlett-Packard UV−visible HP 8453 spectrophotometer (Palo Alto, CA, USA), using 10 mm path length quartz cells. The whole UV−visible spectrum (190−770 nm) was recorded ($\Delta\lambda$ = 1 nm) at pH 1 (0.1 M HCl aqueous solution) and at pH 3.2 (model wine solution: 5 g/L tartaric acid in 12% EtOH/H_2O v/v adjusted at pH 3.2 with 1 M NaOH). The CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, and h_{ab}) and color differences (ΔE^*_{ab}) were determined by using CromaLab software.[38] The illuminant D_{65} and CIE 1964 10° standard observer were used as references in the calculations, following the recommendations of the Commission International de L'Eclairage.[39]

RESULTS AND DISCUSSION

Hemisynthesis and Structural Characterization. The reaction mixture between vescalagin and myrtillin initially showed a dark red color that turned into a deep purple color over time. The reaction progress was monitored by HPLC-DAD-MS^n to determine the optimum reaction time. After 24 h, the degradation of the starting materials and the formation of secondary products were more important than the formation of direct condensation vescalagin−myrtillin products, and therefore this time was set as final time reaction. Nevertheless, high amounts of the starting vescalagin and myrtillin remained in the reaction medium, and they could be partially recovered (Figure 1). The reaction (Figure 2) was repeated 10 times. Different products were obtained (Figure 1), but among them, only two showed a band of absorption in the visible region of the spectrum. These two pigments were isolated by semipreparative HPLC, hence obtaining 13 mg of product **1** (molar yield of 4.3%) and 1.5 mg of product **2** (molar yield of 0.5%). Their purities (higher than 95 and 90% for products **1** and **2**, respectively) were determined by HPLC-DAD-MS analysis.

The characterization of these hybrid pigments was carried out by HPLC-DAD-ESI$^+$-MS (full mass and MS^n analyses) and by NMR spectroscopy. The UV−visible spectra of these hybrid pigments showed two important bands (Figure 3a): one, the most important, in the UV region with a maximum around 230 nm (characteristic of the ellagitannins) and a shoulder around 280 nm (characteristic of the anthocyanin) and another in the visible region with a maximum around 540 nm, which is characteristic of the flavylium form of some purple anthocyanin derivatives.[40]

Mass spectrometry analysis allowed the corroboration of the identity of these products as 1-deoxyvescalagin-myrtillin direct condensation products. MS analyses of both pigments provided in full mass analysis a molecular ion at m/z 1381 (Figure 3b) with the same fragmentation pattern (Figure 3c,d): a main fragment in MS^2 analysis at m/z 1219 ([M$^+$ − 162]), corresponding to the loss of the glucose moiety of myrtillin, and a main fragment in MS^3 analysis at m/z 917 ([M$^+$ − 162 − 302]), which could correspond to the 1-deoxyvescalagin moiety formed by the loss of the myrtillin residue. However, this fragment might also correspond to the 1-deoxyvescalin-delphinidin moiety formed by the loss of the hexahydroxydiphenoyl (HHDP) residue of the ellagitannin, because it also represents the loss of 302 units of mass. The MS^4 analysis showed a complicated fragmentation pattern that could originate from the fragmentation of the 1-deoxyvescalagin moiety, as well as from the 1-deoxyvescalin-delphinidin moiety.

Both hybrid pigments (products **1** and **2**) resulted from an acid-catalyzed nucleophilic substitution at the C-1 center of vescalagin with retention of configuration.[28] The anthocyanin molecule has two potential nucleophilic positions (6′-C and 8′-C),[15,41] but due to the higher net negative charge at the ground state of the anthocyanin 8′-C, this position is more likely to be involved in the linkage than 6′-C.[4] This hypothesis can be supported by the fact that in most of the anthocyanin condensation products characterized to this day, the 8′-C of the anthocyanin is involved in the linkage.[13] Nevertheless, Atanasova and co-workers[42] have reported polymeric acetaldehyde-mediated self-condensation products of malvidin-3-*O*-glucoside in which the 6′-C of the anthocyanin is also involved in the linkage. Thus, the 6′-C position of the anthocyanin seems to be also reactive, although to a lesser extent than the 8′-C position. For this reason, and taking into account the rate at which the compounds were obtained, product **1**, the major one, was identified as 1-deoxyvescalagin-(1β→8)-myrtillin (8′-C isomer), whereas product **2**, obtained in a much lower proportion, was identified as 1-deoxyvescalagin-(1β→6)-myrtillin (6′-C isomer). Although some of the ^{1}H NMR signals of the ellagitannin glucose part of these compounds could not be fully assigned due to the presence of minor impurities caused by partial degradation in the NMR solutions, the signals corresponding to their main protons were attributed with the help of the analysis of the HMQC and HMBC correlation data maps of the major product **1** (data not shown) and comparison with the NMR data of the oenin− and malvidin−vescalagin adducts.[28,29] The main proton signals were thus assigned as follows.

Product **1** (Figure 2): ^{1}H NMR (800 MHz, MeOH-d_4/TFA [99:1]) δ 3.55−4.03 (m, 5H, H-2‴, H-3‴, H-4‴, H-5‴, H-6‴), 3.99 (m, 1H, H-6α), 4.63 (s, 1H, H-1), 4.85 (bs, 1H, H-3), 5.21 (m, 1H, H-4), 5.29 (bs, 1H, H-2), 5.33 (d, J = 7.6 Hz, 1H, H-1‴), 5.61 (d, J = 7.3, 1H, H-5), 5.74 (s, 1H, H-2′(**V**)), 6.50 (s, 1H, H-2′(**III**)), 6.77 (s, 1H, H-2′(**IV**)), 6.79 (s, 1H, H-6′), 7.47 (s, 2H, H-2″, H-6″), 9.14 (s, 1H, H-4′).

Product **2** (Figure 2): 1H NMR (800 MHz, MeOH-d_4/TFA [99:1]) δ 3.55−4.03 (m, 5H, H-2‴, H-3‴, H-4‴, H-5‴, H-6‴), 5.29 (s, 1H, H-2), 5.33 (d, J = 7.7 Hz, 1H, H-1‴), 5.61 (d, J = 7.3 Hz, 1H, H-5), 5.74 (s, 1H, H-2′(**V**)), 6.49 (s, 1H, H-2′(**III**)), 6.77 (s, 1H, H-2′(**IV**)), 6.70 (s, 1H, H-8′), 7.47 (s, 2H, H-2″, H-6″), 9.14 (s, 1H, H-4′).

The reaction between vescalagin and myrtillin showed some important differences from that between vescalagin and oenin.[28,29] Whereas the reaction between oenin and vescalagin yielded only one hybrid product, the 8′-C isomer, two isomers could be isolated from the reaction involving myrtillin and vescalagin. Moreover, the reaction between myrtillin and vescalagin generated more side-products than the reaction between oenin and vescalagin. This is in accordance with

previous studies carried out in our laboratory about the formation of pyranoanthocyanins in wine model solutions that evidenced a higher reactivity for myrtillin than for oenin (unpublished results). Among the side-products detected, one of them (product **3**) could be isolated with a purity >90%. The HPLC-DAD-MS^n analysis of this side-product confirmed that it was indeed a product derived from the reaction between vescalagin and myrtillin. The UV−visible spectrum showed a band at 230 nm, which is characteristic of the ellagitannins, and another at 280 nm, which is characteristic of the anthocyanin, but no band in the visible region could be detected, evidencing that this product was not colored. The mass spectra showed a signal at m/z 1397 ($[M + H]^+$), which could correspond to the pseudomolecular ion of this compound. The fragmentation pattern confirmed that this product was formed from the condensation of vescalagin and myrtillin, because the main ion fragment detected in the MS^2 analysis (m/z 1235, $[M + H - 162]^+$) originated from the loss of the glucose moiety of myrtillin, and the main ion fragment in the MS^3 analysis was detected at m/z 917 ($[M + H - 162 - 318]^+$), which corresponds to the 1-deoxyvescalagin moiety. Unfortunately, NMR analyses of this side-product **3** did not provide enough information to allow its full structural determination.

Regioisomerization Process. Two regioisomers thus resulted from the acid-catalyzed nucleophilic substitution reaction between vescalagin and myrtillin. In the case of the malvidin 3-*O*-glucoside(oenin)−vescalagin and malvidin−vescalagin hybrid pigments, Quideau and co-workers detected only the 8′-C regioisomer,[28] although they proposed that the 6′-C regioisomer could in principle derive from a regioisomerization process.[29] Anthocyanins and related compounds in mildly acidic aqueous solutions are involved in a series of chemical reactions leading to mixtures of colored and colorless species.[43,44] Thus, anthocyanin derivatives could undergo a series of reversible transformations that might lead to a partial transformation of product **1** into product **2** following the regioisomerization process shown in Figure 4.[29] The hydration of the major pigment (1-deoxyvescalagin-(1β→8)-myrtillin), followed by ring-opening, can lead to the *E*-chalcone, in which rotation around the C-4′−C-4′a bond is possible. Ring reclosure and dehydration after rotation around this bond would cause the regioisomerization of the 1-deoxyvescalagin-(1β→8)-myrtillin into the 1-deoxyvescalagin-(1β→6)-myrtillin. To confirm if the 6′-C isomer could thus be formed, the evolution of an aqueous solution of the 8′-C isomer adjusted to pH 3 was monitored over time by means of HPLC-DAD-MS. Figure 5 shows the chromatograms obtained at four different times (0, 3, 8, and 16 days). At the third day, the percentage of the area of the peak corresponding to the 8′-C isomer started to

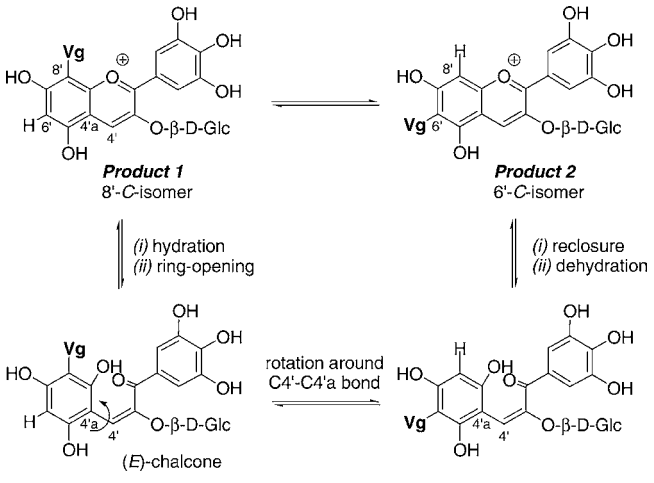

Figure 4. Putative regioisomerization of 1-deoxyvescalagin-(1β→8)-myrtillin (product **1**) into 1-deoxyvescalagin-(1β→6)-myrtillin (product **2**) in a mildly acidic aqueous solution (pH 3). Vg = 1-deoxyvescalagin moiety.

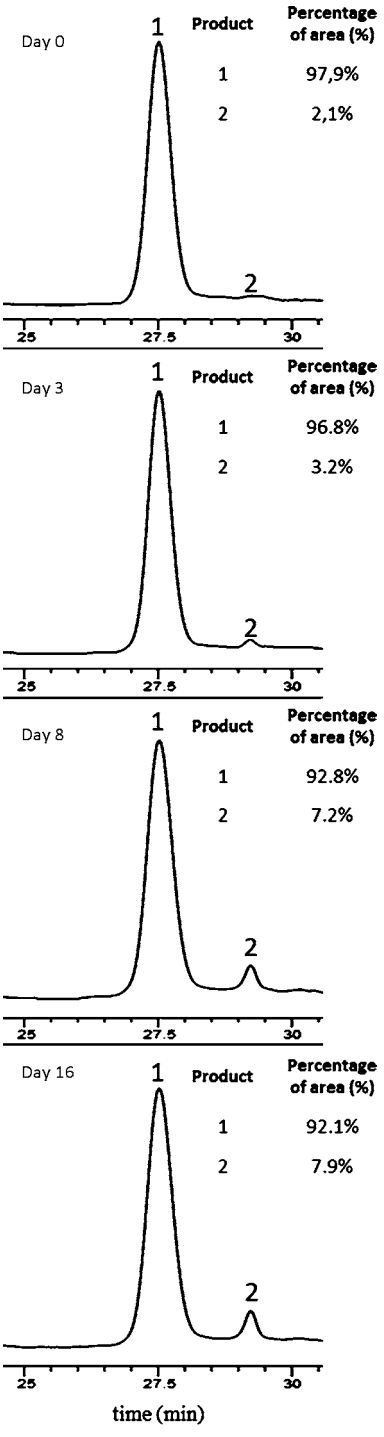

Figure 5. HPLC monitoring of the evolution of a mildly acidic aqueous solution (pH 3, HCl) of product **1**, confirming the regioisomerization of 1-deoxyvescalagin-(1β→8)-myrtillin (**1**) into 1-deoxyvescalagin-(1β→6)-myrtillin (**2**).

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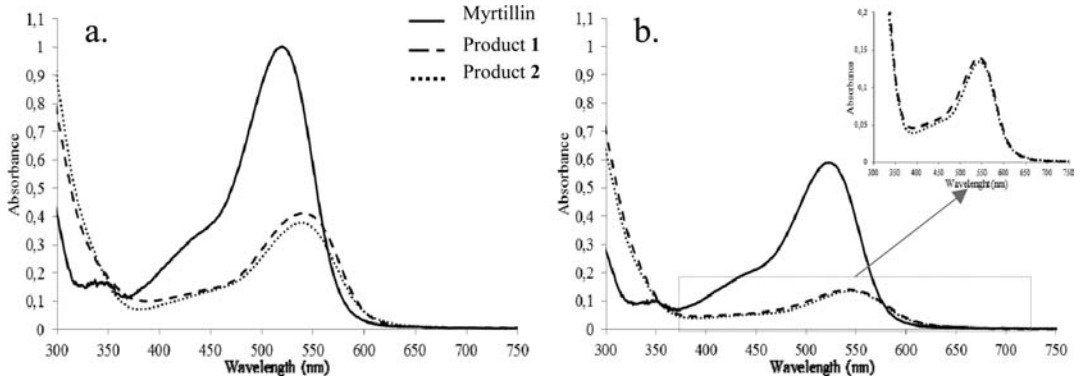

Figure 6. Visible spectra of myrtillin (4.6×10^{-5} M), 1-deoxyvescalagin-(1β→8)-myrtillin (product **1**, 9.2×10^{-5} M), and 1-deoxyvescalagin-(1β→6)-myrtillin (product **2**, 9.2×10^{-5} M) at pH 1: (a) aqueous 1 M HCl solution) and pH 3.2; (b) wine model solution composed of 12% EtOH/H_2O (v/v) and tartaric acid (5g/L) adjusted to pH 3.2 with 1 M NaOH.

decrease concomitantly with an increase of that corresponding to the 6′-C isomer. At the end of the experience, a clear formation of the 6′-C isomer was detected (the percentage of the area of the corresponding peak changed from 2.1% at day 3 to 7.9% at day 16). These changes of the percentages of the peak areas indicate that the regioisomerization is a slow process, because no change could be detected in the chromatogram during the first day (data not shown), and 3 days was necessary to observe a noticeable increase of the peak corresponding to the 6′-C isomer. Between days 8 and 16, changes were only minor, suggesting that equilibrium was reached and that it was slightly shifted toward a preferred formation of the 8′-C isomer. The thermodynamic and kinetic properties of the 8′-C isomer[45] support that the regiomerization occurs.

Thus, the 6′-C isomer (1-deoxyvescalagin-(1β→6)-myrtillin) could be formed in two different ways, either by a direct nucleophilic substitution reaction during which the anthocyanin attacks the 1-deoxyvescalagin carbocationic intermediate from its nucleophilic 6′-C center or by an isomerization of the 8′-C isomer (1-deoxyvescalagin-(1β→8)-myrtillin). During the reaction, both isomers were detected even in the first hours, thus suggesting that the direct nucleophilic attack of the anthocyanin 6′-C center is operational. However, the chromatographic peak corresponding to the 6′-C isomer was much less important than that corresponding to the 8′-C isomer. It is also possible that the 8′-C to 6′-C regioisomerization could in part take place during the purification of the products, because this purification was carried out using a mildly acidic aqueous mobile phase (H_2O/HCOOH 999:1). Thus, in our opinion, most of the 6′-C isomer isolated for further characterization might have been formed through this regioisomerization process.

Chromatic Characterization. The chromatic properties of these pigments were also determined. The UV−visible spectra of these compounds (9.2×10^{-5} M) were measured and compared to the UV−visible spectra of myrtillin (4.6×10^{-5} M) both in aqueous solutions at pH 1 and in wine model solutions at pH 3.2 (Figure 6). An important bathochromic shift is observed in the visible band of the hybrid pigment spectra relative to that of the native myrtillin, which is in agreement with the violet hue exhibited by these hybrid compounds. This shift is comparable to that observed in the acetaldehyde-mediated flavanol−anthocyanin derivative pigments,[12,13,16] which show blue hues. The maximum absorption wavelength is shifted by ca. 20 nm toward higher wavelengths in the hybrid pigments (Table 1) as compared with myrtillin at both pH values.

Table 1. Maximum Absorption Wavelength of Myrtillin, 1-Deoxyvescalagin-(1β→8)-myrtillin (8′-C Isomer) and 1-Deoxyvescalagin-(1β→6)-myrtillin (6′-C Isomer) at pH 1 and 3.2

λ_{max} (nm)	pH 1	pH 3.2
myrtillin	520	524
8′-C isomer	539	543
6′-C isomer	539	545

The molar absorption coefficients (ε) at pH 1 of myrtillin and the major pigment (**1**, 1-deoxyvescalagin-(1β→8)-myrtillin) have been calculated from the absorbance at the maximum absorbance wavelength measured at five different concentrations ranging between 7.6×10^{-6} and 9.2×10^{-5} M. The value obtained for the hybrid pigment **1** (ε = 9040 cm^{-1} M^{-1}, λ = 539 nm) was much lower than that corresponding to the native myrtillin (ε = 21900 cm^{-1} M^{-1}, λ = 520 nm). The molar absorption coefficient at pH 1 of the minor pigment (**2**, 1-deoxyvescalagin-(1β→6)-myrtillin) was estimated from the absorbance at the maximum absorbance wavelength measured at two different concentrations (9.2×10^{-5} and 4.6×10^{-5} M). The value obtained (ε = 8570 cm^{-1} M^{-1}, λ = 539 nm) was similar to that determined for product **1**. This is in accordance with the results obtained for the oenin− and malvidin−vescalagin hybrid pigments, which also showed molar absorptivities lower than those of their corresponding native pigments.[29] Thus, the presence of the vescalagin moiety linked to the anthocyanin A-ring through a C−C linkage affects the molar absorption coefficient of the resulting hybrid pigments. Moreover, the resulting data perfectly fit with the Lambert−Beer law, and no effect on the maximum absorbance wavelength was detected when the pigment concentration was increased, indicating that this pigment did not form self-association complexes in the range of concentration studied (7.6×10^{-6} and 9.2×10^{-5} M). The lack of self-association may be related to the involvement of the chromophore in intramolecular copigmentation, as shown for the hybrid pigment formed with oenin.[29]

The CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, and h_{ab}) were determined from the whole visible spectra of the hybrid pigments and myrtillin at the same concentration (4.6×10^{-5} M) at two different pH values: pH 1 (0.1 N HCl aqueous

solution) and pH 3.2 (wine model solution). Table 2 shows the CIELAB parameters determined, which were employed to objectively evaluate the color of the hybrid pigments and also to make comparisons with the myrtillin color. At both pH values, the lightness (L^*) of the hybrid pigments was higher than that determined for myrtillin, in accordance with the lower molar absorption coefficient. The hues (h_{ab}) determined for the hybrid pigments were likewise significantly lower than those determined for myrtillin, in accordance with the bluish hues exhibited by these pigments.

Table 2. Chromatic Parameters (CIELAB Space) of Myrtillin (4.6×10^{-5} M), 1-Deoxyvescalagin-(1β→8)-myrtillin (8′-C Isomer, 4.6×10^{-5} M), and 1-Deoxyvescalagin-(1β→6)-myrtillin (6′-C Isomer, 4.6×10^{-5} M) at pH 1 and 3.2

	pH	L^*	a^*	b^*	C^*_{ab}	h_{ab}
myrtillin	1	73.42	54.82	7.26	55.30	7.54
	3.2	80.30	41.76	−1.52	41.79	−2.08
8′-C isomer	1	80.49	31.99	−11.32	33.93	−19.49
	3.2	91.98	10.66	−4.55	11.59	−23.09
6′-C isomer	1	82.12	28.86	−9.88	30.51	−18.90
	3.2	92.43	10.02	−4.65	11.05	−24.87

Moreover, differences in the behavior of the hybrid pigments and myrtillin with pH changes were evidenced by the variations in the values of the CIELAB parameters. Whereas lightness increased around 7 units in the case of myrtillin, the increase in the L^* values of the hybrid pigments with the pH change was >11 units in both cases. On the contrary, the change in the hue values was less important in the case of the hybrid pigments than in the case of myrtillin.

To evaluate the importance of these changes and the differences of the color of the different pigments, color differences (ΔE^*_{ab}) between the different pigments at the same pH value and for the same pigment at different pH values were calculated. Table 3 shows the values obtained. The most important color differences were found between myrtillin and the hybrid pigments at both pH values, being higher at pH 3.2 than at pH 1. The color differences between the two hybrid pigments were detectable by the human eye only at pH 1 (because the ΔE^*_{ab} value is ≥3), but these pigments had indistinguishable colors at pH 3.2, even when using analytical methods ($\Delta E^*_{ab} \leq 1$). Thus, at wine pH, these hybrid pigments showed no difference in their color. Moreover, the color differences determined for the pigments at different pH values were higher for the myrtillin−vescalagin pigments than for myrtillin, the highest color difference being observed in the case of the 8′-C isomer.

Table 3. Color Differences (ΔE^*_{ab}) Obtained in the Solutions of Myrtillin, 1-Deoxyvescalagin-(1β→8)-myrtillin (8′-C Isomer), and 1-Deoxyvescalagin-(1β→6)-myrtillin (6′-C Isomer) at pH 1 and 3.2

color differences calculated between	pH	ΔE^*_{ab}
6-′C isomer and 8′-C isomer	1	3.80
	3.2	0.79
8′-C isomer and myrtillin	1	30.27
	3.2	33.36
6′-C isomer and myrtillin	1	32.30
	3.2	34.12
color differences calculated between	pigment	ΔE^*_{ab}
pH 1 and 3.2	6′-C isomer	22.11
	8′-C isomer	25.15
	myrtillin	17.17

Distinctive physicochemical properties of the hybrid pigment[45] support the variations of the color parameters upon the pH change relative to those observed for the native anthocyanin.

In conclusion, two new regioisomeric anthocyano-ellagitannin hybrid pigments have been obtained by hemisynthesis from vescalagin and myrtillin: 1-deoxyvescalagin-(1β→8)-myrtillin (8′-C isomer, major) and 1-deoxyvescalagin-(1β→6)-myrtillin, (6′-C isomer, minor). This study has also demonstrated that, in mildly acidic solutions, a partial transformation of the 8′-C isomer into the 6′-C occurs via a slow regioisomerization process. These hybrid pigments showed important differences in their chromatic properties relative to those of myrtillin: a bathochromic shift (ca. 20 nm) in the maximum absorbance wavelength at both pH values used, lower molar absorption coefficients (ε = 9040 cm^{-1} M^{-1} for the 8′-C isomer and ε = 8570 cm^{-1} M^{-1} for the 6′-C isomer in comparison with ε = 21900 cm^{-1} M^{-1} for myrtillin), and differences in their behavior upon pH changes. Color differences between the two hybrid pigments were detectable by the human eye only at pH 1, but these pigments had indistinguishable colors at pH 3.2.

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Notes

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