Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 1344–1351
Food Chemistry
www.elsevier.com/locate/foodchem

Structural and chromatic characterization of a new Malvidin 3-glucoside–vanillyl–catechin pigment

Carlos Sousa [a], Nuno Mateus [a], Artur M.S. Silva [b], Ana M. González-Paramás [c], Celestino Santos-Buelga [c], Victor de Freitas [a,*]

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

Received 4 January 2004; accepted 4 April 2006

Abstract

A new pigment, resulting from the reaction between malvidin 3-glucoside and catechin in the presence of vanillin, was detected in a model solution. This newly formed anthocyanin-aryl-flavanol adduct was structurally characterized by 1D and 2D NMR and mass spectrometry, and its chromatic characteristics were studied by UV–Vis techniques. The new pigment was shown to have a wavelength of maximum absorption in the visible region (λ_{max}) of 549 nm, conferring on it a purple colour, and a molar extinction coefficient value (ε) of 12,247 $l \cdot mol^{-1} \cdot cm^{-1}$.

Keywords: Anthocyanin; Oenin; Catechin; Vanillin; Wine; Colour

1. Introduction

Colour is one of the most important wine sensory characteristics, being strongly influenced by the phenolic content of the grapes, as well as oenological practices and storage conditions. In fact, the initial purple-red colour of young wines arises from the original grape anthocyanins extracted from grape skins, while during ageing this colour shifts to a more reddish brown hue, mainly due to progressive structural changes of anthocyanins (Jurd, 1969; Somers, 1971). These changes may occur through different mechanisms, one of which is the aldehyde-mediated association of anthocyanins and flavanols, which has been extensively studied in model solutions (Bakker, Picinelli, & Bridle, 1993; Dallas, Ricardo da Silva, & Laureano, 1996a, Dallas, Ricardo da Silva, & Laureano, 1996b; Escribano-Bailón, Dangles, & Brouillard, 1996; Fulcrand, Doco, Es-Safi, Cheynier, & Moutounet, 1996; Pissarra, Mateus, Rivas-Gonzalo, Santos-Buelga, & de Freitas, 2003; Rivas-Gonzalo, Bravo-Haro, & Santos-Buelga, 1995). More recently pigments resulting from these reactions have been detected in red wines (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Mateus, Pascual-Teresa, Rivas-Gonzalo, Santos-Buelga, & de Freitas, 2002; Revilla, Perez-Magariño, Gonzalez-San Jose, & Beltran, 1999; Vivar-Quintana, Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo, 1999). Some of the aldehydes involved in these reactions originate from the wood used in the ageing process. In fact, ageing in oak barrels is a common practice in red wine production. The different varieties of oak used around the world affect the composition of the mature wine, and the extraction of volatile and non-volatile compounds from wood during ageing contribute to the wines taste, directly or by interactions with other wine components (Escalona, Birkmyre, Piggott, & Paterson, 2002).

* Corresponding author. Tel.: +351 226082858; fax: +351 226082959.
E-mail address: vfreitas@fc.up.pt (V.de Freitas).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.04.050

The extraction of volatile compounds from oak barrels depends on the quantity of compounds that are potentially extractable, and the period of contact between wine and wood. The factors which affect the pool of oak extractives are the species and geographical origin of the wood (Chatonnet, 1991; Miller, Howell, Michaelis, & Dickmann, 1992), the seasoning of the staves (Sefton, Francis, Pocock, & Williams, 1993; Vivas, Glories, Doneche, & Gueho, 1991; Vivas & Glories, 1993), the toasting of the barrel (Chatonnet, Boidron, & Pons, 1989) and the age of the barrel (Chatonnet, 1991).

Phenolic aldehydes (vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde) represent an important group of potentially extractable compounds. Vanillin is reported to be at sub-threshold concentrations in wines, with no significant contribution to the wine aroma, unlike in other wood-matured alcoholic drinks, such as spirits (Escalona et al., 2002). Nevertheless, it can interact with other wine components during maturation in oak barrels, possibly reducing their impact in wine and contributing to colour changes.

This work studies the formation of a new pigment, resulting from the vanillin-mediated reaction between malvidin 3-glucoside and catechin in model solutions. The newly formed compound was structurally and chromatically characterised.

2. Materials and methods

2.1. Standards

Vanillin was obtained from Fluka Chemika (Buchs, Switzerland) and (+)-catechin was purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Extraction and purification of malvidin-3-glucoside

Anthocyanins were extracted from red grape skins (*Vitis vinifera*) with 40% aqueous ethanol. Grape skin extract was applied onto a TSK Toyopearl gel HW-40(S) (Tosoh, Japan) column (300 × 25 mm) and eluted with water acidified with 2% HCl, at a flow rate of 0.8 ml/min, in order to yield a fraction of non-acylated anthocyanins, with malvidin 3-glucoside being the predominant one.

2.3. Synthesis of malvidin 3-glucoside-vanillyl-catechin

A solution (520 ml) composed of malvidin-3-glucoside (2 mM):catechin:vanillin (molar ratio of 1:4:10) was prepared in 12% ethanol, pH 1.5. This solution was kept at 35 °C and protected from light, to obtain the maximum amount of adduct. The appearance of the pigment was monitored by HPLC-DAD.

2.4. HPLC conditions

Samples were analysed by HPLC-DAD using a reversed-phase C18 column (250 × 4.6 mm i.d.) at 25 °C. Solvents were (A) water/formic acid (95:5), and (B) acetonitrile. The elution gradient was performed using a K-1001 Knauer pump from 10% to 35% B over 50 min at a flow rate of 1.5 ml/min. Detection was carried out at 520 nm (K-2800, Knauer).

2.5. Isolation of the malvidin 3-glucoside-vanillyl-catechin pigment

After a seven-day reaction period, the solvent was concentrated from the model solution using a rotary evaporator at 30 °C, to a volume of ~5 ml. The solution was applied to a 300 × 25 mm TSK Toyopearl gel HW-40(S) column (Tosoh, Japan), and the pigment was eluted with acidified (2% HCl) water/methanol (1:1), at a flow rate of 0.8 ml/min. The isolated pigment was then submitted to further purification, which consisted of a final elution on silica gel 100 C18-reversed phase, using a 10 cm diameter medium-porosity sintered glass funnel, connected to standard vacuum filtration glassware. The sample was applied on the top of the funnel and the elution was firstly performed with deionized water in order to remove inorganic salts and any other organic impurities. The purified pigment was then recovered with distilled methanol, acidified with 2% HCl. The solvent was partially evaporated using a rotary evaporator at 30 °C, and the sample was then freeze-dried and stored at −18 °C until use.

2.6. LC-MS conditions

A Hewlett-Packard 1100 series liquid chromatograph, equipped with an AQUA™ (Phenomenex, Torrance, CA, USA) reversed-phase C18 column (150 × 4.6 mm, 5 μm) thermostatted at 35 °C, was used. Solvents were (A) aqueous 0.1% trifluoroacetic acid, and (B) acetonitrile, using the gradient reported by Pissarra et al. (2003). The capillary voltage was 3 V and the capillary temperature 190 °C. Spectra were recorded in positive ion mode between m/z 120 and 1500. 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 an MS–MS spectrum of the most intense ion, using a relative collision energy of 30 and 60.

2.7. 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-DRX500 spectrometer at 298 K, with TMS as internal standard. ^{1}H chemical shifts were assigned using 1D and 2D ^{1}H NMR (gCOSY) while ^{13}C resonances were assigned using 2D NMR (gHMBC, gHSQC and gNOESY) techniques (Bax & Subramanian, 1986; Bax & Summers, 1986; Hurd, 1990; Kay, Keifer, & Saarinen, 1992; Wagner & Berger, 1996). The delay for the long range C/H coupling constant was optimised to 7 Hz.

2.8. Chromatic assays

For the pH assay, solutions of malvidin 3-glucoside-vanillyl-catechin (0.08 mM) were prepared in 90% distilled methanol at different pH values over a range between 1 and 11, adjusted with HCl or NaOH.

To study bleaching by SO_2, a solution of the pigment at pH 1.0 was used and different aliquots of an aqueous solution of sodium bisulphite (5 mg/L) were added to this solution, in order to achieve SO_2 concentrations over a range between 0 and 200 ppm.

The extinction molar coefficient (ε) was also determined using solutions of malvidin 3-glucoside-vanillyl-catechin over a concentration range between 0.01 and 0.08 mM in methanol/HCl (99.9:0.01). The coefficient was calculated from the slope of the graphic of colour intensity (at the λ_{max} of the pigment), as a function of the concentration of the pigment.

Spectral absorbance curves were recorded for all of these solutions from 360 to 700 nm with a 1 nm sampling interval, using a 10 mm path length cell and a UV-3101 Shimadzu spectrophotometer.

3. Results and discussion

3.1. Identification of the newly formed malvidin 3-glucoside-vanillyl-catechin

The vanillin-mediated reaction between malvidin 3-glucoside and catechin led to the formation of two major red pigments. The compounds were numbered 1 or 2, according to their order of elution by HPLC (Fig. 1). The peaks of these compounds have similar intensities in the HPLC chromatogram, which indicates that both isomers are present in similar quantities. MS analysis of both of these new pigments showed a molecular ion consistent with the structure of malvidin 3-glucoside-vanillyl-catechin ($[M]^+$ at m/z 917) (Fig. 2), by analogy with the anthocyanin-alkyl/aryl-flavanol adducts obtained in the aldehyde-induced reactions reported in the literature (Escribano-Bailón et al., 1996; Pissarra et al., 2003). The proposed mechanism for this type of reaction (Fig. 3) starts with the protonation of the aldehyde (I), forming the respective carbocation (II) which rapidly induces an electrophilic attack to the phloroglucinol ring of the flavanol (III), preferentially at position C8 (Bendz, Martensson, & Nilsson, 1967). The formed adduct (IV) then reacts with anthocyanins (V) to form new coloured pigments (VI). The fact that two pigments with identical molecular ions are formed is explained by the existence of two diastereoisomers, which are supposed to differ in the stereochemistry of the asymmetric carbon (C9) of the interflavanolic linkage, as demonstrated by Escribano-Bailón et al. (1996). MS^2 fragmentation of this newly-formed pigment showed a typical pattern, with a loss of the catechin moiety ($[M-290]^+$, fragment at m/z627) and a loss of the glucose moiety ($[M-(290+162)]^+$, fragment at m/z 465).

The ^1H and ^{13}C NMR chemical shifts of both isomers of the isolated pigment in CD_3OD/TFA (98:2) are indicated in Tables 1 and 2. The ^1H chemical shifts were assigned using 1D and 2D NMR techniques (COSY and NOESY), and the assignment of the carbon resonances was made using 2D techniques (HSQC and HMBC). The HMBC spectra were optimised, in order to visualize long distance coupling ^1H–^{13}C in $^2J_{C,H}$, and $^3J_{C,H}$, in order to assign most of the carbons, especially the ones of the vanillyl linkage.

3.2. Anthocyanidin moiety

All of the protons of the anthocyanidin moiety were easily assigned by comparison with those of anthocyanin-aryl/alkyl-catechin adducts reported in the literature (Pissarra et al., 2004). Protons H-4C and H-6A were attributed to singlets around 8.80 ppm and 6.74 ppm,

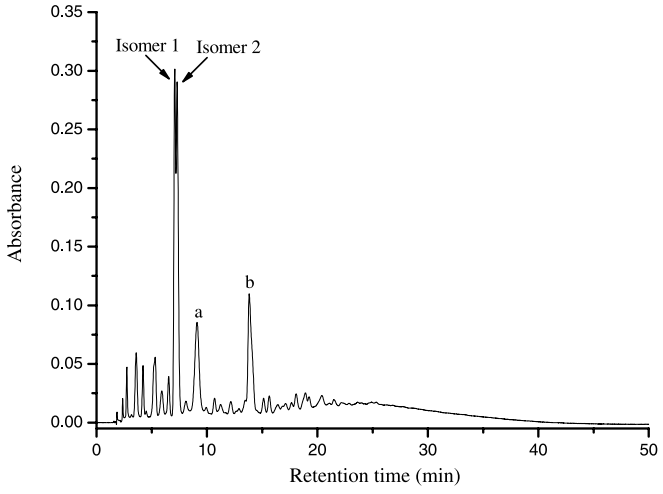

Fig. 1. HPLC chromatogram at 520 nm of the model solution (pH 1.5, 35 °C) containing malvidin 3-glucoside, catechin and vanillin, after 7 days of reaction (a, malvidin 3-glucoside; b, unknown)

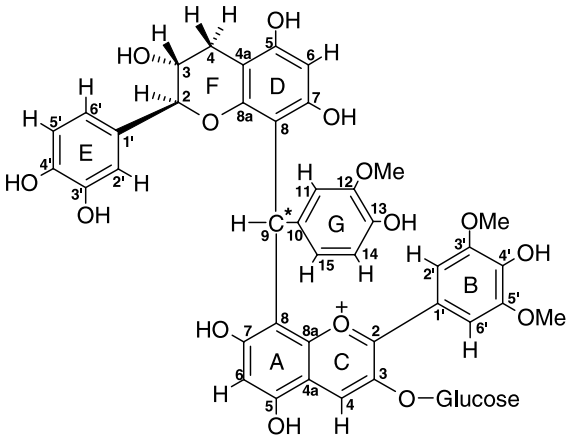

Fig. 2. Structure proposed for the malvidin 3-glucoside-vanillyl-catechin pigment.

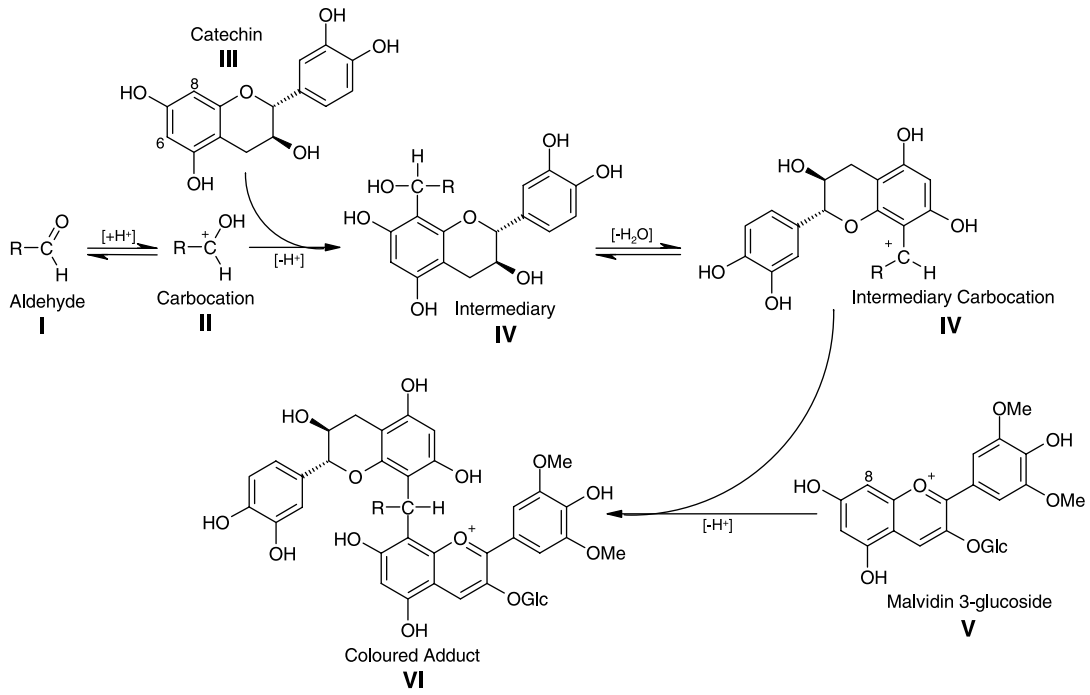

Fig. 3. Proposed mechanism for the formation of anthocyanin-alkyl/aryl-flavanol adducts. R, different alkyl/aryl groups.

respectively, as well as the equivalent protons H-2′,6′B, attributed around 7.60 ppm. The two methoxyl groups of ring B were also attributed to singlets around 3.90 ppm for the two isomers.

The assignments of carbon C-4C around 134.2 ppm, carbon C-6A around 103.9 ppm, carbons C-2′,6′B around 110.8 ppm and the methoxyl carbons around 57.2 ppm were obtained by HSQC. The remaining carbons were assigned through long range ^{1}H–^{13}C connectivities obtained in the HMBC spectrum.

3.3. Flavanol moiety

The protons H-4αF and H-4βF of the flavanol moiety were assigned through the characteristic AMX spin system of the flavanol pyran ring observed in the COSY spectrum. Thus, protons H-4αF and H-4βF correspond, respectively, to the double doublets at around 3.00 and 2.50 ppm. The proton H-3F was assigned as a multiplet at 3.57 and 3.66 ppm for each isomer, through its correlations with the protons H-4αF and H-4βF. The proton H-3F is also correlated with proton H-2F, which is attributed to the doublets at 4.0 and 4.3 ppm for each isomer ($J = 9.1$ Hz). The relative 2,3-stereochemistry is concluded to be *trans*, as proton H-2F appears as a large doublet ($J_{2-3} = 7$–9 Hz) corresponding to (+)-catechin, instead of a broad singlet or a doublet with a small coupling constant (0–3 Hz), characteristic of a (−)-epicatechin unit (Fletcher, Porter, Haslam, & Gupta, 1977). The only proton detected in ring D (H-6D) was assigned as a singlet at 6.04 ppm, whereas proton H-2′E was attributed to the singlet at 6.02 ppm. Protons H-5′E and H-6′E were assigned to the doublets at around 6.30 and 5.90 ppm ($J = 6.6$ Hz), respectively.

All the carbons related to these protons were attributed through the correlations observed in the HSQC spectrum, while the assignment of most of the other carbons of the flavanol moiety was obtained by HMBC, as can be seen in Tables 1 and 2.

3.4. Vanillyl linkage

The assignment of proton H-4C (anthocyanidin moiety) proves that the vanillyl group is not linked to this position and so the vanillyl linkage is assumed to be situated between ring A of the anthocyanidin moiety and ring D of the catechin moiety. The position of the vanillyl linkage was elucidated by NOESY experiments. In fact, spatial couplings were observed between protons H-2′6′B of ring B and proton H-9 of the vanillyl linkage for both isomers. This indicates a spatial proximity between the B ring of the anthocyanidin and the vanillyl bridge, which is only possible in the case of a C8 linkage, rather than a C6 linkage.

The NMR data do not clarify the position of the vanillyl linkage in ring D of the catechin moiety. Nonetheless, according to the proposed mechanism, the interflavonoid linkage is more likely attached to carbon C-8 of the flavanol moiety, which possesses a higher negative ground state charge than at carbon C-6 (Bendz et al., 1967).

Proton H-9 appears as a singlet at 6.62 and 6.86 ppm for isomer 2 and isomer 1, respectively. Proton H-11G was attributed to the singlet around 6.60 ppm for each isomer, whereas proton H-14G was assigned as a doublet at

Table 1
^{1}H and ^{13}C NMR data and HMBC and HSQC correlations of isomer 1 of malvidin 3-glucoside-vanillyl-catechin, determined in CD_3OD/TFA (98:2)[a]

Position	δ ^{1}H (ppm); J (Hz)	δ ^{13}C (ppm)	HMBC	HSQC
Anthocyanin moiety				
2C	–	162.3	H-2′,6′B, H-4C	–
3C	–	145.8	H-2′,6′B, H-4C, H-G1-1	–
4C	8.80; s	134.2	–	H-4C
4aA	–	114.2	H-6A	–
5A	–	157.2	H-6A, H-4C	–
6A	6.74; s	103.9	–	H-6A
7A	–	167.8	H-6A, H-9CH	–
8A	–	109.1	H-6A	–
8aA	–	154.6	H-4C	–
1′B	–	110.4	H-2′,6′B	–
2′,6′B	7.70; s	110.8	–	H-2′,6′B
3′,5′B	–	149.2	H-2′,6′B	–
4′B	–	145.4	H-2′,6′B	–
OMe	3.89; s	57.2	–	OCH_3 ant
Flavanol moiety				
2F	4.33; d, 9.1	83.8	–	H-2F
3F	3.57[b]	70.7	–	H-3F
4αF	3.05; dd, 6.8/16.8	30.4	–	H-4α,βF
4βF	2.45; dd, 6.8/9.5			
4aD	–	102.2	H-4α,βF	–
5D	–	156.6	H-4αF	–
6D	6.04; s	115.8	–	H-6D
7D	–	na	–	–
8D	–	na	–	–
8aD	–	155.9	H-4α,βF	–
1′E	–	130.4	H-2′E	–
2′E	6.02; s	114.5	–	H-2′E
3′E	–	146.1	H-5′E	–
4′E	–	146.1	H-6′E	–
5′E	6.29; d, 6.6	116.3	–	H-5′E
6′E	5.93; d, 6.6	119.2	–	H-6′E
Sugar moiety				
G1-1	5.28[b]	101.5	–	H-G1-1
G1-2	3.71[b]	74.9	–	H-G1-2
G1-3	3.54[b]	78.4	–	H-G1-3
G1-4	3.46[b]	71.0	–	H-G1-4
G1-5	3.69[b]	78.4	–	H-G1-5
G1-6a	4.06[b]	62.0	–	H-G1-6a
G1-6b	3.91[b]	62.0	–	H-G1-6b
Vanillyl bridge				
9CH	6.86; s	35.0	–	H-9CH
10G	–	133.4	H-14G	–
11G	6.59; s	115.8	–	H-11G
12G	–	146.1	H-15G	–
13G	–	149.2	H-14G, OCH_3 brid	–
14G	6.55; d, 8.5	115.8	–	H-14G
15G	6.43[b]	120.0	–	H-15G
OMe	3.57; s	56.3	–	OCH_3 brid

na, not attributed; s, singlet; d, doublet; dd, double doublet; m, multiplet; ant, anthocyanin; brid, bridge.
[a] Key.
[b] Unresolved.

Table 2
^{1}H and ^{13}C NMR data and HMBC and HSQC correlations of isomer 2 of malvidin 3-glucoside-vanillyl-catechin, determined in CD_3OD/TFA (98:2)[a]

Position	δ ^{1}H (ppm); J (Hz)	δ ^{13}C (ppm)	HMBC	HSQC
Anthocyanin moiety				
2C	–	162.3	H-2′,6′B, H-4C	–
3C	–	145.8	H-2′,6′B, H-4C, H-G1-1	–
4C	8.89; s	134.2	–	H-4C
4aA	–	113.6	H-6A	–
5A	–	157.2	H-6A, H-4C	–
6A	6.73; s	103.9	–	H-6A
7A	–	167.8	H-6A, H-9CH	–
8A	–	109.1	H-6A	–
8aA	–	153.7	H-4C	–
1′B	–	110.4	H-2′,6′B	–
2′,6′B	7.59; s	110.8	–	H-2′,6′B
3′,5′B	–	149.2	H-2′,6′B	–
4′B	–	145.4	H-2′,6′B	–
OMe	3.85; s	57.2	–	OCH_3 ant
Flavanol moiety				
2F	4.01[b]	83.0	–	H-2F
3F	3.66[b]	69.1	–	H-3F
4αF	2.96; dd, 6.8/16.8	29.1	–	H-4α,βF
4βF	2.50; dd, 6.8/9.5			
4aD	–	102.2	H-4α,βF	–
5D	–	156.6	H-4αF	–
6D	6.04; s	115.8	–	H-6D
7D	–	na	–	–
8D	–	na	–	–
8aD	–	155.5	H-4α,βF	–
1′E	–	131.3	H-2′E	–
2′E	6.02; s	114.5	–	H-2′E
3′E	–	146.1	H-5′E	–
4′E	–	146.1	H-6′E	–
5′E	6.32; d, 6.6	116.3	–	H-5′E
6′E	5.94; d, 6.6	119.2	–	H-6′E
Sugar moiety				
G1-1	5.37; d, 7.5	101.5	–	H-G1-1
G1-2	3.60[b]	74.9	–	H-G1-2
G1-3	3.63[b]	78.4	–	H-G1-3
G1-4	3.58[b]	71.0	–	H-G1-4
G1-5	3.59[b]	78.4	–	H-G1-5
G1-6a	4.02[b]	62.0	–	H-G1-6a
G1-6b	3.78[b]	62.0	–	H-G1-6b
Vanillyl bridge				
9CH	6.62; s	35.8	–	H-9CH
10G	–	134.0	H-14G	–
11G	6.68; s	115.8	–	H-11G
12G	–	146.1	H-15G	–
13G	–	149.2	H-14G, OCH_3 brid	–
14G	6.63[b]	115.8	–	H-14G
15G	6.45; d, 9.2	120.0	–	H-15G
OMe	3.66; s	56.3	–	OCH_3 brid

na, not attributed; s, singlet; d, doublet; dd, double doublet; m, multiplet; ant, anthocyanin; brid, bridge.
[a] Key.
[b] Unresolved.

6.55 ppm ($J = 8.5$ Hz) for isomer 1 and as an unresolved signal at 6.63 for isomer 2. Furthermore, proton H-15G was attributed as an unresolved signal at 6.43 for isomer 1 and as a doublet at 6.45 ppm ($J = 9.2$ Hz) for isomer 2. The methoxyl group of ring G was assigned to the singlet around 3.6 ppm for both isomers.

Carbons C-9CH, C-11G, C-14G, C-15G and of the methoxyl group were attributed at 35.5, 115.8, 115.8, 120.0 and 56.3 ppm, respectively, according to the correlations found in the HSQC spectrum. Carbons C-10G, C-12G and C-13G were assigned at 134.0, 146.1 and 149.2 ppm, respectively, through their long distance ^{1}H–^{13}C coupling observed in the HMBC spectrum.

3.5. Chromatic assays

The wavelength of the maximum absorption in the visible region (λ_{max}) of the newly formed pigment was found to be equal to 549 nm in acidulated methanol, conferring on it a purple colour, which is in agreement with the results already reported in literature for some of the malvidin-alkyl/aryl-catechin adducts (Escribano-Bailón, Alvarez-Garcia, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001; Pissarra et al., 2003). The increase of pH from 1 to 11 induced colorimetric changes in the malvidin 3-glucoside-vanillyl-catechin pigment, as can be seen in Fig. 4. This was expected since, similarly to malvidin 3-glucoside and depending on the pH of the solution, the coloured flavylium cation of the anthocyanin-vanillyl adduct co-exists with other forms. Proton transfer reactions lead to the purple/blue quinonoidal bases, whilst hydration of flavylium ions gives rise to the colourless hemiacetal structures in equilibrium with small amounts of yellowish chalcones. In fact, the increase of pH from 1 to 9 led to a displacement of the λ_{max} from 549 to 580 nm (bathochromic effect), whereas at pH 11 this effect was even more pronounced ($\lambda_{max} = 642$ nm). This displacement also occurred with the original anthocyanin, but at a minor scale. This behaviour concurs with the results described in the literature for the malvidin 3-glucoside-ethyl-catechin adduct (Escribano-Bailón et al., 2001), and could be explained by the equilibrium displacement towards the formation of the blue quinonoidal structures, which occurs at lower pH values for the malvidin-vanillyl-catechin adduct comparatively to malvidin 3-glucoside. The stabilisation of the quinonoidal structure at lower pH could be explained by intramolecular copigmentation between the flavanol moiety and the chromophore group. The existence of a shoulder at around 450 nm in the visible spectrum of the newly formed pigment, more noticeable as the pH drops, may be explained by the equilibrium displacement towards the formation of the ionised chalcone form, as was proposed for malvidin 3-glucoside (Asenstorfer, Iland, Tate, & Jones, 2003).

Additionally, the increase of pH from 1 to 5 also induced a clear decrease of absorbance intensity (hypochromism), an effect also observed from pH 7 to 9. This fact is essentially due to the hydratation of flavylium ions of the anthocyanin moiety (Brouillard & Delaporte, 1977). However, the solution containing the new adduct displayed a higher resistance to discoloration when compared with malvidin 3-glucoside, which can be explained by a greater protection of the chromophore moiety, namely carbon 2 of the pyranic ring, against nucleophilic attack by water.

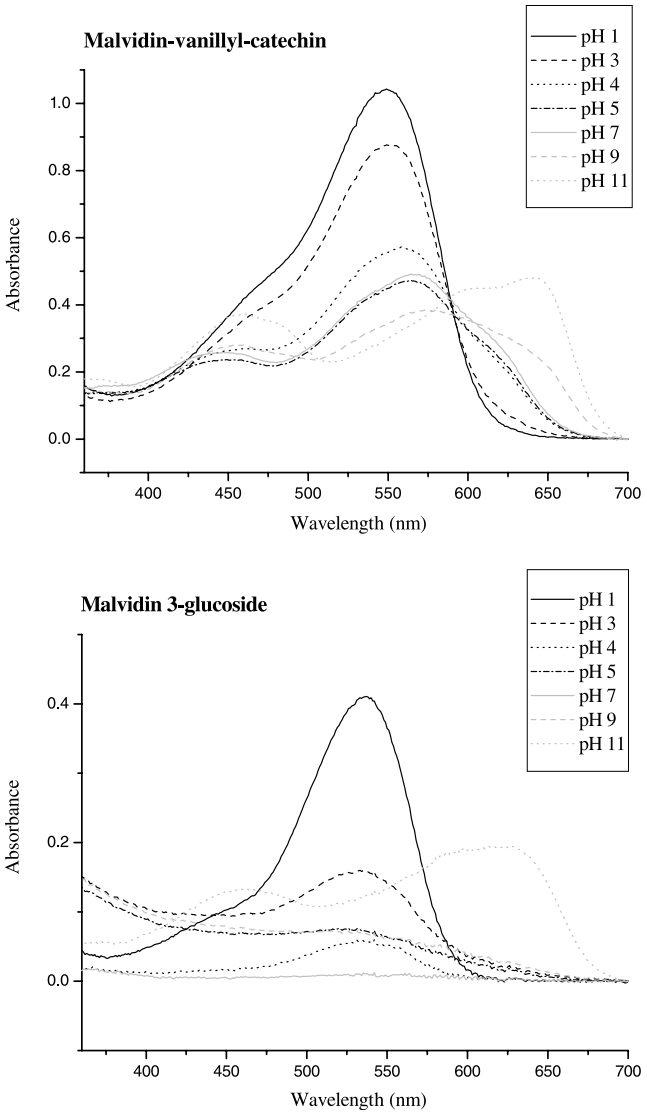

Fig. 4. UV–Vis spectra of malvidin 3-glucoside and malvidin-vanillyl-catechin pigments in 90% methanol solutions at different pH values.

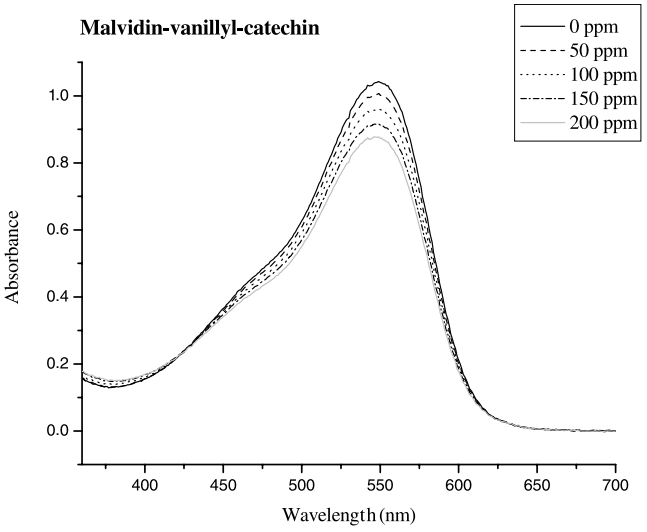

Fig. 5. UV–Vis spectrum of the malvidin-vanillyl-catechin pigment in a 90% methanol solution (pH 1) at different concentrations of SO_2.

The solution at pH 1.0 was used in the study of bleaching by SO_2 across a range between 0 and 200 ppm (Fig. 5). A decrease of absorbance intensity was observed for the malvidin 3-glucoside-vanillyl-catechin adduct with increasing concentrations of SO_2. However, this pigment was less affected by SO_2 than the original anthocyanin, which is almost completely decolorized at the highest concentration of SO_2, (Escribano-Bailón et al., 2001). This effect could be due to the protection promoted by the catechin-vanillyl moiety, which partially prevents the nucleophilic attack of the bisulphite anion at the positively charged carbon 2 or 4 (in lower extension) of the pyranic ring of the anthocyanin, preventing the formation of a colourless adduct.

The molar extinction coefficient (ε) was determined by measuring the absorbance of four solutions with different concentrations of the malvidin 3-glucoside-vanillyl-catechin pigment at its λ_{max} (549 nm in acidulated methanol). ε was found to be equal to 12,247 L mol^{-1} cm^{-1}, in comparison to 16,000 L mol^{-1} cm^{-1} for malvidin 3-glucoside and 17,100 L mol^{-1} cm^{-1} for malvidin 3-glucoside-ethyl-catechin, both values obtained from the literature (Escribano-Bailón et al., 2001; Mateus & de Freitas, 2001). The difference observed between the values of malvidin 3-glucoside-vanillyl-catechin and malvidin 3-glucoside-ethyl-catechin could be due to the presence of a bulky vanillyl group that can reduce the depth of penetration of radiation and therefore the ability to absorb light, reducing the colouring capacity of the adduct.

4. Conclusion

The present work points out the importance of vanillin, one of the most widely used and important flavouring materials worldwide, as well as one of the main aldehydes present in oak wood, as a mediator of the reaction that leads to the formation of the anthocyanin-catechin pigment. A new malvidin 3-glucoside-vanillyl-catechin pigment was synthesised and structurally characterized by NMR, and its structure corresponds to the one expected, according to the proposed mechanism for this type of reaction. In addition, the vanillin-derived adduct showed different chromatic features, when compared to the original anthocyanin. In fact, the newly-formed adduct showed higher protection against the nucleophilic attack of water at different pH values and bisulphite, when compared with the malvidin 3-glucoside. This is probably due to steric hindrances, which obstruct the hydration of the chromophore, thereby reducing color attenuation. This compound may exist in wine stored in oak barrels during the first years of ageing, and may somehow contribute to the colour changes observed under these conditions.

Acknowledgement

Thanks are due to the Portuguese/Spanish Programme ''Acciones Integradas Hispano-Portuguesas'' and ''Acções Integradas Luso-Espanholas'' (ref. HP01-24 and E-23/02).

References

Asenstorfer, R. E., Iland, P. G., Tate, M. E., & Jones, G. P. (2003). Charge equilibria and pK_a of malvidin-3-glucoside by electrophoresis. *Analytical Biochemistry, 318*, 219–299.

Atanasova, V., Fulcrand, H., Cheynier, V., & Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta, 458*, 15–27.

Bakker, J., Picinelli, A., & Bridle, P. (1993). Model wine solutions: colour and composition changes during ageing. *Vitis, 32*, 111–118.

Bax, A., & Subramanian, S. (1986). Sensitivity enhanced two-dimensional heteronuclear shift correlation NMR spectroscopy. *Journal of Magnetic Resonance, 67*, 565–569.

Bax, A., & Summers, M. F. (1986). H and C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. *Journal of the American Chemical Society, 108*, 2093–2094.

Bendz, G., Martensson, O., & Nilsson, E. (1967). Studies of flavilium compounds. I. Some flavilium compounds and their properties. *Arkiv fur Kemi, 27*, 67–77.

Brouillard, R., & Delaporte, B. (1977). Chemistry of anthocyanin pigments. 2. Kinetics and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. *Journal of the American Chemical Society, 99*, 8461–8468.

Chatonnet, P. (1991). Incidences du bois de chêne sur la composition chimique et les qualités organoleptiques des vins: applications technologiques. Thesis, Université de Bordeaux II, U.F.R. Institut d'Oenologie.

Chatonnet, P., Boidron, J. N., & Pons, M. (1989). Effect of heat on oak wood and its chemical composition. Part 2. Variations of certain compounds in relation to toasting intensity. *Connaissance de la Vigne et du Vin, 23*, 223–250.

Dallas, C., Ricardo da Silva, J. M., & Laureano, O. (1996a). Products formed in model wine solutions involving anthocyanins, procyanidin B2 and acetaldehyde. *Journal of Agricultural and Food Chemistry, 44*, 2402–2407.

Dallas, C., Ricardo da Silva, J. M., & Laureano, O. (1996b). Interactions of oligomeric procyanidins in model wine solutions containing malvidin 3-glucoside and acetaldehyde. *Journal of the Science of Food and Agriculture, 70*, 493–500.

Escalona, H., Birkmyre, L., Piggott, J. R., & Paterson, A. (2002). Effect of maturation in small oak casks on the volatility of red wine aroma compounds. *Analytica Chimica Acta, 458*, 45–54.

Escribano-Bailón, T., Alvarez-Garcia, M., Rivas-Gonzalo, J. C., Heredia, F. J., & Santos-Buelga, C. (2001). Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin 3-*O*-glucoside and (+)-catechin. *Journal of Agricultural and Food Chemistry, 49*, 1213–1217.

Escribano-Bailón, T., Dangles, O., & Brouillard, R. (1996). Coupling reactions between flavylium ions and catechin. *Phytochemistry, 41*, 1583–1592.

Fletcher, A. C., Porter, L. J., Haslam, E., & Gupta, R. K. (1977). Plant proanthocyanidins. Part 3. Conformational and configurational studies of natural procyanidins. *Journal of Chemical Society, Perkin Transactions, 1*, 1628–1637.

Fulcrand, H., Doco, T., Es-Safi, N. E., Cheynier, V., & Moutounet, M. (1996). Study of the acetaldehyde induced polymerisation of flavan-3-ols by liquid chromatography-ion spray mass spectrometry. *Journal of Chromatography A, 752*, 85–91.

Hurd, R. E. (1990). Gradient-enhanced spectroscopy. *Journal of Magnetic Resonance, 87*, 422–428.

Jurd, L. (1969). Review of polyphenol condensation reactions and their possible occurrence in the ageing of wines. *American Journal of Enology and Viticulture, 20*, 191–195.

Kay, L. E., Keifer, P., & Saarinen, T. (1992). Pure absorption gradient enhanced heteronuclear single quantum correlation spectroscopy with improved sensitivity. *Journal of the American Chemical Society, 114*, 10663–10665.

Mateus, N., & de Freitas, V. A. P. (2001). Evolution and stability of anthocyanin-derived pigments in Port wine during ageing. *Journal of Agricultural and Food Chemistry, 49*, 5217–5222.

Mateus, N., Pascual-Teresa, S., Rivas-Gonzalo, J. C., Santos-Buelga, C., & de Freitas, V. (2002). Structural diversity of anthocyanin-derived pigments detected in Port wines. *Food Chemistry, 76*, 335–342.

Miller, D. P., Howell, G. S., Michaelis, C. S., & Dickmann, D. I. (1992). The contents of phenolic acid and aldehyde flavor components of white oak as affected by site and species. *American Journal of Enology and Viticulture, 43*, 333–338.

Pissarra, J., Lourenço, S., González-Paramás, A. M., Mateus, N., Santos Buelga, C., Silva, A. M. S., et al. (2004). Structural characterization of new malvidin 3-glucoside-catechin aryl/alkyl-linked pigments. *Journal of Agricultural and Food Chemistry, 52*, 5519–5526.

Pissarra, J., Mateus, N., Rivas-Gonzalo, J. C., Santos-Buelga, C., & de Freitas, V. (2003). Reaction between malvidin 3-glucoside and (+)-catechin in model solutions containing different aldehydes. *Journal of Food Science, 68*(2), 476–481.

Revilla, I., Perez-Magariño, S., Gonzalez-San Jose, M. L., & Beltran, S. (1999). Identification of anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with diode array and mass spectrometry detection. *Journal of Chromatography A, 847*, 83–90.

Rivas-Gonzalo, J. C., Bravo-Haro, S., & Santos-Buelga, C. (1995). Detection of compounds formed through the reaction of malvidin-3-monoglucoside and catechin in the presence of acetaldehyde. *Journal of Agricultural and Food Chemistry, 43*, 1444–1449.

Sefton, M. A., Francis, I. L., Pocock, P. J., & Williams, P. J. (1993). The influence of natural seasoning on the concentrations of eugenol, vanillin, and *cis*- and *trans*-b-methyl-g-octalactone extracted from French and American oakwood. *Sciences des Aliments, 13*, 629–643.

Somers, T. C. (1971). The polymeric nature of wine pigments. *Phytochemistry, 10*, 2175–2216.

Vivar-Quintana, A. M., Santos-Buelga, C., Francia-Aricha, E., & Rivas-Gonzalo, J. C. (1999). Formation of anthocyanin-derived pigments in experimental red wines. *Food Science and Technology International, 5*(4), 347–352.

Vivas, N., & Glories, Y. (1993). Étude de la flore fongique du chêne (Quercus sp.) caractéristique du séchage naturel des bois destinés à la tonnellerie. *Cryptogamie-Mycologie, 14*, 127–148.

Vivas, N., Glories, Y., Doneche, B., & Gueho, E. (1991). Observations sur la flore fongique du bois de chêne (*Quercus* sp.) au cours de son séchage naturel. *Annales des Sciences Naturelles. (Botanique et Biologie vegetale), 13*(11), 149–153.

Wagner, R., & Berger, S. (1996). Gradient-selected NOESY-A fourfold reduction of the measurement time for the NOESY experiment. *Journal of Magnetic Resonance, 123A*, 119–121.