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Role of Vinylcatechin in the Formation of Pyranomalvidin-3-glucoside−(+)-Catechin

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Reactions between malvidin-3-glucoside (mv3glc) and 8-vinylcatechin were carried out to synthesize pyranomv3glc−(+)-catechin pigment and to study the formation of intermediates. A rapid decrease of mv3glc content concomitant with the formation of more complex structures such as mv3glc−vinylcatechin [precursor of pyranomv3glc−(+)-catechin pigment] and mv3glc−divinylcatechin was observed. On the other hand, 8-vinylcatechin undergoes acid-catalyzed dimerization in model wine solution, giving rise to 8-vinylcatechin dimers. These compounds were also found in the reaction between mv3glc and (+)-catechin mediated by acetaldehyde, which provides evidence for the formation of 8-vinylcatechin and its involvement in the formation of pyranoanthocyanins in aged red wines.

KEYWORDS: Malvidin-3-glucoside; 8-vinylcatechin; pyranoanthocyanins; pigments; red wine; mass spectrometry; NMR

INTRODUCTION

Anthocyanins and proanthocyanidins (condensed tannins) are two important families of polyphenolic compounds widespread in food plants and beverages (*1*).

In red wines, anthocyanins are mainly responsible for the color, whereas proanthocyanidins are associated with flavor (astringengy and bitterness) and may also act as noncolored copigments stabilizing anthocyanins by non-covalent interactions improving the wine color. Proanthocyanidins are generally mixtures of oligomers and polymers of flavan-3-ol units [e.g., (+)-catechin] linked through a carbon−carbon bond.

Red wine aging is a complex process that occurs during storage in which anthocyanins and flavan-3-ols undergo several chemical transformations giving rise to new and more stable pigments with different physical−chemical features that change the sensorial characteristics of wines, such as color and flavor. During the aging process the red-purple color of the young red wines progressively changes to a more brick-red hue.

Different mechanisms have been proposed to explain the formation of new pigments in wines, namely, reactions between anthocyanins and flavanols directly (*2*−*4*) or mediated by aldehydes (*5*−*10*).

Recently, several cycloaddition reactions of anthocyanins with other small compounds such as acetaldehyde (*11*), 4-vinylphenols (*12*−*14*), pyruvic acid (*11, 15*), phenolic acids (*16*), and acetoacetic acid (*17*) have been described that occur in wines or in model solutions yielding new families of anthocyanin-derived pigments called pyranoanthocyanins. One of the most important pyranoanthocyanin groups in wines is the carboxypyranoanthocyanins, which have an orange color at wine pH, and their formation results from the cycloaddition of pyruvic acid onto position C4 and the 5-OH group of the anthocyanin (*18, 19*).

Another important group of anthocyanin-derived pigments corresponds to pyranoanthocyanins directly linked to flavan-3-ols (catechins and procyanidins) presenting also a red-orange color (*20*−*23*). Their formation in wines is thought to arise from the cycloaddition reaction between the C4 position and the 5-OH of anthocyanins and the vinyl group of 8-vinylflavanol, through a mechanism identical to that proposed by Fulcrand et al. for

Table 1. Mass Data of the Products Obtained from the the Reaction between Malvidin-3-glucoside and 8-Vinylcatechin after 90 min and 2 Days[a]

m/*z* $[M]^+$	fragments
	90 min
809	647 (-glc), 519 (-cat), 357 (-glc-cat)
1123	971 (-RDA), 961 (-glc), 833 (-cat), 807 (-vinylcat), 671 (-glc-cat), 543 (-cat-cat)
807[b]	645 (-glc), 493 (-glc-RDA)
1301[b]	1139 (-glc), 1011 (-cat), 987 (-glc-RDA), 977 (-glc-glc), 849 (-glc-cat), 493 (-mv3glc-vinyl-cat)
	2 days
805	653 (-RDA), 643 (-glc), 491 (-RDA-glc)
1121	969 (-RDA), 959 (-glc), 831 (-cat), 807 (-glc-RDA), 679 (-cat-RDA)

[a] glc, glucose; RDA, retro-Diels−Alder; cat, catechin. [b] Also detected after 2 days.

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Table 2. Mass Data of the Products Obtained from the Reaction between Malvidin-3-glucoside and (+)-Catechin Mediated by Acetaldehyde after 12 Days[a]

peak	*m/z* $[M]^+$	fragments
A	809	647 (-glc), 519 (-cat), 357 (-glc-cat)
B	805	643 (-glc), 491 (-glc-RDA)
C	633	481 (-RDA), 343 (-cat), 317 (-vinylcat), 165 (-vinylcat-RDA)
D	1125	973 (-RDA), 963 (-glc)
E	607	

[a] glc, glucose; RDA, retro Diels−Alder; cat, catechin.

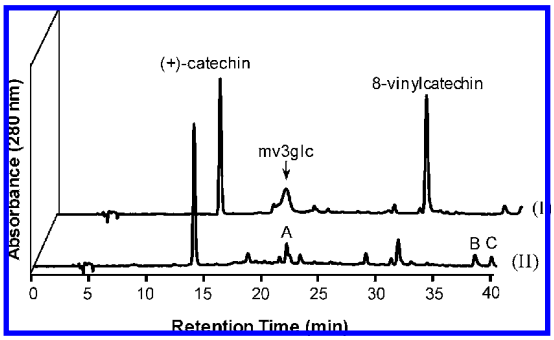

Figure 1. HPLC chromatograms recorded at 280 nm of the reaction of mv3glc (0.48 mM) with 8-vinylcatechin (19 mM) recorded at the initial time (I) and after 90 min (II) of reaction.

the formation of vinylphenol anthocyanin-derived pigments (*12*). The 8-vinylflavanol adducts in wines are not present in grapes and have been proposed to result from the dehydration of the flavanol−acetaldehyde adducts and by the cleavage of ethyl-linked flavanol adducts formed in wines, both from the reaction of flavanols with acetaldehyde (*24*). Nevertheless, these mechanisms remain to be clarified.

A few years ago, a new class of blue anthocyanin derivatives (vinylpyranoanthocyanin−flavanols or *portisins*) was detected and isolated directly from Port red wines (*25*). Their synthesis was already reported in model solutions through the reaction between carboxypyranoanthocyanins and flavanols mediated by acetaldehyde (*26*). Once again, it is suggested that the 8-vinylflavanol adduct is an intermediate in the formation of these compounds in wines (*27*).

Pyranoanthocyanin pigments have attracted great interest because of their unusual spectroscopic properties. However, it has not been possible to isolate sufficient quantities of these compounds from wines to carry out structural characterization (MS, NMR) and chromatic and stability studies.

The aim of this work was to develop a synthesis of pyranomv3glc−(+)-catechin pigment from the reaction between 8-vinyl-(+)-catechin and mv3glc. Additionally, a better understanding of the participation of 8-vinylcatechin in the formation of pyranoanthocyanins in wines mediated by acetaldehyde was developed.

MATERIALS AND METHODS

Reagents. (+)-Catechin was purchased from Sigma-Aldrich (Madrid, Spain). Acetaldehyde was obtained from Fluka Chemika (Buchs, Switzerland). TSK Toyopearl gel HW-40(S) was purchased from Tosoh (Tokyo, Japan). Malvidin-3-glucoside was isolated from a young red table wine (*Vitis vinifera* L. cv. Touriga Nacional) by semipreparative HPLC using a reversed-phase C18 column (250 mm × 4.6 mm i.d.), as reported elsewhere (*28*).

Synthesis of 8-Vinylcatechin. 8-Vinylcatechin was synthesized according to the procedures described elsewhere (*29*). Briefly, the synthesis started with the protection of the OH phenol groups of (+)-catechin by silylation, followed by bromination at C-8 of 3′,4′,5,7-tetra-*O*-*tert*-butyldimethylsilylcatechin. The bromine atom was then replaced by a vinyl group via a cross-coupling Suzuki reaction with subsequent removal of silyl groups to give the 8-vinylcatechin. The final product was a mixture of 8-vinylcatechin and (+)-catechin (1:1) itself, which is a byproduct formed during the Suzuki reaction by debromination of 3′,4′,5,7-tetra-*O*-*tert*-butyldimethylsilyl-8-bromocatechin. Several experiments to purify 8-vinylcatechin were performed by column chromatography techniques without any success.

Synthesis of Pyranomalvidin-3-glucoside−(+)-Catechin Pigment. Pyranomalvidin-3-glucoside−(+)-catechin was obtained through the reaction of mv3glc with 8-vinylcatechin. A solution containing mv3glc (0.48 mM)/8-vinylcatechin (molar ratio of 1:40) was prepared to study the intermediate products of that reaction, whereas a solution containing (1.6 mM)/8-vinylcatechin (molar ratio of 1:10) was set to improve the synthesis of pyranoanthocyanin pigment. Both reactions were performed in 5% ethanol/water solution at pH 3.5 (adjusted with dilute HCl or NaOH) and at 30 °C reaction temperatures. The formation of the new pigments was monitored every day by high-performance liquid chromatography (HPLC) with diode array detection (DAD) at 500 nm. After 20 days, the synthesis reaction was stopped and the pyranoanthocyanin pigment was purified.

Purification of Pyranomalvidin-3-glucoside−(+)-Catechin Pigment. The reaction mixture was directly deposited onto a medium-porosity sintered glass funnel loaded with TSK Toyopearl gel HW-40 (S) (40 mm × 70 mm) connected to standard vacuum filtration glassware and then washed exhaustively with water (~300 mL) and eluted with 30% aqueous methanol acidified with 2% HCl (~200 mL), yielding the first fraction containing the original anthocyanin. The second fraction containing the pyranoanthocyanin pigment was recovered by elution with 60% aqueous methanol acidified with 2% HCl (~100 mL). The solvent was eliminated by evaporation, and this fraction was then applied onto a TSK Toyopearl gel HW-40 (S) column (250 mm × 16 mm i.d.). Flow rate was regulated at 0.8 mL/min using a peristaltic pump. The elution was performed with 40% aqueous methanol acidified with 2% HCl, where the orange band corresponding to the pyranoanthocyanin pigment was collected in different fractions. These fractions were concentrated under vacuum and then applied on a 250 mm × 16 mm i.d. TSK Toyopearl gel HW-40 (S) column, from which the pigment was eluted with 100% acidified methanol for a final purification. After removal of methanol under vacuum and freeze-drying, the pyranoanthocyanin pigment obtained (2.0 mg) was used for NMR analyses. ^{1}H NMR (500.13 MHz, CD_3OD/TFA 98:2), δ 7.64 (s, 1H, H-9D), 7.61 (s, 2H, H-2'B,6'B), 7.09 (d, J = 1.8 Hz, 1H, H-8A), 6.91 (d, J = 1.8 Hz, 1H, H-6A), 6.89 (d, J = 1.6 Hz, 1H, H-2′F), 6.80 (d, J = 8.1 Hz, 1H, H-5′F), 6.78 (dd, J = 1.6, 8.1 Hz, 1H, H-6′F), 6.19 (s, 1H, H-6E), 4.83 (d, J = 6.4 Hz, 1H, H-2G), 4.74 (d, J = 7.7 Hz, 1H, H-Gl1), 4.09−4.15 (m, 1H, H-3G), 3.97 (s, 6H, OCH_3), 3.67 (dd, J = 1.8, 11.7 Hz, 1H, H-Gl6a), 3.54 (t, J = 9.2 Hz, 1H, H-Gl2), 3.36 (*, 1H, H-Gl6b), 3.30 (*, 1H, H-Gl3), 3.17 (*, 1H, H-Gl4), 3.10 (*, 1H, H-Gl5), 2.81 (dd, J = 5.1, 16.1 Hz, 1H, H-4αG), 2.62 (dd, J = 7.0, 16.1 Hz, 1H, H-4βC); ^{13}C NMR (125.77 MHz, CD_3OD/TFA 98:2), δ 169.0 (C-10D), 167.5 (C-7A), 162.8 (C-5E), 161.7 (C-2C), 156.7 (C-8aE), 154.8 (C-5A), 153.3 (C-8aA), 149.0 (C-3′B,5′B), 146.4 (C-4′F), 142.3 (C-4′B), 133.9 (C-3C), 131.3 (C-3′F), 120.7 (C-1′B), 119.4 (C-1′F), 118.8 (C-5′F), 115.6 (C-6′F), 114.9 (C-2′F), 108.4 (C-2′B,6′B), 108.2 (C-4C, C-4aA), 106.5 (C-9D), 103.9 (C-Gl1), 102.0 (C-4aE), 101.5 (C-8E), 100.7 (C-8A), 100.0 (C-6A), 83.0 (C-2G), 78.7 (C-Gl5), 76.7 (C-Gl3), 75.4 (C-Gl2), 70.9 (C-Gl4), 67.9 (C-3G), 62.5 (C-Gl6a, C-Gl6b), 56.7 (OCH_3).

Reaction of Malvidin-3-glucoside with (+)-Catechin Mediated by Acetaldehyde. A solution containing malvidin-3-glucoside (2 mM)/(+)-catechin/acetaldehyde (molar ratio of 1:4:10) was prepared in 12% ethanol/water at pH 3.2 and 30 °C. The formation of the products was monitored every day by HPLC with DAD at 500 nm.

HPLC-DAD Analysis. The samples were analyzed by HPLC (Merck-Hitachi L-7100) on a 150 mm × 4.6 mm i.d. reversed-phase C18 column (Merck) thermostated at 25 °C; detection was carried out at 500 nm using a diode array detector (Merck-Hitachi L-7450A). Solvents were (A) a mixture of water/formic acid 9:1 (v/v) and (B)

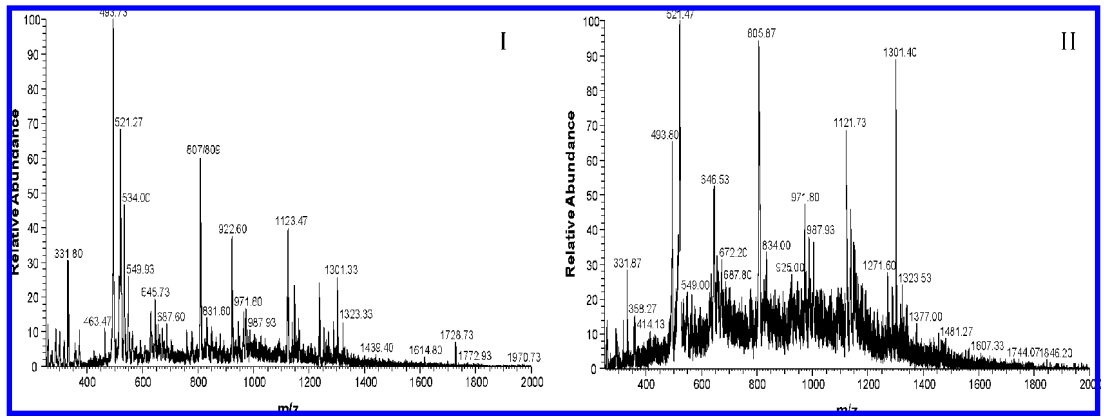

Figure 2. MS spectra obtained from ESI-MS analysis of the reaction of malvidin-3-glucoside (*m/z* 493) with 8-vinylcatechin after 90 min (I) and 2 days (II) of reaction.

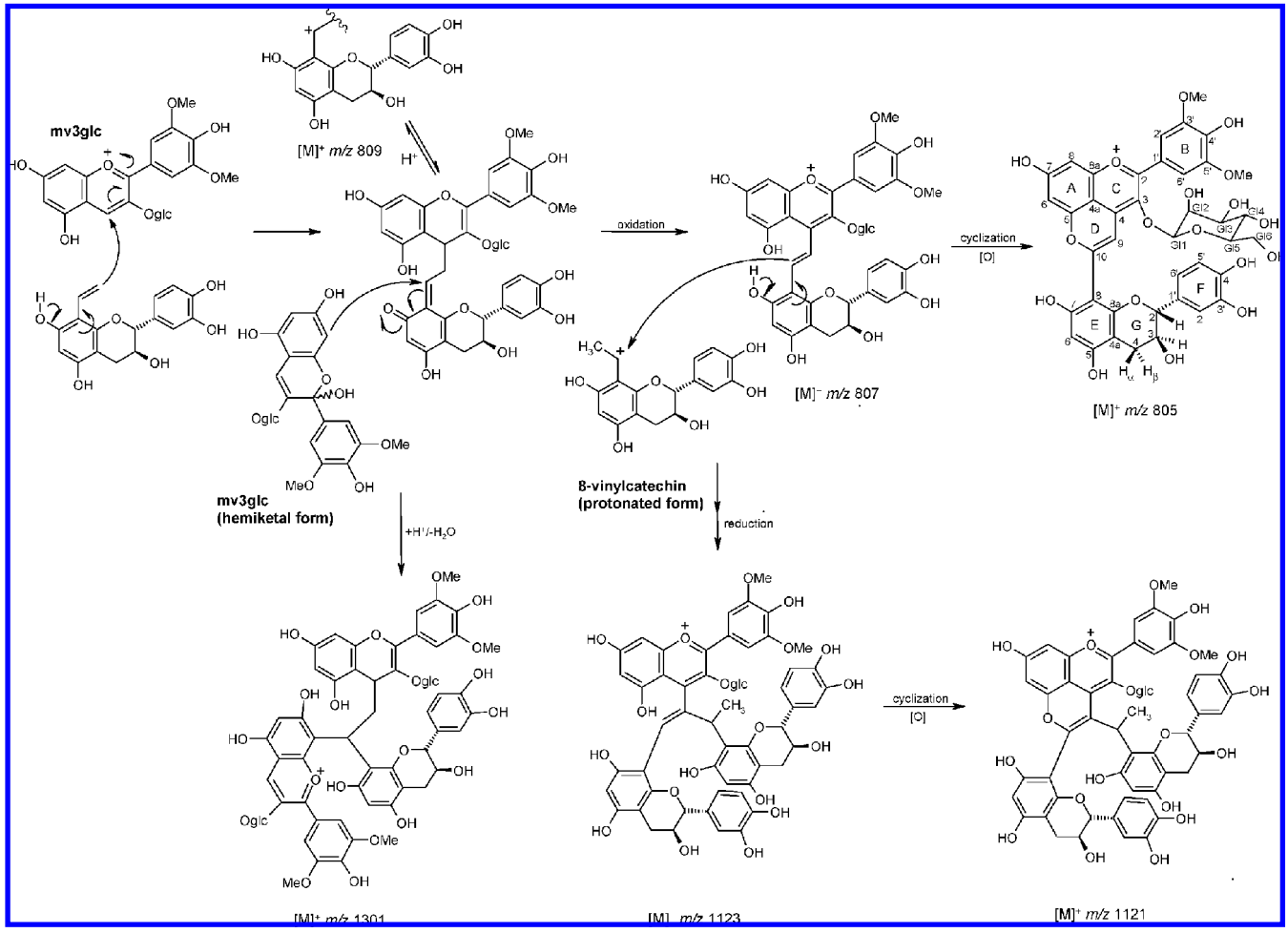

Figure 3. Proposed reaction mechanisms between mv3glc and 8-vinylcatechin.

acetonitrile, with the following gradients: 10−100% B over 60 min (method 1) and 10−35% B over 50 min (method 2), both at a flow rate of 0.5 mL/min. The sample injection volume was 20 μL. The chromatographic column was washed with 100% B for 10 min and then stabilized with the initial conditions for another 10 min.

LC-DAD/ESI-MS and ESI-MS Analyses. A Finnigan Surveyor series liquid chromatograph, equipped with a Thermo Finnigan (Hypersil Gold) reversed-phase column (150 mm × 4.6 mm, 5 μm, C18) thermostated at 25 °C, was used. The samples were analyzed using the same solvents, gradients, injection volume, and flow rate referred to above for HPLC analysis. Double-online detection was done by a photodiode spectrophotometer and mass spectrometry. The mass detector was a Finnigan LCQ DECA XP MAX (Finnigan Corp., San Jose, CA) quadrupole ion trap equipped with an atmospheric pressure ionization (API) source, using an electrospray ionization (ESI) interface. The vaporizer and the capillary voltages were 5 kV and 4 V, respectively. The capillary temperature was set at 325 °C. Nitrogen was used as both sheath and auxiliary gas at flow rates of 90 and 25, respectively (in arbitrary units). Spectra were recorded in positive ion mode between *m/z* 250 and 1500.

The samples were also directly injected into the MS spectrometer with a pump at a flow rate of 3 μL/min. The capillary temperature and voltage used were 275 °C and 15 V, respectively, and spectra were obtained in positive ion mode between *m/z* 150 and 2200. The mass spectrometer was programmed to do a series of three scans: a full mass, a zoom scan of the most intense ion in the first scan, and a MS-MS of the most intense ion using relative collision energies of 30 and 60 V.

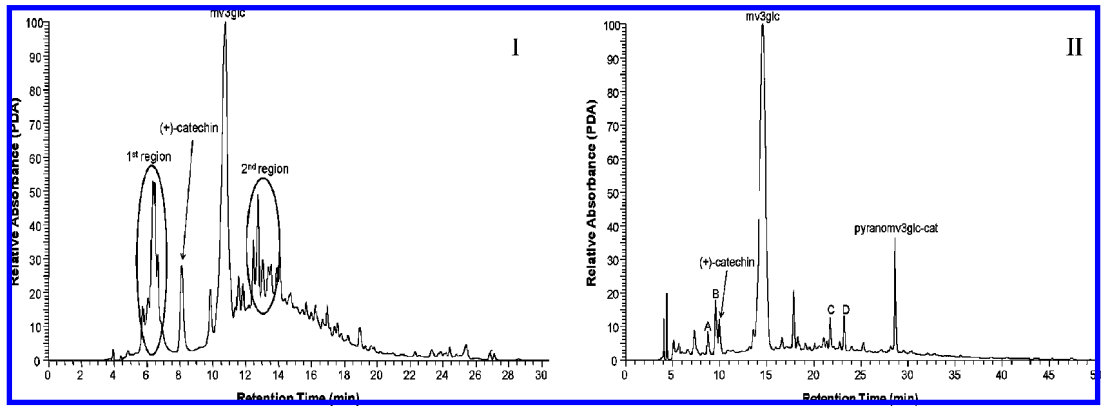

Figure 4. PDA chromatograms obtained from the LC-DAD analysis of the reaction between malvidin-3-glucoside (1.6 mM) and 8-vinylcatechin (16 mM) after 1 h (I) and after 3 days (II) of reaction. Chromatograms I and II were obtained using the gradients of methods 1 and 2, respectively (See Materials and Methods).

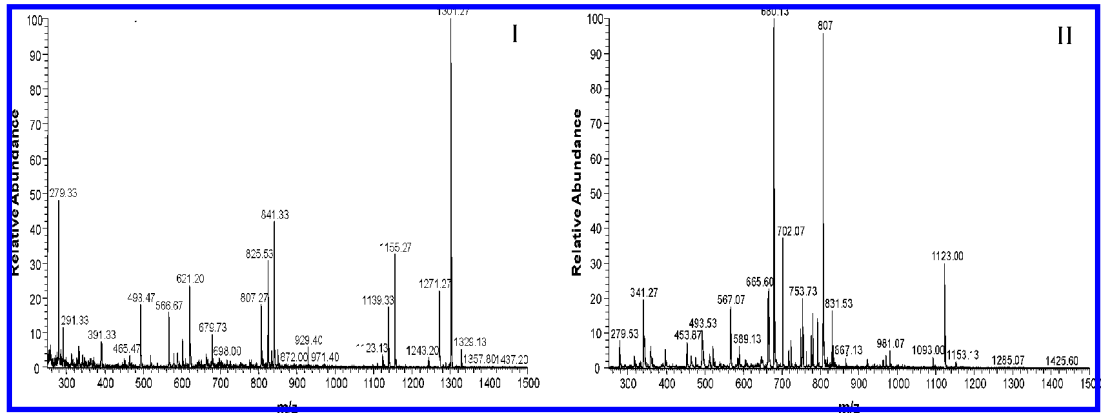

Figure 5. MS spectra of the first (I, 5.7−6.7 min) and second (II, 13.0−13.8 min) regions obtained from LC-ESI/MS analysis of the reaction of mv3glc with 8-vinylcatechin after 1 h of reaction.

NMR Analysis. ^{1}H NMR (500.13 MHz) and ^{13}C NMR (125.77 MHz) spectra were recorded in CD_3OD/TFA (98:2) on a Bruker-Avance 500 spectrometer at 303 K and with TMS as an internal standard [chemical shifts (δ) in parts per million, coupling constants (J) in hertz]. Multiplicities are recorded as singlets (s), doublets (d), triplets (t), doublets of doublets (dd), multiplets (m), and unresolved (*). ^{1}H chemical shifts were assigned using 2D NMR (COSY) experiment, whereas ^{13}C resonances were assigned using 2D NMR techniques (*g*HMBC and *g*HSQC) (*30, 31*). The delay for the long-range C/H coupling constant was optimized to 7 Hz.

RESULTS AND DISCUSSION

Study of the Reaction between Malvidin-3-glucoside and 8-Vinylcatechin. The initial chromatogram of the reaction between malvidin-3-glucoside and 8-vinylcatechin revealed the peaks of the reagents and the one of (+)-catechin, a byproduct of synthesis of 8-vinylcatechin (**Figure 1**) (*29*). After 90 min of reaction, the concentrations of mv3glc and 8-vinylcatechin had decreased significantly. No chromatographic peak corresponding to any pyranoanthocyanin or other anthocyanin-derived pigment that absorbs between 500 and 550 nm was detected. Nevertheless, the chromatographic peaks A−C, which absorbed at 280 nm, were formed during the reaction.

The appearance of these chromatographic peaks, which were already perceptible in the beginning of the reaction (chromatogram I), were also observed in previous studies regarding 8-vinylcatechin stability performed in 5% EtOH/H_2O solution at pH 3.5 and 30 °C, without mv3glc. This suggests that the anthocyanin is not involved in the formation of such compounds.

Analysis by LC-DAD/MS in positive ion mode revealed that peak A had the same molecular weight and identical MS data as 8-vinylcatechin. Thus, peak A showed the pseudomolecular ion $[M + H]^+$ at *m/z* 317, a fragment ion $[M + H - 152]^+$ at *m/z* 165 corresponding to a retro Diels−Alder (RDA) fission of the (+)-catechin moiety, and the fragment ion $[M + H - 152 - 18]^+$ at *m/z* 147 resulting from a further loss of water. This compound could result from the intramolecular cyclization of the 7-OH group and the vinyl group to give a new dihydrofuran ring, the formation of which is expected to be favored in acidic media. Peaks B and C revealed the same pseudomolecular ion $[M + H]^+$ at *m/z* 633, which is consistent with the structure of an 8-vinylcatechin dimer. Their formation could be explained by the acid-catalyzed dimerization of vinyl compounds. The mass spectra of peak B showed fragment ions $[M + H - 316]^+$ at *m/z* 317 and $[M + H - 152]^+$ at *m/z* 481, corresponding to a loss of one vinylcatechin unit and to a retro-Diels−Alder (RDA) fission of the (+)-catechin moiety, respectively. The two dimers B and C could be isomers differing in the stereochemistry of the asymmetric carbon in the interflavonoid linkage.

To study the formation of compounds involving mv3glc that could justify its decrease, the reaction solution was analyzed by mass spectrometry (MS) through direct injection at different times (90 min and 2 days). After 90 min of reaction, the direct ESI-MS analysis in positive ion mode showed several molecular ions that could indicate the rapid formation of compounds between malvidin-3-glucoside and 8-vinylcatechin (**Figure 2**).

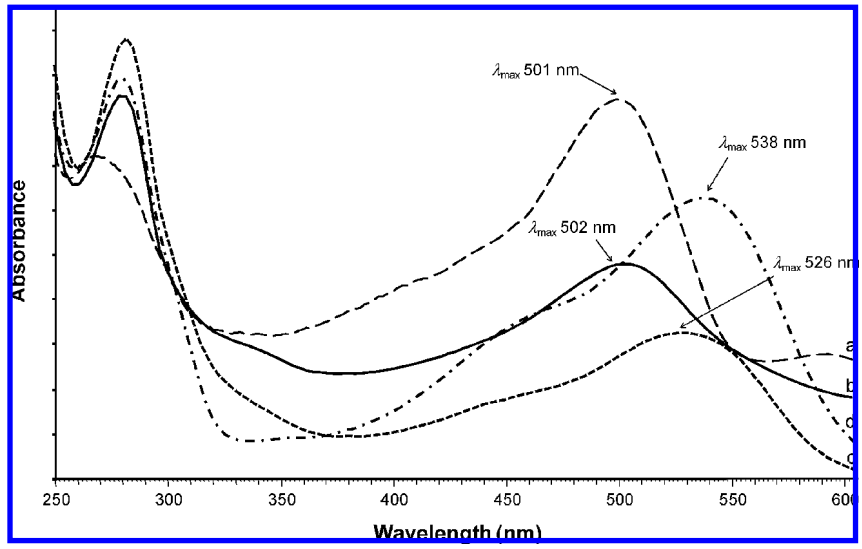

Figure 6. UV−vis spectra recorded from the LC-DAD analysis: (a) pyranomv3glc−catechin; (b) *m*/*z* 807; (c) *m*/*z* 1123; (d) *m*/*z* 1301.

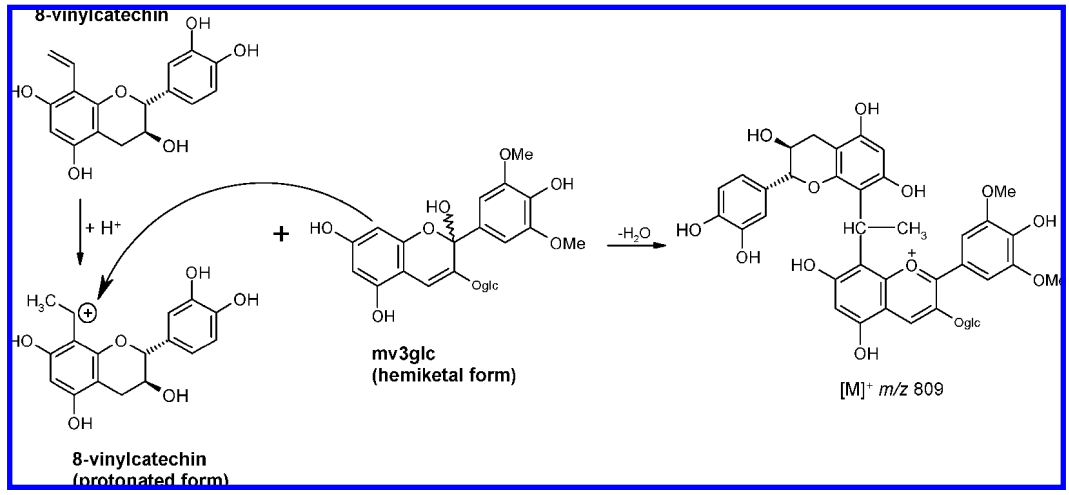

Figure 7. Proposed formation mechanism of 8,8-methylmethine catechin−mv3glc pigments from reactions of mv3glc with 8-vinylcatechin.

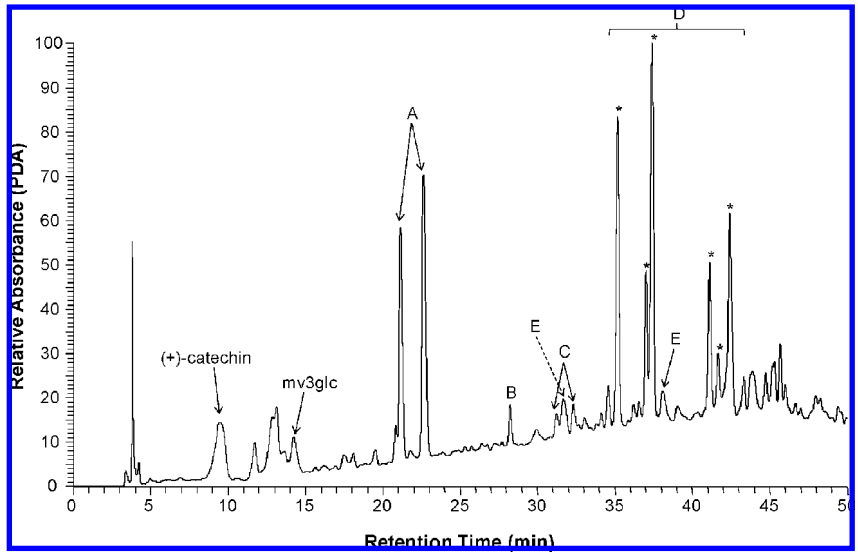

Figure 8. PDA chromatogram obtained from LC-DAD analysis of the reaction of malvidin-3-glucoside, (+)-catechin, and acetaldehyde (1:4:10) after 12 days.

The molecular ions $[M]^+$ and the respective MS^2 and MS^3 fragment ions are shown in **Table 1**. Two ion peaks $[M]^+$ at *m*/*z* 809 and *m*/*z* 807 were detected.

The ion $[M]^+$ at *m*/*z* 809 and the characteristic fragmentation are consistent with a direct condensation between malvidin-3-glucoside and 8-vinylcatechin, probably resulting from the

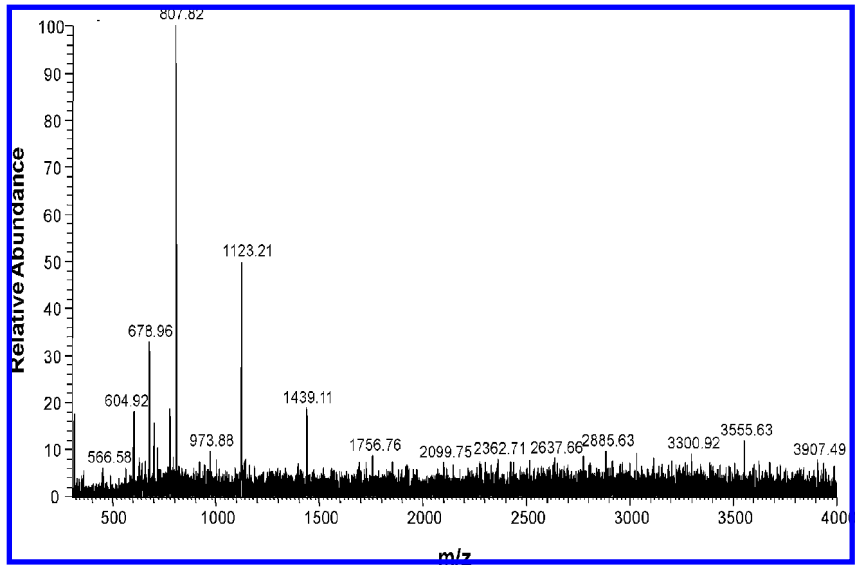

Figure 9. Selected MS spectrum obtained from ESI-MS analysis of the reaction of malvidin-3-glucoside, (+)-catechin, and acetaldehyde (1:4:10) after 12 days.

nucleophilic attack of the vinyl group to the electron-deficient position C-4 of the anthocyanin (**Figure 3**). The formation of this kind of carbocation structure (stabilized by their benzylic character), as intermediaries in the synthesis of pyranoanthocyanins, was already proposed by Fulcrand et al. (*32*).

The ion $[M]^+$ at *m/z* 807 could result from the oxidation of the compound at *m/z* 809, yielding the structure corresponding to a malvidin-3-glucoside linked to a (+)-catechin through a vinyl linkage.

The ion $[M]^+$ with *m/z* 1123 and its mass spectra are consistent with the structure of one malvidin-3-glucoside linked to two 8-vinylcatechins. This compound could result from the reaction of the compound with $[M]^+$ at *m/z* 807 with the protonated form of 8-vinylcatechin, through a possible mechanism depicted in **Figure 3**.

On the other hand, the signal detected at *m/z* 1301 is in agreement with a complex formed by two malvidin-3-glucosides with one molecule of 8-vinylcatechin. The formation of this structure could be originated from the nucleophilic attack of mv3glc in its hydrated form (hemiketal), to the mv3glc−vinylcatechin complex ($[M]^+$, *m/z* 809) followed by a dehydration.

After 2 days of reaction, the direct ESI-MS analysis in positive ion mode showed two new molecular ions at *m/z* 805 and 1121, as well as the ion $[M]^+$ at *m/z* 1301 already found (**Figure 2**).

The structure of the detected compound at *m/z* 805 agrees with that of a pyranomalvidin-3-glucoside−(+)-catechin pigment, its mass spectral data (**Table 1**) being identical to those described in the literature for this compound (*23*). This pigment could arise from the intramolecular cyclization reaction and oxidation of the mv3glc−vinylcatechin complex ($[M]^+$, *m/z* 807) described above and which already exist after 90 min of reaction. A stable flavylium−vinylphenol derivative identical to that of compound with *m/z* 807 was already reported in the literature (*33*). The stability of the referred compound results from the absence of the OH group in C-5, which is required to form the pyranoanthocyanin structures as occurs in this study.

The compound with the molecular ion $[M]^+$ at *m/z* 1121 detected after 2 days of reaction could derive from the compound at *m/z* 1123 detected after 90 min of reaction, through a mechanism identical to that proposed above for the formation of pyranomalvidin-3-glucoside−(+)-catechin.

The signal at *m/z* 809 is no longer present after 2 days of reaction. This could be explained by its total conversion into the mv3glc−vinylcatechin (*m/z* 807), which is the precursor of pyranomv3glc−(+)-catechin pigment. The 8,8-methylmethine catechin−mv3glc pigment also possess a molecular mass at *m/z* 809. However, if this compound was formed, it should be present at the end of the reaction, which is not the case under the described experimental conditions.

Synthesis of Pyranomv3glc−(+)-Catechin Pigment. The reaction of mv3glc with 8-vinylcatechin to synthesize the pyranomv3glc−(+)-catechin pigment was analyzed by LC-DAD/MS after 1 h of reaction, and the respective chromatogram showed two regions in which the same molecular ions of the compounds previously described were detected (**Figure 4**).

The first region contains the molecular ion $[M]^+$ at *m/z* 1301, whereas the second region revealed the presence of the molecular ions $[M]^+$ at *m/z* 1123 and 807 (**Figure 5**). The UV−vis spectra of the peaks of the two different regions are shown in **Figure 6**.

After 3 days of reaction, the LC-DAD/MS analysis revealed the presence of a new chromatographic peak ($[M]^+$, *m/z* 805), corresponding to pyranomalvidin-3-glucoside−(+)-catechin (**Figure 4**). The UV−vis spectra of the synthesized pigment revealed a λ_{max} at 501 nm (**Figure 6**). The mass and UV−vis spectra of the pyranomv3glc−catechin pigment are in agreement with the previously reported data (*23, 34*). The formation of pyranomv3glc−catechin adduct was followed by HPLC, and the reaction was stopped after 20 days, corresponding to the maximum concentration of compound. This pigment was isolated by liquid chromatography, and its structure was further elucidated by NMR. The full ^1H and ^{13}C NMR characterization of this compound is reported herein for the first time and agrees with the partial data already published (*23*).

The LC-DAD/MS analysis also revealed the presence of the minor compounds A and B, both with the same mass spectra corresponding to *m/z* 1301. Their λ_{max} in the visible region is about 538 nm, which corresponds to a red-purple color.

Two minor compounds, peaks C and D, corresponding to the 8,8-methylmethine catechin−mv3glc pigments (*m/z* 809) were also detected. These compounds were described in the literature to result from the reaction of mv3glc with catechin mediated by acetaldehyde. A proposed mechanism involving

8-vinylcatechin is shown in **Figure 7**. The first step involves the protonation of 8-vinylcatechin resulting in the formation of a benzylic carbocation. The second part of the mechanism is identical to the ones described in the literature for the formation of these pigments (*5*).

Because the pyranomv3glc−catechin pigment is the main product of that reaction, 8-vinylcatechin seems to act as a nucleophilic that attacks preferentially the pyran ring of the flavylium cation (**Figure 3**) rather than undergoing protonation to give the carbocation that would further react with C6/C8 positions of ring A of mv3glc.

Study of the Formation of Pyranomv3glc−(+)-Catechin from the Reaction of Malvidin-3-glucoside with (+)-Catechin Mediated by Acetaldehyde. The presence of pyranoanthocyanin−(+)-catechin compounds is reported in the literature to result from the reactions of anthocyanins with (+)-catechin mediated by acetaldehyde (*20*). To better understand the mechanisms leading to the formation of these orange pigments in the presence of acetaldehyde, and the involvement of 8-vinylcatechin as an intermediate, the formation of the previously identified compounds was monitored in a reaction of malvidin-3-glucoside, (+)-catechin, and acetaldehyde (1:4:10) performed in a wine model solution (12% ethanol/water at pH 3.2) at 30 °C.

After 12 days, the reaction mixture was analyzed by LC-DAD/MS and by ESI-MS direct injection. The chromatogram obtained from the LC-DAD analysis showed traces of (+)-catechin and mv3glc as well as a large number of peaks corresponding to compounds formed during the reaction (**Figure 8**). The molecular ions $[M]^+$ and the respective MS^2 and MS^3 fragment ions of the main chromatographic peaks are shown in **Table 2**.

Peaks A (*m/z* 809), D (*m/z* 1125), and E (*m/z* 607) corresponding, respectively, to the 8,8-methylmethine catechin−mv3glc dimers, 8,8-methylmethine catechin−catechin−mv3glc trimers, and 8,8-methylmethine catechin−catechin dimers were already described to be formed in these reactions (*24*).

The mass and UV−vis spectra of peaks B (*m/z* 805) and C (*m/z* 633) are in agreement with the structures of the pyranomalvidin-3-glucoside−(+)-catechin pigment and 8-vinylcatechin dimers, respectively. The presence of the latter is a sign for the formation of 8-vinylcatechin during that reaction. However, through LC-DAD/MS analysis, it was not possible to detect the intermediate compounds previously described resulting from the reactions of mv3glc with 8-vinylcatechin. For this reason, the reaction mixture was further analyzed by direct injection into the mass spectrometer.

The ESI-MS direct analysis revealed, although in low concentration, the two molecular ions $[M]^+$ at *m/z* 807 (mv3glc−vinylcatechin) and *m/z* 1123 (mv3glc−divinylcatechin) already found in the reactions of malvidin-3-glucoside with 8-vinylcatechin (**Figure 9**).

The other molecular ions $[M]^+$ at *m/z* 1121 and 1301 were also investigated. However, their presence in this reaction was not detected, probably because the formation of vinylcatechin is very slow over the time to form those more complex structures. Opposite the reaction of mv3glc with 8-vinylcatechin, the concentration of pyranomv3glc−(+)-catechin is much lower than that of the 8,8-methylmethine catechin−mv3glc pigments. According to the mechanism described in the literature (*5*), acetaldehyde attacks the phloroglucinol ring of catechin to give the respective catechin−ethanol adduct. Further dehydration gives rise to the formation of the protonated form of the 8-vinylcatechin (carbocation) shown in **Figure 7**, which undergoes a nucleophilic attack from the C6/C8 of the mv3glc. Only a small part of this carbocation seems to be displaced toward the formation of the 8-vinylcatechin to generate the pyranomv3glc−catechin compound.

Conclusion. In the present work, 8-vinylcatechin was shown, on the one hand, to be very unstable in model wine solution, undergoing acid-catalyzed dimerization, and, on the other hand, to be very reactive with mv3glc, yielding a great variety of anthocyanin derivatives. This possibly explains why 8-vinylcatechin formation has never been detected in wines or in model wine solutions. The presence of mv3glc−vinylcatechin (*m/z* 807) and mv3glc−divinylcatechin (*m/z* 1123) compounds in the reactions involving mv3glc, catechin, and acetaldehyde suggests that the formation of pyranoanthocyanins in wines could originate from them. This evidence helps to clarify the importance of 8-vinylcatechin as an intermediate in the formation mechanism of pyranoanthocyanins identified in aged red wines.

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