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A New Class of Blue Anthocyanin-Derived Pigments Isolated from Red Wines

NUNO MATEUS,*,† ARTUR M. S. SILVA,‡ JULIAN C. RIVAS-GONZALO,§ CELESTINO SANTOS-BUELGA,§ AND VICTOR DE FREITAS†

Centro de Investigação em Química, Departamento de Química, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal; Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal; and Unidad de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, E-37007 Salamanca, Spain

Two newly formed anthocyanin-derived pigments that revealed unique spectroscopic features, showing maximum absorption in their UV−vis spectra at 575 nm, were isolated by TSK Toyopearl HW-40 (S) gel column chromatography and semipreparative HPLC from an aged Port red wine. Further characterization by ESI/MS and NMR (^{1}H, *g*COSY) showed them to belong to a new class of pigments described here for the first time, the structure of which consisted of a pyranoanthocyanin moiety linked to a flavanol by a vinyl bridge. The extended conjugation of the π electrons throughout all the pigment molecule is likely to confer a higher stability on it and is probably the origin of the intense blue color. The formation of these pigments was found to arise from the reaction between anthocyanin−pyruvic acid adducts and vinyl−flavanol adducts.

KEYWORDS: **Pyranoanthocyanin; flavanols; red wine; aging; blue color**

INTRODUCTION

The color of red wine changes progressively during its life, due to the substitution of grape anthocyanins by other pigments whose structures, occurrence in wine, and mechanisms of formation are still not completely elucidated. Since the earliest works by Somers (*1*, *2*), different types of processes have been shown or proposed to participate in such changes. Classically, the condensation between anthocyanins and flavanols, either direct (*2*−*4*) or mediated by acetaldehyde (*5*−*7*), was assumed to constitute the main mechanism in the formation of wine pigments. More recently, reactions of anthocyanins and/or flavanols with smaller compounds, such as pyruvic acid (*8*−*13*), vinylphenol (*14*, *15*), or glyoxylic acid (*16*, *17*), have also been demonstrated, and new pigment families (namely, pyranoanthocyanins and xanthylium derivatives) resulting from them have been identified. Until very recently, most of the conclusions obtained regarding the identity of new pigments and their mechanisms of formation derived from studies carried out in model solutions. It has been only recently that the first pigments have been shown to occur directly in wine (*18*−*21*), mainly due to the development of the LC-MS technique. Most of the pigments detected in wines correspond to red-orange pyranoanthocyanin-related structures, and only occasionally much lower levels of other pigment classes have also been detected, in particular the red-bluish pigments resulting from the acetaldehyde-mediated condensation of anthocyanins and flavanols. Furthermore, the formation of products from the anthocyanin−flavanol direct condensation has also been established (*18*, *22*). In previous studies, various pyranoanthocyanin structures bearing different flavan-3-ol substituents were isolated from Port wine and completely characterized by MS and NMR (*23*, *24*). Those pigments were proposed to result from the reaction between anthocyanins and vinyl−flavanol adducts derived either from the cleavage of ethyl-linked flavanol oligomers or from the dehydration of the flavanol−ethanol adduct formed after reaction with acetaldehyde. In this investigation, a new class of blue pigments isolated from Port red wine has been discovered, the structure of which has not been identified or postulated before. These pigments are proposed to result from the reaction between anthocyanin−pyruvic acid adducts and vinyl−flavanols, thus corresponding to a further step in the formation of complex structures involved in the changes of color in red wines.

MATERIALS AND METHODS

Source. The pigments were extracted from 2 L of a 2-year-old Port red wine (pH 3.6, 18.5% alcohol (v/v), total acidity 6.5 g/L, total SO_2 20 mg/L), made from grapes of Touriga Nacional and Touriga Francesa varieties (*Vitis vinifera*) grown in the Douro Demarcated Region (northern Portugal).

Pigment Purification. Port wine samples were applied directly onto a 250-mm × 16-mm-i.d. TSK Toyopearl gel HW-40(S) column (Tosoh, Japan) and eluted with water with an increasing percentage of ethanol

* To whom correspondence should be addressed (E-mail nbmateus@fc.up.pt, fax +351.226082959, telephone +351.226082858).
† Universidade do Porto.
‡ Universidade de Aveiro.
§ Universidad de Salamanca.

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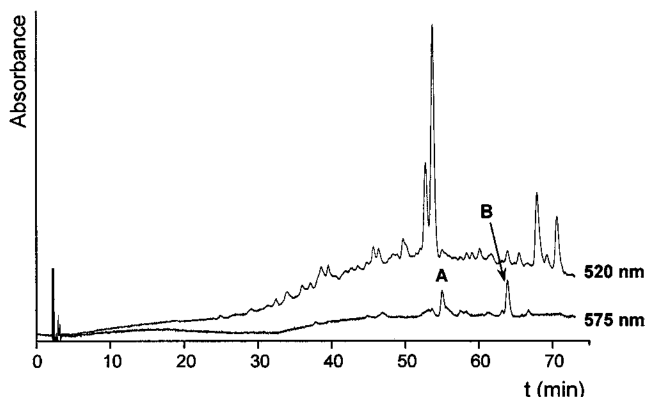

Figure 1. HPLC chromatograms recorded at 520 and 575 nm of the Port wine fraction eluted from TSK Toyopearl gel column with ethanol 99.8% (v/v), showing peaks A and B that correspond to the two new blue pigments isolated.

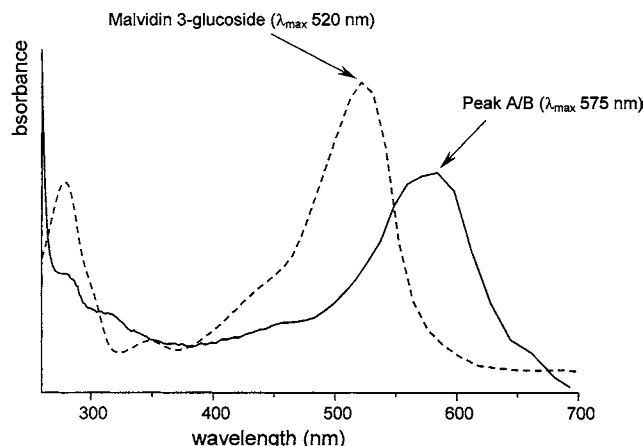

Figure 2. UV–visible spectra of malvidin-3-glucoside and peaks A/B as recorded with the HPLC diode array detector.

up to 40% aqueous ethanol, yielding the original anthocyanidin 3-glucosides and some pyruvic acid adducts of the three major anthocyanidin 3-glucosides (malvidin 3-glucoside, malvidin 3-acetylglucoside, and malvidin 3-coumaroylglucoside) and three anthocyanin−vinyl−flavanol pigments, as reported previously (*12*, *23*). When practically no more colored compounds were eluted from the column, the solvent was changed to 99.8% ethanol (v/v), yielding the coumaroyl derivatives of anthocyanin−vinyl−flavanol pigments (*24*), together with other pigments showing maximum absorption at 575 nm (**Figure 1**). The pH of all the eluents was set to 2.0 with HCl.

Analytical HPLC Conditions. The fraction that eluted with 99.8% (v/v) ethanol from the TSK Toyopearl gel column was analyzed by HPLC (Merck-Hitachi L-7100) on a 250-mm × 4.6-mm-i.d. reversed-phase C18 column (Merck, Darmstadt, Germany); detection was carried out at 520 nm using a diode array detector (Merck-Hitachi L-7450A). The solvents were (A) H_2O/HCOOH (9:1) and (B) CH_3CN/H_2O/HCOOH (3:6:1). The gradient consisted of 20−85% B for 70 min, 85−100% B for 5 min, and then isocratic for 10 min at a flow rate of 1 mL/min (*25*).

Semipreparative HPLC Conditions. The pigments were purified by semipreparative HPLC using the above indicated reversed-phase C18 column using an injection volume of 500 μL and the same gradient program. Each pigment was collected, concentrated under vacuum, and applied on a 150-mm × 16-mm-i.d. TSK Toyopearl HW-40(S) gel column, which was eluted with distilled methanol for a final purification in order to remove the rest of the HPLC solvents and the compounds responsible for the hump situated under their chromatographic peaks in RP-HPLC.

ESI/MS Analysis. Mass spectrometry analyses were performed using a Finnigan LCQ equipped with an API source, using an electrospray ionization (ESI) probe. The pigments were injected directly into the MS spectrometer with a pump at a flow rate of 3 μL/min. The capillary temperature and voltage used were 180 °C and 3 V, respectively, and spectra were obtained in positive-ion mode. When the molecular ion of the pigment was detected, the mass spectrometer was programmed to obtain the MS^2 spectrum using a relative energy of collision of 20.

NMR Analysis. ^1H NMR (500.13 MHz) spectra were measured in CD_3OD/TFA (98:2) on a Bruker-AMX500 spectrometer at 303 K and with TMS as internal standard. ^1H chemical shifts were assigned using 1D and 2D ^1H NMR (COSY).

Formation of Pigments. Formation of the blue pigments was monitored at 35 °C in 20% aqueous ethanol (pH 2.0) in a 2-mL screw-cap vial containing 0.2 mg of malvidin-3-coumaroylglucoside−pyruvic acid adduct, previously isolated (*12*), and 0.33 mg of (+)-catechin. The total volume of the solution was set to 50% of the vial capacity. After 15 days of reaction, the solution was analyzed by HPLC (Merck-Hitachi L-7100) using the reversed-phase C18 column described above; detection was carried out with a diode array detector (Merck-Hitachi L-7450A). The solvents were (A) H_2O/HCOOH (9:1) and (B) $CH_3CN/H_2O/CH_3COOH$ (8:1.95:0.05). The gradient consisted of 10−35% B for 50 min at a flow rate of 1.5 mL/min.

RESULTS AND DISCUSSION

The HPLC chromatogram recorded at 520 nm of the fraction resulting from the elution of several Port wine samples from a TSK Toyopearl gel column with 99.8% ethanol (v/v) showed the presence of four major red pigments (**Figure 1**). Three of these compounds were previously identified as the coumaroyl derivatives of anthocyanin−vinyl−flavanol pigments (*24*). Furthermore, the recording of the HPLC chromatogram at 575 nm revealed the presence of two additional pigments (**Figure 1**) with maximum absorption in the visible region at that wavelength, significantly bathochromically shifted with respect to that of the anthocyanins (**Figure 2**). The two pigments (named A and B) were isolated by semipreparative HPLC for their structural characterization. During the final purification of each pigment by TSK Toyopearl gel column chromatography in acidic conditions (pH 2.0), a blue band was observed, in agreement with the UV−vis spectrum of the pigments. Recently, a pigment with the same λ_{max} was detected in model solutions resulting from the reaction between malvidin-3-glucoside and (+)-catechin in the presence of acetaldehyde (*26*), the structure of which was proposed to be a quinone form of malvidin-3-glucoside−ethyl−(+)-catechin adduct.

Mass Spectrometry. Pigments A and B were analyzed individually by ESI/MS by direct injection into the mass spectrometer. Analysis of pigment A produced a $[M]^+$ ion at m/z 1119 and three fragment ions: $[M - 162]^+$ at m/z 957, corresponding to the loss of a glucoside residue; $[M - 290]^+$ at m/z 829, corresponding to the loss of a catechin unit; and $[M - 452]^+$ at m/z 667, corresponding to the loss of both the glucoside residue and the catechin unit. The pigment B revealed a $[M]^+$ ion at m/z 1265, and its MS^2 spectrum showed a fragment ion $[M - 308]^+$ at m/z 957, corresponding to the loss of a coumaroylglucoside residue (**Figure 3**). These molecular ion masses fit exactly with the structures shown in **Figure 5**.

^{1}H NMR. The ^1H chemical shifts of pigments A and B in CD_3OD/TFA (98:2) are indicated in **Table 1**. The proton chemical shifts were assigned using 1D and 2D NMR techniques (COSY). The protons of the pyranoanthocyanin moiety were easily assigned, whereas the protons of the flavanol moiety were more difficult. The protons of the rings E, F, H, and I of both pigments could not be assigned due to the high complexity of the aromatic region in the respective ^1H NMR spectrum. In general, the NMR data of these pigments are in agreement with

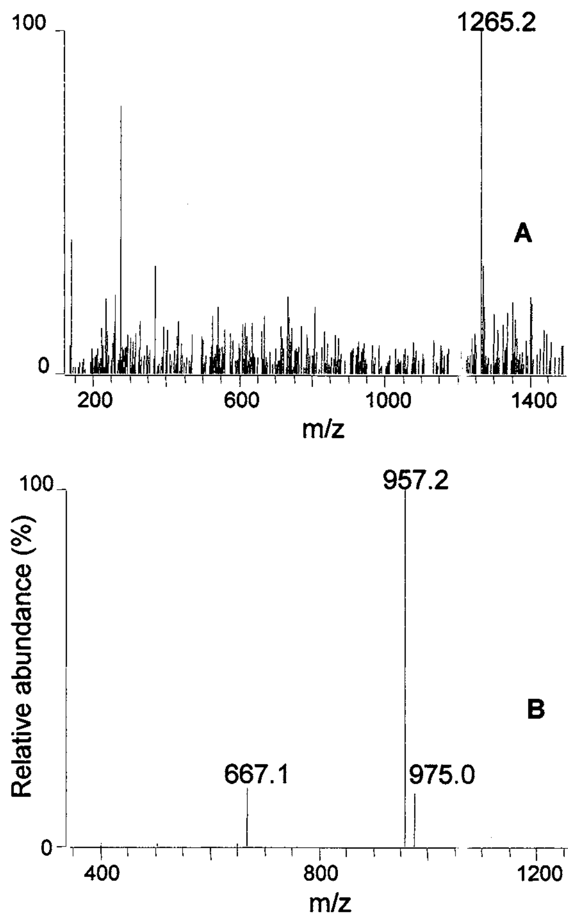

Figure 3. MS analysis of peak B obtained with an ion spray source in the positive-ion mode: (A) MS spectrum; (B) MS^2 spectrum of the ion at *m/z* 1265.

those of anthocyanin−vinyl−flavanol pigments previously described (*23*, *24*).

Pigment A. The spectrum of pigment A revealed the presence of two broad singlets located at 7.33 and 7.43 ppm, corresponding to protons H-6 and H-8 of ring A, respectively. Protons H-2′,6′ and two methoxyl groups of ring B were assigned to the singlets at 7.54 and 3.95 ppm, respectively. Proton H-9 of ring D was located at 8.12 ppm as a singlet. Concerning the flavanol moiety, the protons H-4α and H-4β of ring J were assigned to the unresolved peaks located at 3.08 and 2.39 ppm through the characteristic AMX spin system of the flavanol pyran ring observed in the COSY spectrum. Proton H-3J was assigned to an unresolved peak at 3.82 ppm from its correlation with H-4β found in the COSY spectrum, and proton H-2J was attributed to an unresolved peak located at 3.66 ppm from its correlation with H-3J. The analogous protons of the upper flavanol unit were similarly assigned. Proton H-3G was attributed to an unresolved peak situated at 3.91 ppm that correlates with another peak at 4.12 ppm, which was attributed to H-4G. Proton H-2G was assigned to the broad singlet located at 5.12 ppm. The ^{1}H NMR data obtained did not permit determination of the full identity of the two catechin unit [(+)-catechin or (−)-epicatechin] that constitute the flavanol moiety. Indeed, (+)-catechin units can be distinguished from (−)-epicatechin units by the coupling constant of proton H-2 of the flavanol pyran ring: a large doublet indicates a (+)-catechin moiety, whereas a broad singlet indicates a (−)-epicatechin moiety (*27*). In the case of pigment A, the proton H-2G was found to resonate as a broad singlet, whereas proton H-2J was attributed to an unresolved peak in the ^{1}H spectrum.

Table 1. ^{1}H Chemical Shifts of Pigments A and B Isolated from a 2-Year-Old Port Wine, Determined in CD_3OD/TFA (98:2)

position	δ ^{1}H; *J* (Hz)[a]	
	pigment A	pigment B
pyranoanthocyanin moiety		
6A	7.33; bs	7.30; bs
8A	7.43; bs	7.46; bs
9D	8.12; s	8.06; s
2′B,6′B	7.54; s	7.86; s
OMe	3.95; s	3.98; s
vinyl group		
H_α	8.28; d, 15.8	8.27; d, 15.8
H_β	7.29; d, 15.8	7.28; d, 15.8
flavanol moiety		
2G	5.12; bs	5.31; bs
2J	3.66; *	3.65; *
3G	3.91; *	3.94; *
3J	3.82; *	3.84; *
4G	4.12; *	4.31; *
4αJ	3.08; dd	3.07; dd
4βJ	2.39; dd	2.39; dd
rings E, F, H, I	6.5–7.4, *	6.5–7.4, *
sugar moiety		
Gl-1	4.71; d, 7.7	4.68; d, 7.7
Gl-2	3.61; *	3.62; *
Gl-3, Gl4, Gl-5, Gl-6	3.1–3.8; *	3.1–4.4; *
coumaroyl moiety		
$CH{=}CH_\alpha CO2R$		5.84; d, 15.9
$CH_\beta{=}CHCO2R$		7.29; d, 15.9
2″,6″		7.11; d
3″,5″		6.88; d

[a] Key: *, unresolved; bs, broad singlet; s, singlet; d, doublet; dd, doublet of doublets.

Therefore, these data suggest that the upper flavanol unit is consisted of one (−)-epicatechin molecule, but the identity of the lower flavanol unit cannot be determined. In addition, the interflavanoid linkage cannot be fully ascertained from the data from the NMR experiments, although a C4−C8 interflavanoid linkage is expected, since the C4−C8 procyanidin dimers (B1−B4) are more abundant in grapes and in the resulting wines than their respective C4−C6 counterparts (B5−B8) (*28*, *29*). Thus, the flavanol unit in pigment A could be either B1 or B2, namely those containing (−)-epicatechin as the upper subunit.

With respect to the glucoside protons, the anomeric proton (Gl-1) was detected at 4.71 ppm as a doublet with a large coupling constant (7.7 Hz). Proton Gl-2 was assigned at 3.61 ppm from its correlation with proton Gl-1 in the COSY spectrum. The other glucosyl proton signals were found in the region of 3.1−3.8 ppm.

Concerning the protons of the vinyl group, protons H_α and H_β revealed a clear correlation in the COSY spectrum and were attributed to two doublets located at 8.28 (J = 15.8 Hz) and 7.29 ppm (J = 15.8 Hz), respectively. The large coupling constant of these doublets suggests a trans stereochemistry.

Pigment B. The interpretation of the spectrum of pigment B was done similarly to that of pigment A. Once again, the ^{1}H NMR data obtained did not allow determination of the full identity of the procyanidin dimer that constitutes the flavanol moiety. The spectrum of pigment B showed that the proton H-2G resonates as a broad singlet, whereas proton H-2J was attributed to an unresolved peak. These data suggest that the upper flavanol unit consisted of one (−)-epicatechin molecule, but the identity of the lower flavanol unit cannot be deduced. As previously described for pigment A, the interflavanoid linkage of the procyanidin moiety in pigment B is also expected to be C4−C8.

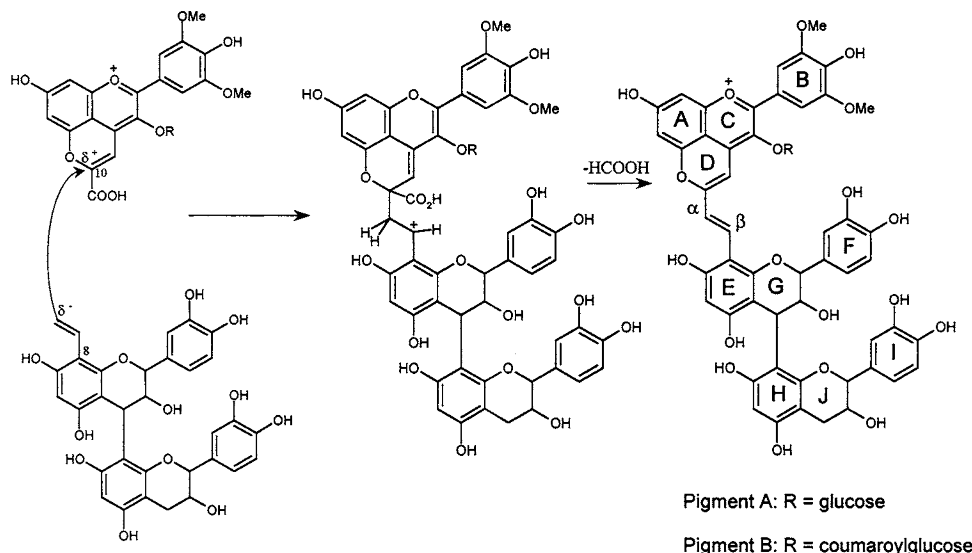

Figure 4. Suggested mechanism for the formation of pigments A and B.

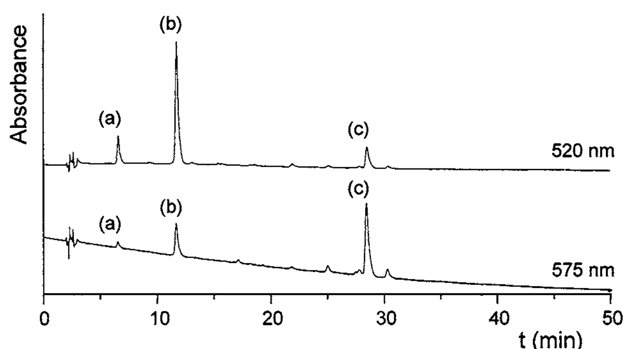

Figure 5. HPLC chromatograms recorded at 520 and 575 nm of the hydroalcoholic solution containing (+)-catechin and malvidin-3-coumaroylglucoside–pyruvic acid adduct after 15 days of reaction: (a) malvidin-3-glucoside–pyruvic acid adduct; (b) malvidin-3-coumaroylglucoside–pyruvic acid adduct; (c) new pigment formed.

The coumaroyl protons were also fully assigned. Two doublets, integrating for one proton each and located at 5.84 and 7.29 ppm, were attributed to the olefinic H_α and H_β of the coumaroyl moiety, respectively, with a large coupling constant ($J = 15.9$ Hz) that suggests a trans stereochemistry. Two other doublets, with a coupling constant of 8.6 Hz and integrating for two protons each, were also observed at 6.88 and 7.11 ppm and were attributed to the four aromatic protons of the coumaroyl ring.

Finally, the protons of the vinyl group that revealed a clear correlation in the COSY spectrum were attributed to two doublets located at 8.28 ($J = 15.8$ Hz) and 7.29 ppm ($J = 15.8$ Hz), corresponding to H_α and H_β, respectively. As previously observed for pigment A, the large coupling constant of these doublets suggests a trans stereochemistry.

Mechanism of Formation. A mechanism of formation of these blue anthocyanin−flavanol pigments is proposed in **Figure 4**. Anthocyanin−pyruvic acid derivatives, formed in a previous step and abundant in Port wines, are thought to react through their C-10 position with the vinyl group of an 8-vinyl−flavanol adduct. The vinyl−flavanol adducts may derive either from the cleavage of ethyl-linked flavanol oligomers resulting from the acetaldehyde-induced condensation of flavanols (*30*), from the dehydration of the flavanol−ethanol adduct formed after reaction with acetaldehyde, or from the cleavage of anthocyanin−ethyl−flavanol pigments. The last step of the formation involves the loss of a formic acid group and oxidation, yielding the new anthocyanin-derived pigment. The extended conjugation of the π electrons in this newly formed structure is likely to confer a higher stability of the molecule and is probably the origin of its blue color.

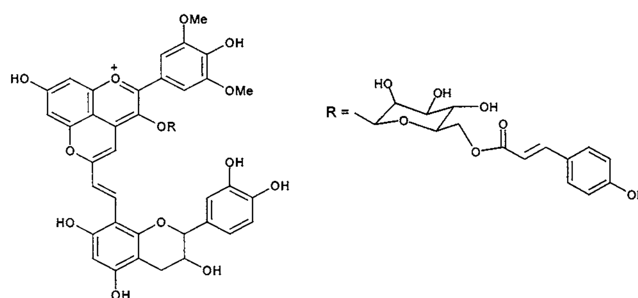

Figure 6. Proposed structure of peak c from **Figure 5**.

To obtain further evidence to support this mechanism, model solutions of malvidin-3-coumaroylglucoside−pyruvic acid adduct and (+)-catechin were prepared in 20% aqueous ethanol (v/v) (pH 2.0) and maintained at 35 °C in oxidative conditions. The HPLC chromatograms of the solution recorded after 15 days of reaction revealed the presence of malvidin-3-coumaroylglucoside−pyruvic acid adduct (b), together with a new peak (c) and malvidin-3-glucoside−pyruvic acid adduct (a), probably resulting from the loss of the coumaroyl group of malvidin-3-coumaroylglucoside−pyruvic acid adduct (**Figure 5**). The UV−vis spectrum of peak c was similar to those of pigments A and B, with λ_{max} at 575 nm. The LC/MS analysis of this newly formed pigment (c) showed a $[M]^+$ ion at *m/z* 977, which fits exactly with the structure in **Figure 6**, identical to that of pigment B, but showing the presence of a catechin unit instead of a procyanidin dimer in the flavanol moiety. Additionally, the MS data revealed one major fragment ion, $[M - 308]^+$ at *m/z* 669, corresponding to the loss of a coumaroylglucoside residue, as observed for pigment B. Overall, the formation of this new class of anthocyanin-derived pigments appears to be the result of the reaction between anthocyanin−pyruvic acid adducts and flavanols, both present in relevant amounts in Port wines.

The detection and structural characterization of these newly formed blue anthocyanin−vinyl−flavanol pigments represent the first evidence of blue pigments occurring in red wines and provide further information regarding the chemical transforma-

tions involved in the complex evolution of the color of red wines. The anthocyanin-derived pigments previously described in the literature arise from the reaction between genuine anthocyanins and other wine components, whereas the formation of these blue pigments involves anthocyanin secondary products. This feature points to new chemical pathways in the formation of red wine pigments, in which anthocyanins are no longer the main precursor. Although these blue pigments were only detected in very small quantities in fortified wines, they present unique spectroscopic features that may somehow contribute to the changing color of aged red wines. Nevertheless, further studies are still required in order to elucidate the factors that govern the formation of the different pigment families and to assess the actual contribution of each of them in the definition of the color of red wines.

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LITERATURE CITED

(1) Somers, T. C. Wine tannins isolation of condensed flavanoid pigments by gel-filtration. *Nature* **1966**, *209*, 368−370.
(2) Somers, T. C. The polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175−216.
(3) Jurd, L. Review of polyphenol condensation reactions and their possible occurrence in the aging of wines. *Am. J. Enol. Vitic*. **1969**, *20*, 191−195.
(4) Liao, H.; Cai, Y.; Haslam, E. Polyphenols interactions. Anthocyanins: Copigmentation and colour changes in red wines. *J. Sci. Food Agric*. **1992**, *59*, 299−305.
(5) Bakker, J.; Picinelli, A.; Bridle, P. Model wine solutions: Colour and composition changes during ageing. *Vitis* **1993**, *32*, 111−118.
(6) Rivas-Gonzalo, J. C.; Bravo-Haro, S.; Santos-Buelga, C. Detection of compounds formed through the reaction of malvidin-3-monoglucoside and catechin in the presence of acetaldehyde. *J. Agric. Food Chem*. **1995**, *43*, 1444−1449.
(7) Timberlake, C. F.; Bridle, P. Interactions between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic*. **1976**, *27*, 97−105.
(8) Bakker J.; Bridle, P.; Honda, T.; Kuwano, H.; Saito, N.; Terahara, N.; Timberlake, C. F. Isolation and identification of a new anthocyanin occurring in some red wines. *Phytochemistry* **1997**, *44*, 1375−1382.
(9) Bakker J.; Timberlake, C. F. Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem*. **1997**, *45*, 35−43.
(10) Fulcrand H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* **1998**, *47*, 1401−1407.
(11) Romero, C.; Bakker, J. Interactions between grape anthocyanins and pyruvic acid, with effect of pH and acid concentration on anthocyanin composition and color in model solutions. *J. Agric. Food Chem*. **1999**, *47*, 3130−3139.
(12) Mateus, N.; Silva, A. M. S.; Vercauteren, J.; De Freitas, V. A. P. Occurrence of anthocyanin-derived pigments in red wines. *J. Agric. Food Chem*. **2001,** *49*, 4836−4840.
(13) Mateus, N.; De Freitas, V. A. P. Evolution and stability of anthocyanin-derived pigments in Port wine during ageing. *J. Agric. Food Chem*. **2001**, *49*, 5217−5222.
(14) Cameira dos Santos, P. J.; Brillouet, J. M.; Cheynier, V.; Moutounet, M. Detection and partial characterisation of new anthocyanin-derived pigments in wine. *J. Sci. Food Agric.* **1996**, *70*, 204−208.
(15) Fulcrand, H.; Cameira dos Santos, P. J.; Sarni-Manchado, P.; Cheynier, V.; Bonvin, J. F. Structure of a new anthocyanin-derived wine pigment. *J. Chem. Soc., Perkin Trans. 1* **1996**, 735−739.
(16) Es-Safi, N. E.; Le Guernevé, C.; Labarbe, B.; Fulcrand, H.; Cheynier, V.; Moutounet M. Structure of a new xanthylium salt derivative. *Tetrahedron Lett*. **1999**, *40*, 5869−5872.
(17) Fulcrand, H.; Cheynier, V.; Oszmiansky, J.; Moutounet, M. An oxidised tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. *Phytochemistry* **1997**, *46*, 223−227.
(18) Atanasova, V.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Anal. Chim. Acta* **2002**, *458*, 15−27.
(19) Revilla, I.; Pérez-Magarino, S.; González-SanJosé, M. L.; Beltrán, S. Identification of anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with diode array and mass spectrometric detection. *J. Chromatogr. A* **1999**, *847*, 83−90.
(20) Vivar-Quintana, A. M.; Santos-Buelga, C.; Francia-Aricha, E.; Rivas-Gonzalo, J. C. Formation of anthocyanin-derived pigments in experimental red wines. *Food Sci. Technol. Int.* **1999**, *5*, 347−352.
(21) Mateus, N.; Pascual-Teresa, S.; Rivas-Gonzalo, J. C.; Santos-Buelga, C.; De Freitas, V. A. P. Structural diversity of anthocyanin-derived pigments in Port wines. *Food Chem*. **2002**, *76*, 335−342.
(22) Remy, S.; Fulcrand, H.; Labarbe, 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.
(23) Mateus, N.; Silva, A. M. S.; Rivas-Gonzalo, J. C.; Santos-Buelga, C.; De Freitas, V. A. P. Identification of anthocyanin−flavanol pigments in red wines by NMR and mass spectrometry. *J. Agric. Food Chem*. **2002**, *50*, 2110−2116.
(24) Mateus, N.; Carvalho, E.; Carvalho, A. R. F.; Melo, A.; González-Paramás, A. M.; Santos-Buelga, C.; Silva, A. M. S.; Rivas-Gonzalo, J. C.; De Freitas, V. A. P. Isolation and structural characterization of new acylated anthocyanin−vinyl−flavanol pigments occurring in aging red wines *J. Agric. Food Chem*. **2003**, *51*, 277−282.
(25) Roggero, J. P.; Coen, S.; Archier, P.; Rocheville-Divorne, C. Étude par C. L. H. P. de la reaction glucoside de malvidine-acétaldéhyde-composé phénolique. *Connaiss. Vigne Vin* **1987**, *21*, 163−168.
(26) Escribano-Bailón, T.; Álvarez-Garcia, M.; Rivas-Gonzalo, J. C.; Heredia, F. J.; Santos-Buelga, C. Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin 3-*O*-glucoside and (+)-catechin. *J. Agric. Food Chem*. **2001**, *49*, 1213−1217**.**
(27) Fletcher, A. C.; Porter, L. J.; Haslam, E.; Gupta, R. K. Plant proanthocyanidins. Part 3. Conformational and configurational studies of natural procyanidins. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1628−1637.
(28) Mateus, N.; Proença, S.; Ribeiro, P.; Machado, J. M.; De Freitas, V. A. P. Grape and wine polyphenolic composition of red *Vitis vinifera* varieties concerning vineyard altitude. *Cienc. Tecnol. Aliment*. **2001**, *3*, 102−110.
(29) Mateus, N.; Marques, S.; Gonçalves, A. C.; Machado, J. M.; De Freitas, V. A. P. Proanthocyanidin composition of red *Vitis vinifera* varieties from the Douro Valley during ripening: Influence of cultivation altitude. *Am. J. Enol. Vitic.* **2001**, *52* (2), 115−121.
(30) Es-Safi, N. E.; Fulcrand H.; Cheynier, V.; Moutounet, M. Studies on the acetaldehyde induced condensation of (−)-epicatechin and malvidin 3-*O*-glucoside. *J. Agric. Food Chem*. **1999**, *47*, 2096−2102.

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