

New Family of Bluish Pyranoanthocyanins

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The use of anthocyanins has been investigated for the preparation of food and beverage natural colorants as they seem to have non-toxic effects. In this context, vinylpyranoanthocyanins were recently found to naturally occur in ageing red wine. This new family of anthocyanin-derived pigments may be obtained directly through the reaction between anthocyanin derivatives and other compounds. Some of these newly formed pigments have been found to exhibit a bluish color at acidic pH. The formation of bluish pigment was obtained through reaction between anthocyanin-pyruvic-acid adducts and flavanols in the presence of acetaldehyde. The formation of similar bluish pigments was attempted using other different precursors. The chromatic features of this kind of pigments bring promising expectations concerning the use of these naturally occurring blue pigments in the food industry.

VITIS VINIFERA ANTHOCYANINS AND DERIVATIVE PIGMENTS IN RED WINE

Anthocyanins are the most important group of water-soluble plant pigments visible to the human eye. These pigments are responsible for a great variety of colours of several fruits, vegetables, and plants. The colour of red wine is mainly due to the presence of these polyphenolic compounds extracted from grape berries during the wine-making process. The general structure of *Vitis vinifera* anthocyanidin monoglucosides is represented in Figure 1. These compounds differ in their hydroxylation and methoxylation patterns of ring B yielding a wide range of colours from orange-red to violet at very acidic pH [1]. The glucosyl moiety linked at the 3-O position of ring C may also be acylated with acetic acid, coumaric acid, or caffeic acid.

These compounds undergo chemical transformations during wine ageing yielding new pigments that become responsible for the changing colour and its longevity [2]. These new pigments were first thought to result mainly from condensation reactions between anthocyanins and flavanols directly or mediated by acetaldehyde [3, 4, 5, 6, 7, 8, 9]. Nevertheless, over the last decade, reactions involving anthocyanins with other compounds such as pyruvic acid [10, 11, 12, 13, 14, 15], vinylphenol [16, 17], vinylcatechol [18], α -ketoglutaric acid [19], acetone [19, 20, 21], and 4-vinylguaiacol [21] have been demonstrated yielding new families of anthocyanin-derived pigments, namely, pyranoanthocyanins, with spectroscopic features that may somehow contribute to a more orange-red colour. This family of pyranoanthocyanins has been extensively investigated over the last years and several

compounds have been recently evidenced in aged Port red wines [22, 23, 24] (see Table 1 and Figure 2).

It is interesting to notice that the anthocyanins-catechin pigments revealed the same λ_{\max} as the pyruvate derivatives, which is hypsochromically shifted from that of original anthocyanins. Strikingly, pigments 9 and 13 which contain a procyanidin dimer unit in their structure revealed an important bathochromic shift (9 nm) from that of their counterparts with a single flavanol monomeric unit ((+)-catechin or (-)-epicatechin). This outcome 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 flavylum chromophore may somehow occur).

PORTISINS—A NEW GROUP OF VINYLPYRANOANTHOCYANINS DETECTED IN PORT WINE

More recently, two new pigments with unique spectroscopic features exhibiting a bluish colour in acidic solution were found to occur in aged Port red wines [25] (see Figure 3, pigments 22 and 23). Indeed, these two pigments with maximum absorption in the visible region at 583 nm were detected by HPLC in two-year-old Port wine samples. These newly formed pigment structures in which anthocyanins are linked to flavanols by a vinyl linkage were named as portisins. Likewise, similar pigments arising from different anthocyanins and flavanols were tentatively detected by LC-DAD-MS in Port wine samples (Table 2).

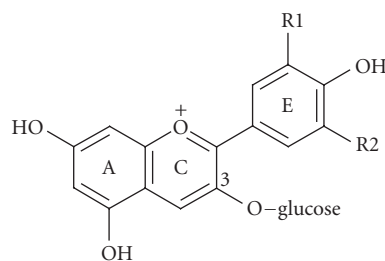


FIGURE 1. Structures of *V. vinifera* anthocyanidin monoglucosides (flavylium form).

Anthocyanidin	R1	R2
Delphinidin	OH	OH
Cyanidin	OH	H
Petunidin	OCH ₃	OH
Peonidin	OCH ₃	H
Malvidin	OCH ₃	OCH ₃

TABLE 1. Pyranoanthocyanins and detected in Port wine fractions. (Mv = malvidin; Dp = delphinidin; Pt = petunidin; Pn = peonidin; py = pyruvic acid derivative; gluc = glucoside; cat = (+)-catechin or (-)-epicatechin; PC = procyanidin dimer.)

Pigment	Pyranoanthocyanin	λ_{\max} (nm)	Structural elucidation
1	Mv 3-gluc-py	511	NMR, MS, UV-Vis
2	Pt 3-(acetyl)gluc-py		MS
3	Mv 3-(acetyl)gluc-py	511	NMR, MS, UV-Vis
4	Pt 3-(coumaroyl)gluc-py		MS
5	Mv 3-(coumaroyl)gluc-py	511	NMR, MS, UV-Vis
6	Pn 3-(coumaroyl)gluc-py		MS
7	Dp 3-gluc-py		MS
8	Dp 3-acetylgluc-py		MS
9	Mv 3-gluc-vinyl-(+)-cat-(+)-cat	520	NMR, MS, UV-Vis
10	Mv 3-(acetyl)gluc-vinyl-PC dimer		MS
11	Mv 3-gluc-vinyl-(+)-cat	511	NMR, MS, UV-Vis
12	Mv 3-(acetyl)gluc-vinyl-cat		MS
13	Mv 3-(coumaroyl)gluc-vinyl(-)-epi-(+)-cat	520	NMR, MS, UV-Vis
14	Mv 3-gluc-vinyl(-)-epi	511	NMR, MS, UV-Vis
15	Mv 3-(coumaroyl)gluc-vinyl-(+)-cat	511	NMR, MS, UV-Vis
16	Mv 3-(coumaroyl)gluc-vinyl(-)-epi	511	NMR, MS, UV-Vis
17	Mv 3-gluc-vinylphenol		MS
18	Mv 3-(caffeoyl)gluc-vinylphenol		MS
19	Pn 3-(coumaroyl)gluc-vinylphenol		MS
20	Mv 3-(coumaroyl)gluc-vinylphenol		MS
21	Mv 3-(acetyl)gluc-vinylphenol		MS

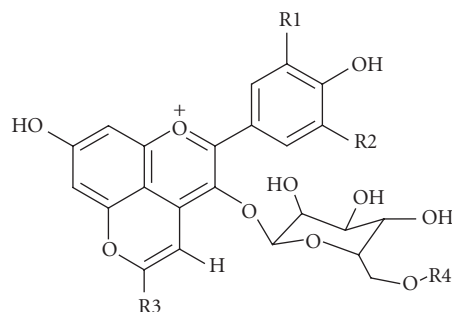
Furthermore, a portisin with a phenol group replacing the flavanol moiety (pigment 26) has also recently been found to occur in aged Port wine (Table 2) (N. Mateus et al, unpublished data). However, the maximum absorption of this pigment in the visible region (538 nm) was found to be quite hypsochromically shifted from that of portisins with a flavanol moiety (Figure 4). The small hydroxylation pattern of the phenol ring probably contributes to this hypsochromic shift more significantly compared to the phloroglucinol ring of flavanols. Effectively, a similar pigment with a phloroglucinol moiety replacing the phenol group resulting from the reaction between malvidin 3-O-glucoside and phloroglucinol in the presence of acetaldehyde was shown to have a λ_{\max} of 565 nm (unpublished data).

Concerning their formation, this new class of anthocyanin-derived pigments may be obtained through a reaction between anthocyanin-pyruvic-acid adducts

and other compounds such as flavanols (eg, catechins, procyanidins) or phloroglucinol in the presence of acetaldehyde (Figure 5) [26], or directly by reaction with *p*-vinylphenol. The last step of their formation is thought to include decarboxylation, dehydration, and oxidation yielding a structure with extended conjugation of the π electrons, which is likely to confer a higher stability of the molecule and is probably at the origin of its blue color. Similar vinylpyranoanthocyanins had previously been synthesized using starting chemicals not found in grapes or in the yeasts [27].

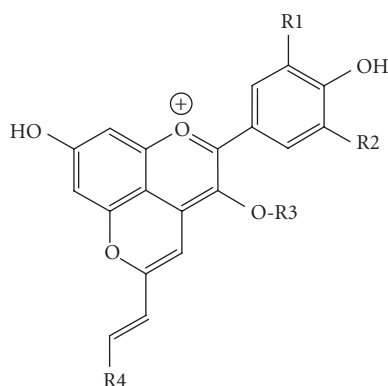
INTEREST AND POSSIBLE APPLICATION OF PORTISINS IN THE FOOD INDUSTRY

The chromatic features of this kind of pigments bring promising expectations concerning the use of these naturally occurring blue pigments in the food industry.



Pigment	R1	R2	R3	R4
1	OMe	OMe	COOH	H
2	OMe	OH	COOH	Acetyl
3	OMe	OMe	COOH	Acetyl
4	OMe	OH	COOH	Coumaroyl
5	OMe	OMe	COOH	Coumaroyl
6	OMe	H	COOH	Coumaroyl
7	OH	OH	COOH	H
8	OH	OH	COOH	Acetyl
9	OMe	OMe	Vinyl-(+)-cat-(+)-cat	H
10	OMe	OMe	Vinyl-PC dimer	Acetyl
11	OMe	OMe	Vinyl-(+)-cat	H
12	OMe	OMe	Vinyl-(+)-cat	Acetyl
13	OMe	OMe	Vinyl-(+)-epi-(+)-cat	Coumaroyl
14	OMe	OMe	Vinyl-(+)-epi	H
15	OMe	OMe	Vinyl-(+)-cat	Coumaroyl
16	OMe	OMe	Vinyl-(+)-epi	Coumaroyl
17	OMe	OMe	Vinylphenol	H
18	OMe	OMe	Vinylphenol	Caffeoyl
19	OMe	H	Vinylphenol	Coumaroyl
20	OMe	OMe	Vinylphenol	Coumaroyl
21	OMe	OMe	Vinylphenol	Acetyl

FIGURE 2. Pyranoanthocyanin structures detected in wine fractions (Table 1); cat = catechin; epi = epicatechin.



Pigment	R1	R2	R3	R4
22	OMe	OMe	Glucose	PC
23	OMe	OMe	Coumaroylglucose	PC
24	OMe	OMe	Glucose	Cat
25	OMe	OMe	Coumaroylglucose	Cat
26	OMe	OMe	Glucose	Phenol
27	OMe	OH	Glucose	Cat
28	OMe	H	Glucose	Cat
29	OMe	OMe	Acetylglucose	Cat

FIGURE 3. Portisin structures detected in Port red wine fractions (Table 2); cat = catechin; PC = procyanidin dimer.

Indeed, despite the extensive colour palette available in nature, pigments exhibiting blue colours are very scarce. For instance, the blue colours displayed by some flowers are mainly due to copigmentation phenomena [28, 29, 30, 31, 32]. Moreover, bluish hues may be obtained by the presence of quinonoidal forms of anthocyanins in high pH media [33, 34].

Therefore, the food industry has been searching for new alternative ways to produce products (foodstuffs and

beverages) with bluish colours. Bearing this in mind, the production of bluish pigments was attempted in the laboratory using different precursors. Firstly, the formation of such pigments requires anthocyanins, which can be obtained using several red fruit extracts. Sweet cherry, bilberry, red apple, plum, blackberry, and elderberry extracts were used as anthocyanin sources for the synthesis of anthocyanin-derived pigments. Following this, the formation of the anthocyanin-pyruvic-acid

TABLE 2. Portisins detected in Port wine fractions. (Mv = malvidin; Pn = peonidin; Pt = petunidin; gluc = glucoside; cat = catechin; PC = procyanidin dimer.)

Pigment	Portisin	λ_{\max} (nm)	Structural elucidation
22	VinylpyranoMv-3-gluc-PC	583	NMR, MS, UV-Vis
23	VinylpyranoMv-3-coumaroylgluc-PC	583	NMR, MS, UV-Vis
24	VinylpyranoMv-3-gluc-cat	572	MS, NMR, UV-Vis
25	VinylpyranoMv-3-coumaroylgluc-cat	577	NMR, MS, UV-Vis
26	VinylpyranoMv-3-phenol	538	MS, NMR, UV-Vis
27	VinylpyranoPt-3-gluc-cat	570	MS
28	VinylpyranoPn-3-gluc-cat	569	MS
29	VinylpyranoMv-3-acetylgluc-cat	577	MS

TABLE 3. Some anthocyanins respective pyruvic acid adducts and portisins obtained from different red fruit extracts. (Cy = cyanidin; Mv = malvidin; py = pyruvic acid derivative; gluc = glucoside; samb = sambubiose; ara = arabinose; rut = rutinose; cat = catechin.)

Source	Anthocyanin	λ_{\max} (nm)	Pyruvic acid adduct	λ_{\max} (nm)	Portisin	λ_{\max} (nm)
Blackberries	Cy-3-gluc	516	Cy-3-gluc-py	505	VinylpyranoCy-3-gluc-cat	567
Sweet cherries	Cy-3-rut	516	Cy-3-rut-py	505	VinylpyranoCy-3-rut-cat	567
Elderberries	Cy-3-samb	516	Cy-3-samb-py	505	VinylpyranoCy-3-samb-cat	567
Red apple	Cy-3-gal	516	Cy-3-gal-py	505	VinylpyranoCy-3-gal-cat	567
Bilberries	Cy-3-ara	516	Cy-3-ara-py	505	VinylpyranoCy-3-ara-cat	567
Bilberries	Mv-3-ara	528	Mv-3-ara-py	511	VinylpyranoMv-3-ara-cat	572
Bilberries	Mv-3-gluc	528	Mv-3-gluc-py	511	VinylpyranoMv-3-gluc-cat	572
Grape berries	Mv-3-coumaroylgluc	532	Mv-3-coumaroylgluc-py	516	VinylpyranoMv-3-coumaroylgluc-cat	578

adduct was achieved through a reaction with pyruvic acid, as previously developed for grape malvidin 3-O-glucoside-pyruvic-acid adduct. The different anthocyanins from the red fruit extracts yielded pyruvic acid adducts with a λ_{\max} hypsochromically shifted from that of genuine anthocyanins, some of which are indicated in Table 3. Consequently, the colour of all the extracts turned to a more orange-like hue. These anthocyanin-pyruvic-acid adducts were used as precursors for the formation of portisins, which was attempted using (+)-catechin in the presence of acetaldehyde.

As an example, Figure 6 shows the anthocyanin profile of an elderberry extract (*Sambucus nigra*) after two days of reaction with pyruvic acid. The anthocyanins of elderberries are two cyanidin monoglucosides (2) (cyanidin 3-O-glucoside and cyanidin 3-O-sambubioside) and a cyanidin 3,5-diglucoside (1) (3-O-sambubioside, 5-O-glucoside). This latter is not likely to react with pyruvic acid as position 5-O of the anthocyanin must be free from any substitution. Therefore, the only two pigments formed are the pyruvic acid adducts of the cyanidin monoglucosides ((3) and (4)), as seen from the respective HPLC chromatogram recorded at 520 nm (Figure 6a). Moreover, the HPLC chromatogram recorded at 570 nm of the purified pyruvate extract further treated with catechin in the presence of acetaldehyde is shown in Figure 6b. This portisin profile of the elderberry extract was obtained when practically all the pyruvic acid derivatives had reacted. The two portisins obtained correspond to

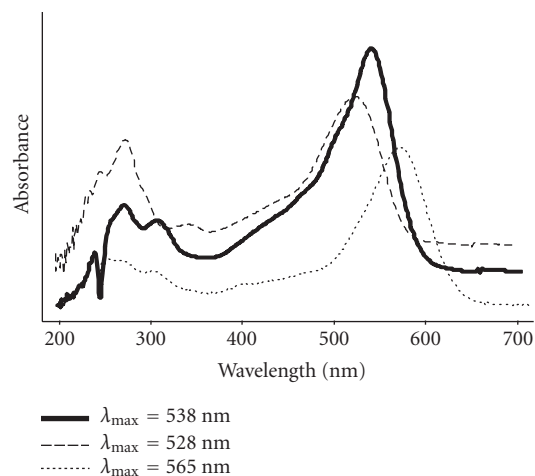


FIGURE 4. (a) UV-Vis spectra of malvidin 3-O-glucoside (solid), (b) vinylpyranoMv-3-gluc-phenol (dashed), and (c) vinylpyranoMv-3-gluc-phloroglucinol (dotted) recorded from the HPLC diode array detector (pH = 1.5).

the vinylpyranoanthocyanins of cyanidin 3-O-glucoside (5) and cyanidin 3-O-sambubioside (6), as confirmed by LC-DAD-MS (data not shown).

Overall, the malvidin monoglucosides and derivatives appeared to be the anthocyanins with the highest λ_{\max} in the UV-Vis spectrum when compared with cyanidin

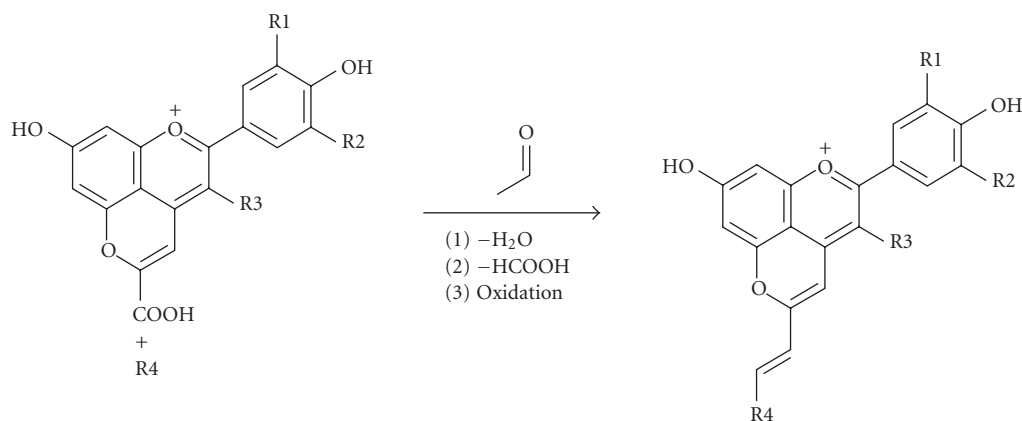


FIGURE 5. Formation reaction of portisins. R_1 and R_2 are H, OH, or OMe, R_3 is an (-O-glycosyl) group which is substituted with one, or more acyl groups, and R_4 is an aryl.

mono- or diglucosides, as seen from Table 3. The type of sugar moiety and the presence of a mono- or disaccharide in the anthocyanin structure did not seem to induce any influence on its λ_{\max} . This behaviour was also observed with regard to the anthocyanin-pyruvic-acid adducts and the respective portisins.

Additionally, acylation of the sugar moiety of malvidin monoglucosides with *p*-coumaric acid yielded in a λ_{\max} higher than its nonacylated counterpart. It has already been reported that, in the case of anthocyanins, acylation of the sugar moiety with hydroxycinnamic acids induces a bathochromic shift, as well as an intensification and stabilization of the colour, probably through intramolecular copigmentation phenomena, as reported elsewhere [35]. This bathochromic shift arising from the acylation of the sugar moiety was also observed for the portisins reported in aged Port red wine (Table 3).

CONCLUSION

The search for new natural food colourings has attracted the interest of several manufacturers over the last years. From the organoleptic point of view and considering the available colours widespread in nature, it can be seen that blue pigments are rare. Therefore, the production of new natural blue colourings for the food industry appears to be a priority. Concerning the food quality and safety, the natural colourings present significant benefits compared to the synthetic ones, even if it may be due to psychological concerns of the consumer. In fact, nature-derived pigments are easily accepted as being healthy and are thus a major issue for the food industry.

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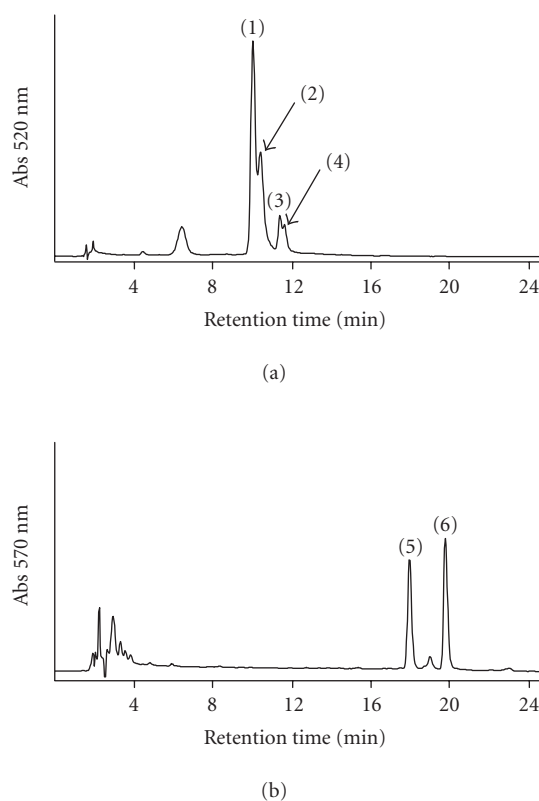


FIGURE 6. HPLC chromatograms of an elderberry extract. (a) anthocyanins after 2 days of reaction with pyruvic acid: Cy-3-(samb)-5-gluc (1), Cy-3-samb + Cy-3-gluc (2), Cy-3-samb-py (3), Cy-3-gluc-py (4); (b) portisins: vinylpyranoCy-3-samb (5), vinylpyranoCy-3-gluc (6).

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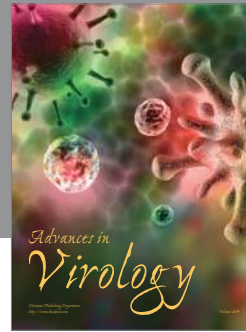
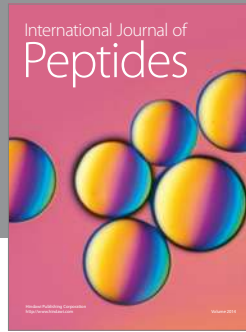
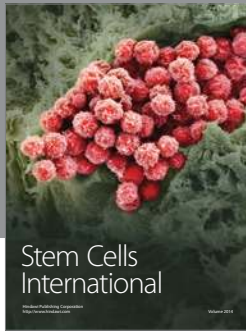
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