Structure of new anthocyanin-derived wine pigments

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Two new malvidin-derived pigments, referred to as A and B, were detected in red wine made from *Vitis vinifera* grapes (var. Carignane) and characterised. The results reported herein indicate that they are formed by covalent binding of major wine anthocyanins [malvidin 3-monoglucoside and malvidin 3-(6-*p*-coumaroyl)monoglucoside] with 4-vinylphenol. Synthetic products obtained by reaction between these reagents were shown to be identical with the natural compounds on the basis of their UV–visible, mass and ^{1}H NMR spectra. Their formation involves cyclisation between C-4 and the hydroxy group at C-5 of the original flavylium moiety and the vinylphenol double bond. Subsequent oxidation leads to pigment A or B. Their structure can yield two flavylium mesomeric forms, one corresponding to the malvidin type and the other to the pelargonidin type. The pigments' colour suggests that the latter is the predominant form under our conditions.

Introduction

Polyphenols contribute to the sensory properties associated with fruit quality (colour, bitterness, astringency, *etc.*).[1] Among them, anthocyanins are responsible for the red, blue or purple colour of most plants (flowers and fruits). The possibility of using them as food colourants has attracted the attention of the food industry.[2,3]

Colour expression of anthocyanins is closely connected to environmental factors such as temperature, light and pH. In addition, the ability of these natural pigments to interact with various molecules is another way to modify their intrinsic colour and, generally, to enhance their stability.[4] Such associations involve either non-covalent interactions or covalent binding. Two famous examples illustrate this concept; the first one is the copigmentation phenomenon (vertical stacking)[5,6] and the other one is the formation of anthocyanin–tannin complexes, occurring in wine ageing.[7,8] In the latter case (*i.e.* covalent binding), several mechanisms have been postulated to explain variation in hue from violet-red to brownish-red as the wine ages.[9–11] The latest hypothesis proposed in this field suggests the copigmentation reaction as the first step leading to covalent binding.[12]

This paper presents the structure of two anthocyanin-derived red-orange pigments discovered in wines, partly characterised in our previous report.[13] Structural elucidation has been achieved using both analytical (including mass spectrometry, ^{1}H NMR spectroscopy) and synthetic methods. The properties of the new molecules are discussed and the mechanism by which they are formed in the course of wine-making proposed.

Results and discussion

Two new pigments, selectively adsorbed on polymeric membranes during cross-flow microfiltration of red wine prepared at the INRA experimental winery (Gruissan, France) from *Vitis vinifera* var. Carignane grapes, were isolated as described earlier.[13] These pigments, referred to as **A** and **B**, exhibited an absorption maximum in the visible region, suggesting that they were related to anthocyanins. However, their UV–visible spectra (Fig. 1), recorded by means of a diode array detector coupled with the HPLC system, showed a particular band around 507 nm, differing from that of typical anthocyanins by having a narrower and more pointed shape. Moreover, the retention times of compounds **A** and **B** suggested that they were less polar and/or larger than the native pigments. The spectrum of pigment **B** displayed an additional shoulder around 310 nm, pointing to acylation with *p*-coumaric acid,[14,†] as in the UV–visible spectrum of malvidin 3-(6-*p*-coumaroyl)-monoglucoside, another grape anthocyanin. Although pigment **A** was more abundant in wine than pigment **B**, the latter was the major product in the membrane methanolic extract, due to its lower polarity and higher affinity for the membrane material. It was thus isolated by HPLC for structural elucidation.

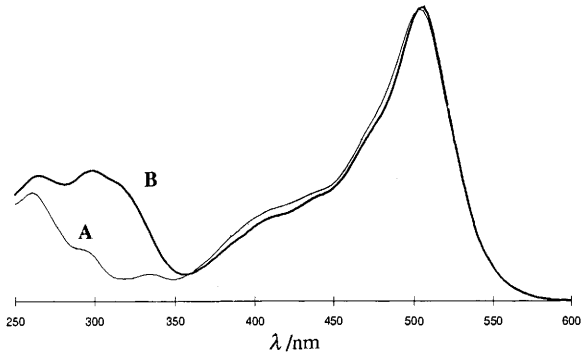


Fig. 1 UV-visible spectra of the new red-orange wine pigments **A** and **B**

Alkaline methanolysis of pigment **B** yielded methyl *p*-coumarate and the second pigment, **A**. Identification of the ester molecule was based on its retention time, UV–visible spectrum and co-chromatography with an authentic sample. Therefore, it was concluded that **B** was the *p*-coumaric ester of **A**.

Complete acid hydrolysis of **B** released glucose, identified by gas chromatography of the alditol acetate derivatives,[15] and a new compound which is likely to be the corresponding aglycone, given its lower polarity and UV–visible spectrum (loss

† *p*-coumaric acid = *p*-hydroxycinnamic acid.

Table 1 ^{1}H NMR chemical shifts (in ppm) of pigments **A**, **B** and their precursors, with *J*-values (in Hz) in parentheses[a]

	Pigment A	Pigment B	Malvidin 3-glucoside	Malvidin 3-(6-*p*-coumaroyl)glucoside
2‴,6‴-H	8.15 (8.8)	8.11 (8.8)		
3‴,5‴-H	7.03 (8.8)	7.03 (8.8)		
>C=C*H*$^{\alpha}$	7.94	7.93		
4-H			9.04	8.92
2′-, 6′-H	7.73	7.70	7.99	7.92
6-, 8-H	7.19	7.15	6.66, 6.97	6.54, 6.89
C*H*$^{\beta}$=CHCO$_2$R		7.21 (15.9)		7.40 (15.9)
2″-, 6″-H		7.34 (8.5)		7.27 (8.5)
3″-, 5″-H		6.81 (8.5)		6.76 (8.5)
CH=C*H*$^{\alpha}$CO$_2$R		5.91 (15.9)		6.19 (15.9)
Anomeric H	under the water signal	4.80 (7.6)	5.35 (7.6)	5.37
Glu-CH$_2$		4.04		4.33–4.49
ArO*Me*	4.00	3.84	4.00	3.97
Glucosyl	3.55–3.9	3.16–3.64	3.39–3.92	3.41–3.86

[a] Values were determined in [^{2}H$_4$]methanol–[^{2}H]TFA (99 : 1, v/v) at 300 K with the central solvent peak as internal reference (δ_H 3.30).

of the 300 nm absorbance and maintainance of the visible band with slight hypsochromic effect). Note that when malvidin 3-monoglucoside, the main anthocyanin of grape and wine, was hydrolysed under the same conditions, the aglycone residue could not be detected because of its spontaneous decomposition. Thus, compound **B** and its aglycone showed a greater resistance towards the drastic hydrolysis conditions than did the native pigments, suggesting a stabilising effect of their structural modification. Among the possible modifications of anthocyanins, the presence of a second sugar residue on the C-5 hydroxy group or that of a 4-substituent is known to increase the stability of the molecules.[16,17]

Further experiments were performed in order to determine the position of the glycosidic linkage on the glucose residue. GLC–MS analysis of the partially methylated alditol acetate derivatives showed that the C-1 of glucose is linked to the anthocyanin nucleus, as in all grape anthocyanins. The conformation of the sugar and the position of *p*-coumaric acid were determined by ^{1}H NMR experiments as discussed later.

Mass spectrometry was performed using an electrospray interface in the positive mode. The spectrum of pigment **B** showed a peak at *m*/*z* 755.5, corresponding presumably to the molecular flavylium cation M$^+$. These data confirmed that the compound cannot be a simple grape anthocyanin as the relative molecular mass of the largest one, malvidin 3-(6-caffeoyl)-monoglucoside,‡ equals 655.6; nor can the compound be a direct dimer. A second peak, more intense, located at *m*/*z* 447.5, was attributed to the aglycone fragment ion as the loss of 755.5 − 447.5 = 308 corresponds to that of a *p*-coumaroyl-glucose moiety. This typical fragmentation has been already reported in an analysis of carrot anthocyanins.[18] The mass of the fragment exceeds that of malvidin, the largest anthocyanin aglycone, indicating that the structural modification involves the anthocyanidin nucleus.

^{1}H NMR analysis of pigment **B** was also performed in acidified deuteriated methanol at 400 MHz. The results allowed us to recognize malvidin 3-(6-*p*-coumaroyl)monoglucoside as the anthocyanin moiety. However, the signal corresponding to 4-H proton of the latter was absent from the spectrum, suggesting that the C-4 position was substituted. On the other hand, additional signals consisting of a singlet and a four-spin system were detected in the aromatic region. Given the mass difference between pigment **B** and its anthocyanin precursor, malvidin 3-(6-*p*-coumaroyl)monoglucoside, these additional signals were tentatively attributed to a 4-vinylphenol substituent doubly linked to the anthocyanidin moiety.

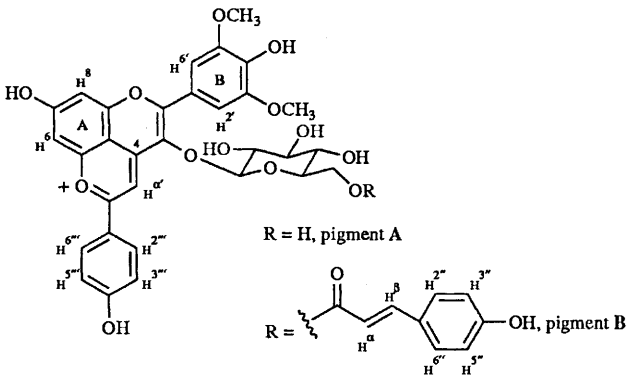
‡ caffeic acid = 3,4-dihydroxycinnamic acid.

To confirm this hypothesis, pigments **A** and **B** were synthesized using 4-vinylphenol with, respectively, malvidin 3-monoglucoside and malvidin 3-(6-*p*-coumaroyl)monoglucoside isolated from grape skins. HPLC traces of each synthetic mixture showed the presence of one product with same retention time and UV–visible spectrum as pigments **A** and **B**, respectively. In order to compare the NMR data of the wine pigments with those of the synthetic products, pigment **A** was synthesized in larger amounts, isolated and purified by HPLC, whereas pigment **B** could not be obtained in sufficient amounts for structural investigations, due to the lower quantity of malvidin 3-(6-*p*-coumaroyl)monoglucoside available.

The mass spectrum of synthetic pigment **A** showed a major peak at *m*/*z* = 609.0 along with the same aglycone fragment ion (*m*/*z* = 447.1) as natural pigment **B**. This confirms that the mass difference between **A** and **B** results from the additional coumaroyl residue in pigment **B** and that they are both malvidin-derived pigments formed by reaction with 4-vinylphenol. Their formation in red wine is believed to proceed by reaction of the wine anthocyanin pigments (malvidin 3-monoglucoside and its *p*-coumaric ester) with 4-vinylphenol. The occurrence of the latter in wines is well documented.[19–21]

Table 1 displays ^{1}H NMR data of synthetic pigment **A** along with those of natural wine pigment **B**. NMR spectra of malvidin 3-monoglucoside and malvidin 3-(6-*p*-coumaroyl)monoglucoside were made for comparison and are included in Table 1. The results confirmed that the synthetic product was identical to the natural one, except for the coumaroyl residue.

Comparison of the aliphatic region between the coumaroylated anthocyanins and the non-coumaroylated ones showed that the glucose methylene protons moved downfield in the acylated pigments. This shift and the integration value of the latter signal confirmed that the substituent is at the C-6 position of the glucose as shown earlier in the case of natural acylated anthocyanins.[22] Besides, the large coupling constant (7.63 Hz) of the anomeric proton allowed us to define the β conformation of the glucosyl residue. Thus, the conformation of the glucose molecule as well as the position of the coumaroyl group are identical in malvidin 3-(6-*p*-coumaroyl)monoglucoside and pigment **B**. In the same way, acylation of the glucose caused an upfield shift on the aglycone protons (6-H, 8-H of A-ring, 4-H, 2′-H and 6′-H of B-ring) in malvidin 3-(6-*p*-coumaroyl)monoglucoside as well as in pigment **B**.[23] Nevertheless, this effect was emphasised in the case of malvidin-derived pigments by the presence of the 4-vinylphenol substituent. In particular, protons 6-H and 8-H became equivalent in the derived pigments, suggesting that the hydroxy group on C-5 is a substitution position. On the other hand, the signal at low field (respectively δ 9.04 and 8.92), attributed to proton 4-H in spectra of the



Scheme 1 Structures of malvidin-derived pigments **A** and **B**

native anthocyanins, was missing in those of pigments **A** and **B**, suggesting that this position was also substituted, as already mentioned.

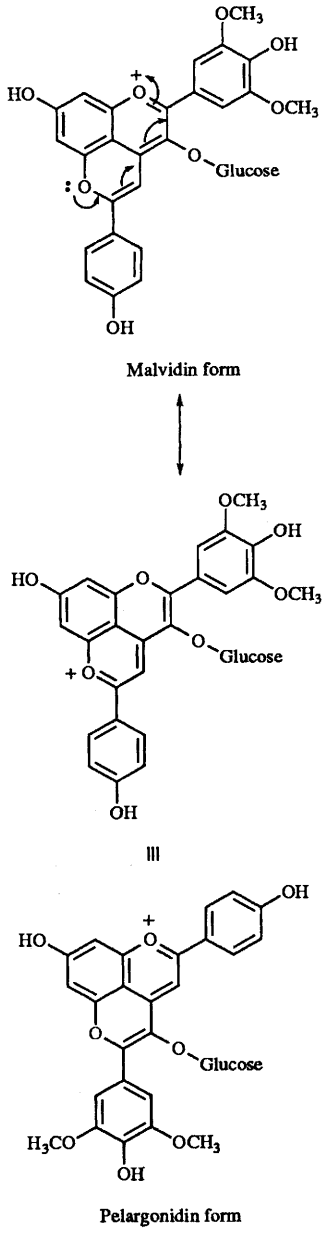
Finally, the set of data obtained by NMR and mass spectrometry allowed us to propose the structures presented above. Their formation may result from a cycloaddition or an electrophilic addition to the anthocyanin nucleus, followed by an oxidation step. The structure of the flavylium cation showing both a nucleophilic site on the 5-hydroxy group and an electrophilic site on the C-4 position is in favour of the electrophilic addition mechanism. Moreover, the polarisability of the vinylphenol double bond (δ^+ for the carbon adjacent to the phenol ring, δ^- for the end carbon) suggests the formation of the regioisomer presented here; although NMR analysis does not allow one to distinguish between the two possibilities.

Another argument in favour of this structural determination is the UV–visible spectra of the new molecules. The ratio of UV to visible absorbance was approximately twice that of malvidin, indicating a larger chromophore for the derived pigments **A** and **B**. In fact, the new pigments are mesomeric as shown below for **A**. Their absorption maxima are much closer to that of pelargonidin 3-monoglucoside (506 nm) than that of malvidin 3-monoglucoside (534 nm),[24] suggesting that pelargonidin form (b) predominates under our conditions. Note that, in addition, a synthetic flavylium [7-hydroxy-2-(4-hydroxystyryl)-benzopyrylium salt] prepared by Jurd 1964,[25] which corresponds to the lower part of pigment **A** (malvidin form) also showed an absorption maximum at 507 nm.

On the basis of this observation, it seems possible to modify the colour of the new pigments by selecting either the anthocyanin or the vinylphenol derivative. The synthesis of a new series of related pigments differing by their substitution patterns on the B-ring or on the vinylphenol ring is under way. The possibility to provide a wide range of tints is attractive, especially as the new compounds revealed an unusual colour stability in the course of our experimentation. As mentioned in the literature,[4,26] substitution at C-4, in the present case by vinylphenol, protects the molecule from hydration, which usually leads to the colourless 'carbinol' form. Thus, the next step of the present work will be a physicochemical study of how anthocyanin equilibria change with pH, since the colour results from the proportion of the four anthocyanin species, namely, the red flavylium cations (AH^+), the blue anhydrobase (A), the colourless 'carbinol' (**B**) and the yellow chalcone (C).[27]

Conclusions

Pigments **A** and **B** seem to be formed by the addition of the vinylphenol ethylenic bond to the anthocyanin nucleus, followed by an oxidation step. It takes place at acidic pH (3–4) prevailing in wine and may participate in the gradual colour changes from red-purple to tawny tints reported to occur in the course of wine ageing. To our knowledge, this is the first report



Scheme 2 Possible mesomeric forms of pigment **A**

of new pigments formed by reaction between anthocyanins and other phenolic constituents in wines.

The presence of 4-vinylphenol has often been reported in wine.[19,28] It proceeds from the decarboxylation of *p*-coumaric acid by a side yeast activity referred to as SCD (substituted cinnamate decarboxylase).[20,29] This volatile phenol, associated with an unpleasant smell and often responsible for off-flavours, is more abundant in white wines than in red ones. Several mechanisms have been postulated to explain this difference. The reaction of 4-vinylphenol with anthocyanins demonstrated herein may also contribute to lowering its concentration in red wines.

Experimental

Mass spectrometry

Mass spectrometric analyses were performed with an electrospray interface in the positive mode by means of a MS Engine mass spectrometer (Hewlett Packard). Percentage values in parentheses refer to the height relative to the highest peak in the spectrum. Partially methylated alditol acetates were analysed by GLC–MS as described below.

NMR spectroscopy

NMR spectra of samples in [$^2H_4$]methanol–(CF_3CO_2D-[^2H]TFA) (99:1, v/v) were run at 300 K on a JEOL EX-400 instrument at 400 MHz. *J* Values are given in Hz. The central solvent signals were used as internal reference (δ_H 3.30).

HPLC analyses

The wines and membrane extracts were analysed by HPLC using a Waters-Millipore (Millipore Corp., Milford, MA, USA) system including two M510 pumps, a U6K manual injector, an automated gradient controller and a 990 diode array detector. UV–visible spectra were recorded on line from 250 to 600 nm. The column was a reversed-phase Lichrospher 100-RP18 (5 μm packing) (250 × 4 mm i.d.) protected with a guard column of the same material (Merck, Darmstadt, Germany). Phenolic compounds were eluted under the following conditions: 1 cm^3 min^{-1} flow rate; oven temperature, 30 °C; solvent A, water–formic acid (98:2, v/v); solvent B, acetonitrile–water–formic acid (80:18:2, v/v); elution with linear gradients from 5 to 30% B in 40 min, from 30 to 50% B in 20 min, and from 50 to 80% B in 10 min, followed by washing and reconditioning of the column. Characterisation of the major grape anthocyanins present in the wines and membrane extracts was achieved by HPLC analysis and comparison of the elution order and UV–visible spectra with those of reference compounds and literature data.[23,30]

Pigment purification

The membrane methanol extracts were prepared as described earlier.[13] They were evaporated to dryness, the residue was dissolved in ethanol (3 cm^3) and the solution was added to aq. NaOH (2 mol dm^{-3}; 2.4 cm^3). The first purification step consisted of liquid chromatography on normal-phase silica gel (Si 60, Merck, Darmstadt, Germany). Elution was carried out successively with ethanol (100 cm^3) (fraction 1), methanol–water (90:10, v/v) (500 cm^3) (fraction 2) and methanol–trifluoroacetic acid (TFA) (99.9:0.1, v/v) (100 cm^3) (fraction 3). Each collected fraction was taken to dryness by rotary evaporation and dissolved in methanol–TFA (99.9:0.1, v/v) (1.5 cm^3) prior to HPLC analysis. The unknown orange pigments were isolated from fraction 2 by HPLC on a semi-preparative scale. The latter separation was performed using the same equipment as described above but under the following conditions: column, μ-Bondapak RP-18 (10 μm packing) (300 × 7.8 mm i.d.) (Millipore Corp., Milford, MA, USA); flow rate, 2 cm^3 min^{-1}; oven temperature, 30 °C; solvent A, 2.5% acetic acid in water; solvent B, acetonitrile–solvent A (80:20, v/v); elution with linear gradients from 5 to 20% B in 20 min, from 20 to 50% B in 30 min and from 50 to 80% B in 10 min. Fractions containing the orange pigments were collected, pooled, concentrated under reduced pressure and lyophilised. The amounts of pigments **A** and **B** obtained were 0.42 mg and 2.12 mg, respectively.

Isolation of malvidin 3-monoglucoside and malvidin 3-(6-*p*-coumaroyl)monoglucoside

Anthocyanin extracts were prepared from skins of *V. vinifera* var. Grenache noir. Skins were peeled from washed berries and extracted with methanol–(1 mol dm^{-3}) hydrochloric acid (99:1, v/v). They were ground in a Warren blender before extraction under magnetic agitation during 2 h at 4 °C. After centrifugation (5000 rpm at 4 °C for 15 min), the methanolic extract was concentrated under reduced pressure and filtered through 0.45 μm HLVP filters (Millipore, Milford, MA) to obtain a crude extract. After dilution with deionised water, the crude extract was partially purified by polyvinylpyrrolidone (PVP) chromatography using a method adapted from Glories (1976).[31] After removal of sugars and acids by washing with water, anthocyanins were eluted with methanol–water–(1 mol dm^{-3}) hydrochloric acid (70:29:1, v/v). Each anthocyanin was then purified by reversed-phase HPLC on a semi-preparative scale.

Purification of malvidin 3-monoglucoside and malvidin 3-(6-*p*-coumaroyl)monoglucoside

The HPLC apparatus was a Kontron Instruments (Milan, Italy) system including a 460 autosampler, a 325 pump system, a 430 dectector and an MST1 450 data system. The semi-preparative column was reversed-phase μ-Bondapack C-18 (Millipore-Waters, Milford, MA) (10 μm packing) (300 mm × 7.8 mm i.d.) protected with a guard column filled with Lichrospher 100RP-18 (Merck). Malvidin 3-monoglucoside and malvidin 3-(6-*p*-coumaroyl)monoglucoside were eluted and collected under the following conditions: 2 cm^3 min^{-1} flow rate; oven temperature, 30 °C; solvent A, water–formic acid (98:2, v/v); solvent B, acetonitrile–water–formic acid (80:18:2, v/v). Elution was with a mixture of solvents A:B (90:10, v/v), isocratic for 10 min, and continuing with linear gradients to 85:15 for 18 min, to 70:30 for 10 min and to 50:50 for 5 min. Characterisation of both anthocyanins was achieved by comparison of their elution order and UV spectra with literature data[30] and with those of reference compounds prepared and identified earlier in our laboratory. After acidification, the isolated compounds were lyophilised and used for NMR and mass analyses.

Synthesis of pigment A

Maldivin 3-monoglucoside (7.9 mg, 15 μmol) was dissolved in 10^{-3} mol dm^{-3} hydrochloric acid (2 cm^3). A solution of 4-vinylphenol (10% in propylene glycol; 0.05 cm^3, 43.5 μmol) was added and the mixture was stirred at 30 °C for 4 h. Reaction progress was monitored by HPLC under the conditions used for wines and membrane extracts analyses. After 24 h, malvidin 3-monoglucoside was completely replaced by pigment **A**. The latter was then purified by reversed-phase HPLC on a semi-preparative scale as described for wine pigments. The amount of pigment **A** finally obtained was 1.16 mg.

Synthesis of pigment B

Pigment **B** was synthesized from a global acylated anthocyanin extract which was first collected in the course of the purification of malvidin 3-monoglucoside. The reaction was conducted with the same molar ratio of reagents as used in the synthesis of pigment **A**. Reaction progess was monitored by HPLC as described above.

Structural analyses

Sugar analysis. After hydrolysis of pigment **B** by heating at 120 °C for 75 min in 2 mol dm^{-3} TFA (Albersheim *et al.*, 1967),[32] neutral sugars were determined by GLC of the alditol acetate derivatives[15] at 210 °C on a fused-silica DB-225 capillary column (30 m × 0.32 mm i.d., 25 μm film; J and W Scientific, Folsom, CA, USA) with hydrogen as the carrier gas.

The positions of glycosidic linkage(s) on the sugar moiety(ies) were determined by methylation of the sugar free hydroxy groups by the Hakomori method as described by Jansson *et al.*, (1976),[33] followed by acid hydrolysis, conversion of the partially methylated sugars into alditol acetates, and analysis on DB-1 and DB-225 capillary columns.[34] Identifications were based on retention times and were confirmed by GLC–MS, using the DB-225 column (on-column injection at 50 °C; injector, temperature gradient from 50 to 250 °C at 180 °C min^{-1}; oven, temperature gradient from 150 to 180 °C at 50 °C min^{-1} for 15 min, then 5 °C min^{-1} to 210 °C; He as carrier gas at 2 cm^3 min^{-1}) coupled to a Finnigan Mat ITD 700 mass spectrometer.

Saponification reaction. Pigment **B** (9.8 mm^3 of a 0.53 g dm^{-3} methanolic solution) was added to 2 mol dm^{-3} methanolic sodium methanolate (25 mm^3) and kept for 2 h at ambient

temperature under argon. After acidification with methanol–TFA (99.5:0.5, v/v; 265 mm^3), the released compounds were analysed by HPLC as described at the beginning of this Experimental section.

Mass and NMR data of pigment A. *m/z* 609.0 (34.8%, M^+) and 447.1 (100, M − glucose), δ_H 3.55–3.9 (6 H, m, glucosyl protons), 4.00 (6 H, s, B-ring 2 × MeO), 7.03 (2 H, d, *J* 8.8, vinylphenol ring 3‴- and 5‴-H), 7.19 (2 H, br s, A-ring 6- and 8-H), 7.73 (2 H, br s, B-ring 2′- and 6′-H), 7.94 (1 H, s, >C=CH$^{\alpha'}$) and 8.15 (2 H, d, *J* 8.8, vinylphenol ring 2‴- and 6‴-H).

Mass and NMR data of pigment B. *m/z* 755.5 (100%, M^+) and 447.5 (25, M − *p*-coumaroyl glucose), δ_H 3.16–3.4 (3 H, m, glucosyl protons), 3.64 (1 H, t, glucosyl proton), 3.84 (6 H, s, B-ring 2 × MeO), 4.04 (2 H, br d, glucosyl-CH_2), 4.80 (1 H, d, *J* 7.6, anomeric glucosyl proton), 5.91 (1 H, d, *J* 15.9, $HOC_6H_4CH{=}CHCO_2$), 6.81 (2 H, d, *J* 8.5, *p*-coumaroyl ring 3″- and 5″-H), 7.03 (2 H, d, *J* 8.8, vinylphenol ring 3‴- and 5‴-H), 7.15 (2 H, br s, A-ring 6- and 8-H), 7.21 (1 H, d, *J* 15.9, $HOC_6H_4CH{=}CHCO_2$), 7.34 (2 H, d, *J* 8.5, *p*-coumaroyl ring 2″- and 6″-H), 7.70 (2 H, br s, B-ring 2′- and 6′-H), 7.93 (1 H, s, >C=CH$^{\alpha}$) and 8.11 (2 H, d, *J* 8.8, vinylphenol ring 2‴- and 6‴-H).

NMR data of malvidin 3-monoglucoside. δ_H 3.39–3.92 (6 H, m, glucosyl protons), 4.00 (6 H, s, B-ring 2 × MeO), 5.35 (1 H, d, *J* 7.6, anomeric glucosyl proton), 6.66 (1 H, br s, A-ring 6-H), 6.97 (1 H, br s, A-ring 8-H), 7.99 (2 H, br s, B-ring 2′- and 6-H) and 9.04 (1 H, s, benzopyrilium ring 4-H).

NMR data of malvidin 3-(6-*p*-coumaroyl)monoglucoside. δ_H 3.41–3.86 (4 H, m, glucosyl protons), 3.97 (6 H, s, B-ring 2 × MeO), 4.33–4.49 (2 H, br d, glucosyl-CH_2), 5.37 (1 H, d, *J* 7.6, anomeric glucosyl proton), 6.19 (1 H, d, *J* 15.9, $HOC_6H_4CH{=}CHCO_2$), 6.54 (1 H, br s, A-ring 6-H), 6.76 (2 H, d, *J* 8.5, *p*-coumaroyl ring 3″- and 5″-H), 6.89 (1 H, br s, A-ring 8-H), 7.27 (2 H, d, *J* 8.5, *p*-coumaroyl ring 2″- and 6″-H), 7.40 (1 H, d, *J* 15.9, $HOC_6H_4CH{=}CHCO_2$), 7.92 (2 H, br s, B-ring 2′- and 6′-H) and 8.92 (1 H, s, benzopyrylium ring 4-H).

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