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Isolation and Structural Characterization of New Acylated Anthocyanin−Vinyl−Flavanol Pigments Occurring in Aging Red Wines

NUNO MATEUS,*,† ELISABETE CARVALHO,† ALEXANDRE R. F. CARVALHO,‡ ANDRÉ MELO,‡ ANA M. GONZÁLEZ-PARAMÁS,§ CELESTINO SANTOS-BUELGA,§ ARTUR M. S. SILVA,|| 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, CEQUP, 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

Three newly formed pigments were detected and isolated from a 2-year-old Port wine through TSK Toyopearl HW-40(S) gel column chromatography and characterized by UV−visible spectrophotometry, NMR, and mass spectrometry (ESI/MS). ^{1}H NMR and ^{13}C NMR data for these pigments obtained using 1D and 2D NMR techniques (COSY, NOESY, *g*HSQC, and *g*HMBC) are reported for the first time. The structure of the pigments was found to correspond to the vinyl cycloadducts of malvidin 3-coumaroylglucoside bearing either a procyanidin dimer or a flavanol monomer ((+)-catechin or (−)-epicatechin). Additionally, conformational analysis was performed for one of these newly formed pigment using computer-assisted model building and molecular mechanics. A chemical nomenclature is proposed to unambiguously name this new family of anthocyanin-derived pigments.

KEYWORDS: Red wine; flavanol; aging; pigments; anthocyanins; isomers; NMR; mass spectrometry

INTRODUCTION

The color evolution of red wines during aging is a complex process, which is supposed to involve progressive structural changes of the polyphenolic compounds extracted from the grape berries, namely anthocyanins and proanthocyanidins. During winemaking and storage, these compounds undergo several chemical transformations due to associations or complexation reactions with other compounds such as proteins, polysaccharides, and metals (*1*−*3*) or oxidation−reduction reactions (*4*−*6*), which lead to their polymerization (*7*, *8*). The great diversity of chemical transformations which occur in wine give rise to more stable compounds with different physical−chemical features that change the organoleptic properties of the wine (color and flavor). Recently, it has been reported that anthocyanins may give rise to other pigments through the reaction with small molecules such as pyruvic acid (*9*, *10*), *p*-vinylphenol (*11*), α-ketoglutaric acid (*12*), acetone (*12*−*14*), and 4-vinylguaiacol (*14*). The anthocyanin−pyruvic acid adducts, which were found to be the major newly formed pigments detected in red wine so far, were shown to be more stable than their anthocyanin precursors during wine aging (*15*) and present spectroscopic features that may contribute to the orange-red color of aging red wines (*16*−*18*). Despite the fact that most of the transformation mechanisms involving anthocyanins and 3-flavanols (e.g. proanthocyanidins) are practically unknown, several studies have been performed over the last years, bringing new insights into this matter (*19*−*23*). The first major structural transformations yield newly formed pigments resulting from the reaction between anthocyanins and 3-flavanols directly (*7*, *24*, *25*) or mediated through acetaldehyde (*8*, *19*, *26*−*28*). Copigmentation phenomena have been suggested to be the first step of the reactions involving anthocyanins (*29*, *30*).

The recent advances in mass spectrometry coupled to HPLC and the use of NMR allowed the structural elucidation of some anthocyanin−flavanol-derived pigments. A new family of anthocyanin−vinyl−flavanol pigments previously detected in red wines (*22*, *31*−*32*) has been very recently characterized using 1D and 2D NMR techniques (*23*). The present work deals with the detection, isolation, and characterization of new acylated anthocyanin−vinyl−flavanol pigments occurring in aging Port wines by ESI/MS and UV−visible and NMR spectroscopy.

* To whom correspondence should be addressed. E-mail: vfreitas@fc.up.pt (V.d.F.); nbmateus@fc.up.pt (N.M.). Fax: +351.226082959. Tel: +351.226082858.
† Centro de Investigação em Química.
‡ CEQUP.
§ Universidad de Salamanca.
|| Universidade de Aveiro.

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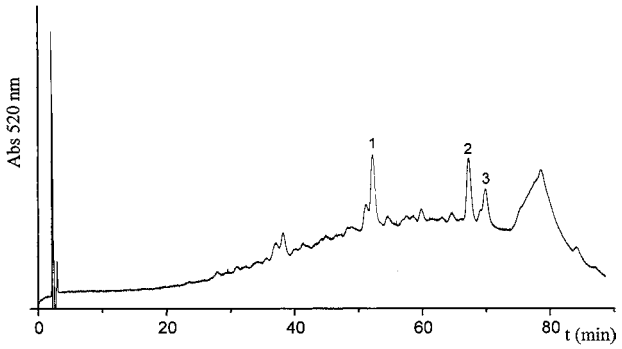

Figure 1. HPLC chromatogram recorded at 520 nm of the fraction eluted from TSK Toyopearl HW-40(S) gel column with 99.8% ethanol (v/v) in the fractionation of a Port red wine. Peaks 1–3 correspond to newly formed pigments.

MATERIALS AND METHODS

Source.

The pigments were extracted from a 2-year-old Port 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 (*Vitis vinifera*) grown in the Douro Demarcated Region (Northern Portugal).

Pigment Purification.

Port wine samples were applied on a TSK Toyopearl gel HW-40(S) column and eluted with water with increasing percentage of ethanol until 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 (*18*, *23*). When practically no more colored compounds were eluted from the column, the solvent was changed to 99.8% ethanol (v/v) yielding three major pigments (**Figure 1**) showing a retention time on RP-HPLC longer than that of the anthocyanin-derived pigments previously identified. These three pigments were purified by semi-preparative HPLC on a 250 × 4.6 mm i.d. reversed-phase C18 column (Merck, Darmstadt, Germany) using an injection volume of 500 μL. Each pigment was collected, concentrated under vacuum, and applied on a 150 × 16 mm i.d. TSK Toyopearl HW-40(s) gel column (Tosoh, Japan), which was eluted with distilled methanol for a final purification to remove the rest of the HPLC solvents and the compounds responsible for the hump situated under their chromatographic peaks in RP-HPLC (**Figure 1**).

Semipreparative and Analytical HPLC Conditions.

The fraction eluted with 99.8% (v/v) ethanol from the TSK Toyopearl gel column was analyzed by HPLC (Merck-Hitachi L-7100) using the above indicated reversed-phase C18 column; detection was carried out at 520 nm using a diode array detector (Merck-Hitachi L-7450A) (**Figure 1**). 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 (*27*).

ESI/MS Analysis.

Mass spectrometry analysis was performed using a Finnigan LCQ equipped with an API source, using an electrospray ionization (ESI) probe. Pigments 1−3 were injected directly into the mass 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, its MS^2 spectrum was obtained using a relative energy of collision of 20. MS^3 of the main fragment ion in the MS^2 spectrum was also obtained using a relative energy of collision of 30.

NMR Analysis.

^{1}H NMR (500.13 MHz) and ^{13}C NMR (125.77 MHz) spectra were measured in CD_3OD/TFA (98:2) on a Bruker-AMX500 spectrometer at 303 K and with TMS as internal standard. ^{1}H chemical shifts were assigned using 1D and 2D ^{1}H NMR (COSY and NOESY), while ^{13}C resonances were assigned using 2D NMR techniques (*g*HMBC and *g*HSQC) (*33*, *34*). The delay for the long-range C/H coupling constant was optimized to 7 Hz.

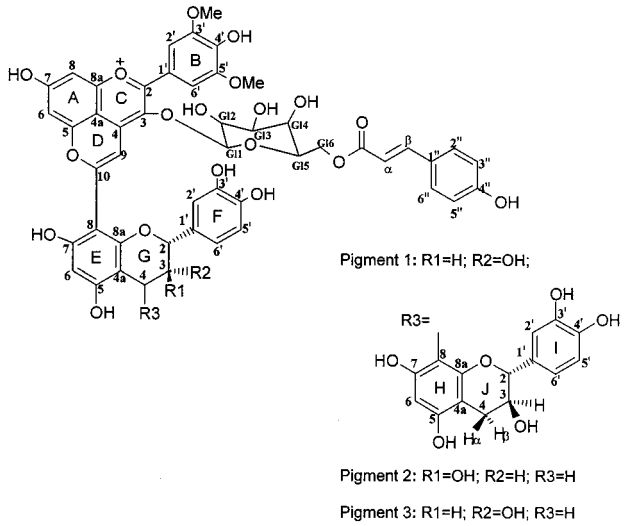

Figure 2. Structures of pigments 1–3.

Conformational Analysis.

Theoretical calculations were performed on pigment 2 using computer-assisted model building (MacroModel) (*35*−*37*) and molecular mechanics (MM3) (*35*).

RESULTS AND DISCUSSION

Mass Spectrometry.

The three pigments isolated (1−3) were analyzed individually by ESI/MS by direct injection into the mass spectrometer. Analysis of pigment 1 produced a $[M]^+$ ion at *m*/*z* 1239 and a fragment ion $[M - 308]^+$ at *m*/*z* 931, corresponding to the loss of a coumaroylglucoside residue. The other two pigments (2 and 3) revealed similar mass spectrometry data, yielding a $[M]^+$ ion at *m*/*z* 951 and a fragment ion $[M - 308]^+$ at *m*/*z* 643, also corresponding to the loss of a coumaroylglucoside residue. These molecular ion masses fit exactly with the structures shown in **Figure 2**. The fact that pigments 2 and 3 present the same mass with different retention times on RP-HPLC suggests that they are isomers probably differing in the flavanol moiety, either a (+)-catechin or a (−)-epicatechin unit.

UV−Visible Spectroscopy.

The UV−vis spectra of the pigments recorded with the HPLC diode array detector are shown in **Figure 3**. Their λ_{max} are hypsochromically shifted with regard to those of the anthocyanins: pigment 1 has λ_{max} at 512 nm, while the other two pigments (2 and 3) show λ_{max} at 503 nm, similar to the λ_{max} of the pyruvic acid adducts (*18*). These λ_{max} coincide with those obtained for their analogous nonacylated structures previously reported (*23*). Additionally, the spectra of the three pigments present a noteworthy shoulder around 310 nm, which is characteristic of acylation with *p*-coumaric acid (*38*). It is interesting to notice that the pigment structure (acylated or nonacylated) that contains a procyanidin dimer unit revealed an important bathocromic shift (9 nm) comparatively to the structures that contain a flavanol monomeric unit ((+)-catechin or (−)-epicatechin), despite possessing the same flavylium moiety. This observation highlights the importance of the type of flavanol moiety on the color characteristics of the pigments, and it suggests that some kind of intramolecular copigmentation between the flavanol residue and the flavylium chromophore may exist. On the other hand, the existence of a *p*-coumaroyl acyl residue does not show any influence on the λ_{max} of the pigments.

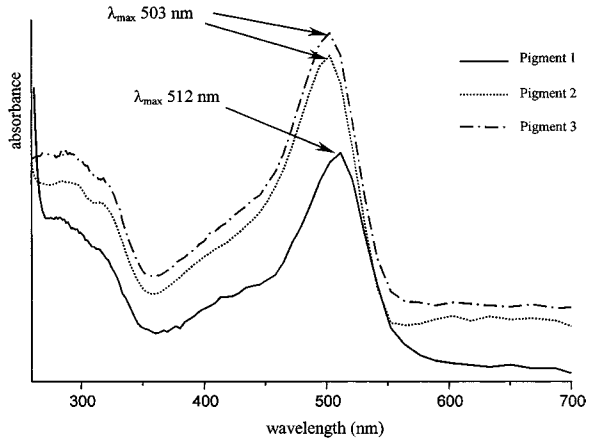

Figure 3. UV–visible spectra of pigments 1–3 recorded with the HPLC diode array detector, showing the λ_{max} values for pigment 1 (512 nm) and pigments 2 and 3 (503 nm).

^{1}H NMR.

The ^{1}H chemical shifts of pigments 1−3 in CD_3OD/TFA (98:2) are indicated in **Tables 1**−**3**, respectively. The proton chemical shifts were attributed using 1D and 2D NMR techniques (COSY and NOESY). All the protons were assigned for pigments 2 and 3, while the protons of the rings E, F, H, and I of pigment 1 could not be assigned due to the low quantity of product and the high complexity of the aromatic region in the respective ^{1}H NMR spectrum. In general, the NMR data of these pigments are in agreement with the ones of the analogous nonacylated species described in the literature (*23*).

Pigment 1: Malvidin 3-Coumaroylglucoside−Vinyl−(−)-Epicatechin−(+)-Catechin.

Concerning the flavanol moiety, the relative 2,3-stereochemistry that distinguishes (+)-catechin from (−)-epicatechin was deduced from the coupling constant of the H-2 proton of the flavanol pyran ring: a large doublet indicates a (+)-catechin moiety, whereas a broad singlet indicates an (−)-epicatechin moiety (*39*). The broad singlet and the doublet with a high coupling constant (J = 11.5 Hz) obtained respectively for the protons H-2G and H-2J suggest that the flavanol moiety is comprised of one (−)-epicatechin molecule (upper unit) linked through an interflavanoid bond to a (+)-catechin molecule (lower unit). Therefore, the flavanol moiety of pigment 1 seems to correspond to either procyanidin dimers B1 or B7 depending on the interflavanoid linkage (C4−C8 or C4−C6). This interflavanoid linkage cannot be fully ascertained from the data obtained from the NMR experiments. Nevertheless, a C4−C8 interflavanoid linkage is expected for pigment 1, since the C4−C8 procyanidin dimers (B1−B4) are more abundant in grapes and in the resulting Port wines than their respective C4−C6 counterparts (B5−B8) (*40*, *41*).

Pigment 2: Malvidin 3-Coumaroylglucoside−Vinyl−(+)-Catechin.

The ^{1}H NMR data obtained led us to conclude that the flavanol moiety consisted of a (+)-catechin molecule, as the proton H-2G resonates as a doublet (J = 7.3 Hz).

Sufficient quantity of pigment 2 was isolated to perform another two-dimensional experiment, NOESY, which confirmed the assignment of several proton chemical shifts and yielded important information concerning the spatial proximity of different protons within the structure (**Figure 4**). The nOe correlations observed suggest that the sugar moiety is located closely to the flavylium moiety as anticipated correlations were observed between the protons Gl-1 and Gl-2 and the proton H-9 of ring D. Additionally, the proton Gl-2 revealed another expected correlation with the proton H-2′,6′ of ring B. Additionally, the protons Gl-6 of the sugar moiety were found to correlate with two protons of the pyran ring of the catechin unit (H-2G and H-4αG). To determine the spatial conformation of this pigment, theoretical calculations were performed on this pigment using computer-assisted model building (MacroModel) (*35*−*37*) and molecular mechanics (MM3) (*35*). A systematic conformational study was performed, yielding 39 relevant conformations, which were analyzed. Data from nOe and J-coupling constants obtained from NMR experiments, coupled with the results of theoretical calculations, allowed refinement of the structure, and the most probable conformation in solution was deduced (**Figure 5**).

Table 1. ^{1}H and ^{13}C NMR Data and HMBC and HSQC Correlations of Pigment 1 {8-Vinyl-epi-(4–8)-cat-[1V,2V:5*O*,4]-malvidin-3-coumaroylglucoside} Isolated from a 2-year-Old Port Wine, Determined in CD_3OD/TFA (98:2)[a]

posn	δ(^{1}H); J (Hz)	δ(^{13}C)	HMBC	HSQC
	Pyranoanthocyanin Moiety			
2C		158.5	H-2′B, H-6′B	
3C		131.0	H-9D	
4C		108.7		
4aA		108.0	H-6A, H-8A, H-9D	
5A		153.0	H-6A	
6A	6.85; d, 1.8	100.1		H-6A
7A		na		
8A	6.95; d, 1.8	100.3		H-8A
8aA		146.0	H-6A	
9D	7.54; s	107.0		H-9D
10D		168.2	H-9D	
1′Bh		121.0	H-2′B,6′B	
2′B, 6′B	7.60; s	109.0		H-2′B,6′B
3′B, 5′B		148.0	OMe, H-2′B,6′B	
4′B		142.0	H-2′B,6′B	
OMe	4.00; s	57.8		OCH_3
	Flavanol Moiety			
2G	5.42; bs	77.8		H-2G
2J	3.95; d, 11.5	82.6		H-2J
3G	4.09; bs	72.0		H-3G
3J	3.78; m	69.6		H-3J
4G	4.61; bs	37.1		H-4G
4αJ	3.08; dd, 5.8/16.2	30.2		H-4J
4βJ	2.46; dd, 9.6/16.2	30.2		
rings E, F, H, I	6.6–7.1*			
	Sugar Moiety			
Gl-1	4.91; d, 7.7	104.2		H-Gl-1
Gl-2	3.67*	75.9		H-Gl-2
Gl-3	3.42*	75.5		H-Gl-3
Gl-4	3.12	72.0		H-Gl-4
Gl-5	3.36	77.8		H-Gl-5
Gl-6a	4.19*	64.2		H-Gl-6a
Gl-6b	4.31*	64.2		H-Gl-6b
	Coumaroyl Moiety			
R1CO_2R2		168.0	H_α, H_β	
CH=$CH_\alpha CO_2$R	5.88; d, 15.5	115.0		H_α
CH_β=CHCO$_2$R	7.37; d, 15.5	146.8	H_α, H-2″,6″	H_β
1″		127.0	H_α, H-3″,5″	
2″, 6″	7.36; d	132.0	H_β, H-4″	H-2″,6″
3″, 5″	6.87; d	117.8	H-4″	H-3″,5″
4″		161.0	H-2″,6″, H-3″,5″	-

[a] Key: *, unresolved; s, singlet; bs, broad singlet; m, multiplet; d, doublet; dd, double doublets; na, not attributed.

In general, the NOESY data points to a relatively closed structure with the coumaroyl group being oriented toward the outside of the bulk structure, supposedly without any interactions with the pyranoanthocyanin chromophore. This feature could explain why the acylation of these pigments did not induce any change on their λ_{max}.

Pigment 3: Malvidin 3-Coumaroylglucoside−Vinyl−(−)-Epicatechin.

Table 2. ^{1}H and ^{13}C NMR Data and HMBC and HSQC Correlations of Pigment 2 {8-Vinylcat-[1V,2V:5*O*,4]-malvidin-3-coumaroylglucoside} Isolated from a 2-year-Old Port Wine, Determined in CD_3OD/TFA (98:2)[a]

posn	δ(^{1}H); *J* (Hz)	δ(^{13}C)	HMBC	HSQC
Pyranoanthocyanin Moiety				
2C		160.8	H-2′B,6′B	
3C		134.2	H-9D	
4C		na		
4aA		107.8	H-6A, H-9D	
5A		154.9	H-6A	
6A	6.74; d, 1.9	100.8	H-8A	H-6A
7A		166.5	H-6A, H-8A	
8A	6.78; d, 1.9	100.9	H-6A	H-8A
8aA		153.8	H-8A	
9D	7.55; s	107.0		H-9D
10D		169.1	H-9D	
1′B		120.3	H-2′B,6′B	
2′B, 6′B	7.51; s	108.9		H-2′B,6′B
3′B, 5′B		149.6	OMe, H-2′B,6′B	
4′B		142.5	H-2′B,6′B	
OMe	3.94; s	57.1		OCH_3
Flavanol Moiety				
2G	4.74; d, 7.3	82.0	H-3G, H-4αG, H-2′F	H-2G
3G	4.07; m	68.8	H-2G	H-3G
4αG	2.85; dd, 5.4/16.2	28.8	H-2G, H-6E	H-4G
4βG	2.55; dd, 7.9/16.2	28.8	H-6E	
4aE		na		
5E		na		
6E	6.26; s	103.0	H-4αG, H-4βG	
7E		na		
8E		101.2	H-9D	
8aE		157.0	H-4αG	
1′F		120.0	H-2G	
2′F	6.96; d, 1.7	116.5	H-2G	H-2′F
3′F		131.2	H-2G, H-2′F	
4′F		145.8	H-5′F, H-6′F	
5′F	6.87; d, 8.1	120.8	H-6′F	H-5′F
6′F	6.81; dd, 8.1/1.7	115.8	H-5′F	H-6′F
Sugar Moiety				
Gl-1	4.63; d, 7.7	104.1		H-Gl-1
Gl-2	3.54*	75.7		H-Gl-2
Gl-3	3.35*	72.5		H-Gl-3
Gl-4	na	70.2		H-Gl-4
Gl-5	3.30*	78.9		H-Gl-5
Gl-6a	4.06*	64.0		H-Gl-6a
Gl-6b	4.27*	64.0		H-Gl-6b
Coumaroyl Moiety				
R1CO_2R2		168.8	H_α, H_β	
CH═$CH_\alpha CO_2$R	5.70; d, 15.9	114.2		H_α
CH_β═CHCO_2R	7.14; d, 15.9	146.1	H_α, H-2″,6″	H_β
1″		126.3	H_α, H-3″,5″	
2″, 6″	7.06; d, 8.6	131.1	H_β, H-4″	H-2″,6″
3″, 5″	6.78; d, 8.6	116.9	H-4″	H-3″,5″
4″		161.0	H-2″,6″, H-3″,5″	

[a] Key: *, unresolved; s, singlet; m, multiplet; d, doublet; dd, double doublets; na, not attributed.

Table 3. ^{1}H and ^{13}C NMR Data and HMBC and HSQC Correlations of Pigment 3 {8-Vinyl-epi-[1V,2V:5*O*,4]-malvidin-3-coumaroylglucoside} Isolated from a 2-year-Old Port Wine, Determined in CD_3OD/TFA (98:2)[a]

posn	δ(^{1}H); *J* (Hz)	δ(^{13}C)	HMBC	HSQC
Pyranoanthocyanin Moiety				
2C		161.0	H-2′B,6′B	
3C		133.8	H-9D	
4C		107.8	H-9D	
4aA		107.9	H-6A	
5A		153.0	H-6A	
6A	6.83; d, 1.9	100.7	H-8A	H-6A
7A		167.2	H-8A	
8A	6.85; d, 1.9	100.8	H-6A	H-8A
8aA		155.1	H-8A	
9D	7.65; s	107.2		H-9D
10D		169.8	9D	
1′B		120.6	H-2′B,6′B	
2′B, 6′B	7.57; s	108.6		H-2′B,6′B
3′B, 5′B		149.0	H-2′B,6′B	
4′B		142.0	OMe, H-2′B,6′B	
OMe	3.95; s	57.1		OCH_3
Flavanol Moiety				
2G	4.92; s	81.2	H-2′F, H-6′F, H-3G	H-2G
3G	4.26*	67.5	H-2G	H-3G
4αG	2.90*	29.7		H-4G
4βG	2.83*			
4aE		102.0	H-4αG, H-4βG	
5E		163.0	H-4βG	
6E	6.20; s	na		
7E		na		
8E		102.8	H-9D	
8aE		na		
1′F		120.2	H-2′F	
2′F	7.02; s	114.9		H-2′F
3′F		131.8	H-2G	
4′F		146.1	H-2′F, H-5′F, H-6′F	
5′F	6.80; d, 8.3	115.8		H-5′F
6′F	6.86; dd, 8.3/1.9	119.2	H-2G	H-6′F
Sugar Moiety				
Gl-1	4.69; d, 7.7	104.0		HG1-1
Gl-2	3.60*	75.7		HG1-2
Gl-3	3.30*	72.1		HG1-3
Gl-4	3.28*	71.0		HG1-4
Gl-5	3.32*	77.6		HG1-5
Gl-6a	4.15*	64.0		HG1-6a
Gl-6b	4.26; dd, 11.6/1.9	64.0		HG1-6b
Coumaroyl Moiety				
R1CO_2R2	-	168.5	H_α	
CH═$CH_\alpha CO_2$R	5.74; d, 15.9	114.1		H_α
CH_β═CHCO_2R	7.17; d, 15.9	146.5	H_α, H-2″,6″	H_β
1″		126.8	H_α, H-3″,5″	
2″, 6″	7.10; d, 11.1	131.0	H_β, H-4″	H-2″,6″
3″, 5″	6.78; d, 11.1	116.7	H-4″	H-3″,5″
4″		161.7	H-2″,6″, H-3″,5″	

[a] Key: *, unresolved; s, singlet; d, doublet; dd, double doublets; na, not attributed.

The ^{1}H NMR spectrum of pigment 3 was interpreted as previously for pigment 2. The main difference between these two isomers concerns the coupling constant of proton H-2G of the pyran ring of the flavanol moiety. Indeed, proton H-2G of pigment 3 resonates as a singlet, whereas proton H-2G of pigment 2 resonates as a doublet (J = 7.3 Hz). This feature indicates that the flavanol moiety of pigment 3 consists of a (−)-epicatechin unit.

^{13}C NMR.

The ^{13}C chemical shifts found for pigments 1−3 in CD_3OD/TFA (98:2) are indicated in **Tables 1**−**3**, respectively. Practically all the carbon resonances of these pigments were attributed, especially for pigments 2 and 3. In general, the assignment of most of the carbon resonances of the pigments were obtained using two-dimensional techniques (HSQC and HMBC). The ^{13}C chemical shifts of rings E, F, H, and I of the flavanol moiety of pigment 1 could not be assigned due to the high complexity of the spectra in that region and also because of the low quantities of product isolated. The carbons 4aE, 5E, and 7E for pigment 2 and the carbons 6E, 7E, and 8aE for pigment 3 could not be assigned since no correlations were observed in the respective HSQC and HMBC spectra.

Mechanism of Formation.

The mechanism of formation of these anthocyanin−flavanol derivatives is supposed to be similar to that described elsewhere (*23*). Hence, their structures are thought to arise from the reaction between malvidin 3-coumaroylglucoside and a 8-vinylflavanol adduct following a mechanism proposed by Fulcrand et al. (*11*) for the formation of 4-vinylphenol anthocyanin-derived pigments. The vinyl−flavanol adducts may derive either from the cleavage of ethyl-linked flavanol oligomers resulting from the acetaldehyde-induced condensation of flavanols (*42*),

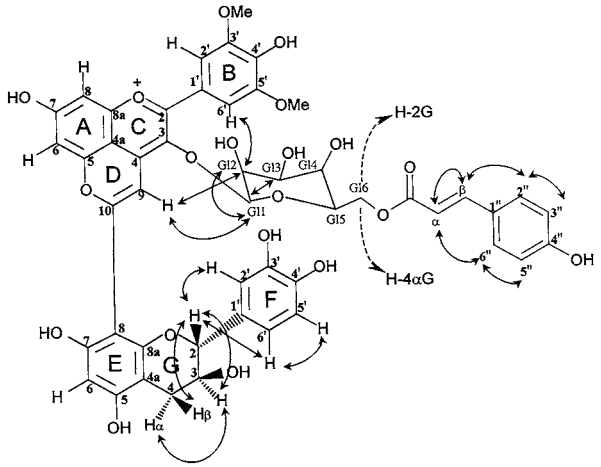

Figure 4. Correlations ^{1}H–^{1}H found in the NOESY spectrum of pigment 2.

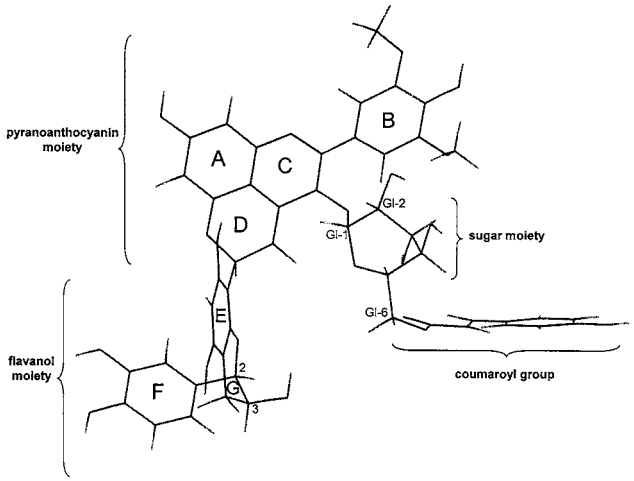

Figure 5. Preferred conformation of pigment 2 determined by computer-assisted model building (MacroModel) and molecular mechanics (MM3).

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 an oxidative process whereby the vinyl−flavanol adduct binds to the flavylium moiety, giving rise to the aromatization of ring D. The resulting extended conjugation of the π electrons in this newly formed structure is likely to confer a higher stability of the molecule.

Chemical Nomenclature of the Pigments.

This family of anthocyanin-derived pigments has been relatively recently characterized, and as a result of its high structural complexity, the literature lacks in chemical nomenclature systematic rules to unambiguously name them. Therefore, some nomenclature rules are proposed herein to name these complex structures using IUPAC rules and some trivial names. By analogy with the mechanism of formation previously proposed (*23*), the 8-vinylflavanol adduct is considered to be the side group of the main unit, which is the anthocyanin flavylium ion (**Figure 6**). The linkage between the carbons of the vinyl group (indicated by 1V and 2V) and oxygen 5 (5O) and carbon 4 of the anthocyanin is represented between brackets and separated by a colon. The order in which the atoms are indicated between square brackets explains how the linkage is made: carbon 1V of the vinyl group is linked to the oxygen atom at the position 5O of the anthocyanin, whereas carbon 2V of the vinyl group is linked to carbon 4 of the anthocyanin.

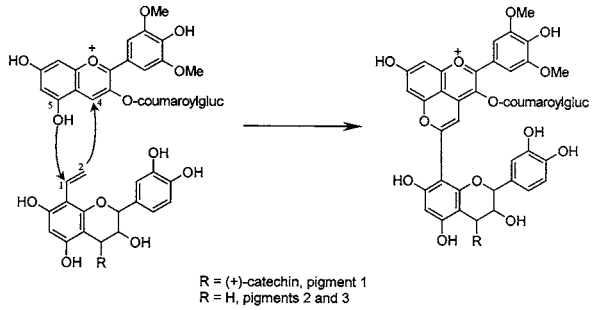

Figure 6. Mechanism proposed for the formation of pigments 1–3.

On the basis of these rules, the following chemical names were established:

pigment 1, 8-vinyl-epi-(4−8)-cat-[1V,2V:5*O*,4]-malvidin-3-coumaroylglucoside;

pigment 2, 8-vinylcat-[1V,2V:5*O*,4]-malvidin-3-coumaroyl-glucoside;

pigment 3, 8-vinyl-epi-[1V,2V:5*O*,4]-malvidin-3-coumaroyl-glucoside.

Similarly, other pyranoanthocyanins previously detected by authors could be named using this chemical nomenclature. Thus, the pyruvic acid and acetaldehyde adducts of malvidin-3-glucoside (i.e., vitisins A and B in the trivial name given by Bakker and Timberlake (*17*)) would be respectively 1-(carboxy-vinyl)-[1V,2V:5*O*,4]-malvidin-3-glucoside and vinyl-[1V,2V:5*O*,4]-malvidin-3-glucoside.

The detection and structural characterization of these anthocyanin−vinyl−flavanol pigments provides important information regarding the chemical transformations involved in the complex evolution of the color of red wines. The formation of vinyl−flavanol adducts and their reaction with genuine anthocyanins appear to be crucial for the formation of newly formed pigments, which present spectroscopic features that may contribute to the changing color of aging red wines. Nevertheless, further studies are needed to assess the real contribution of these anthocyanin-derived pigments to the resulting wine color.

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