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New pyranoanthocyanins from blackcurrant seeds

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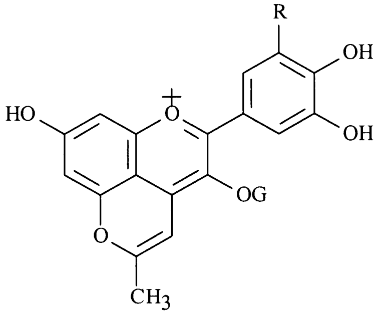
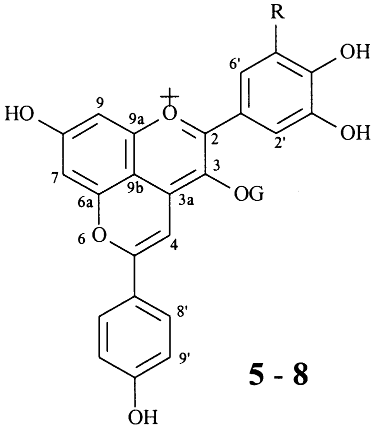
Abstract—Four new pyranoanthocyanins, namely pyranocyanin C and D and pyranodelphinin C and D, possessing the pyrano[4,3,2-*de*]-1-benzopyrylium core structure were isolated from an extract of blackcurrant seeds. Their chemical structures were elucidated by spectral methods and confirmed by chemical synthesis involving blackcurrant anthocyanins and *p*-coumaric acid. It is postulated that this condensation reaction plays a significant part in the evolution of red wine colour. © 2002 Elsevier Science Ltd. All rights reserved.

In order to better understand the chemistry of the antioxidative compounds involved in the stabilisation of the polyunsaturated γ-linolenic acid[1,2] that is present at high level in blackcurrant (*Ribes nigrum*) seeds we investigated the phenolics which resulted in the isolation and structural identification of four novel pyranoanthocyanins **1**–**4**, namely pyranocyanin A and B and pyranodelphinin A and B (see Fig. 1 for chemical structures).[3] These pyranoanthocyanins, in parallel to those derived from malvidin glycosides found in red wines,[4,5] were understood to be formed via oxidative addition followed by cyclisation between the blackcurrant anthocyanins, the rutinosides and glucosides of cyanidin and delphinidin[6–9] and acetone used as the extracting solvent. This unusual condensation reaction was subsequently demonstrated by chemical synthesis.[10] In a continuation of this work we wish to report the isolation and identification of another set of four compounds, which showed similar on-line HPLC–UV absorption with λ_{max} at ca 503 nm; this value being 20 nm higher than those of the pyranoanthocyanins we previously identified and having longer retention times.

The acetone–water (7:3) extract of blackcurrant seed residue was first fractionated on a Polyamide column. HPLC examination of the fraction containing the



1 - 4



5 - 8

	R	G
1/5	H	rut
2/6	OH	rut
3/7	H	glc
4/8	OH	glc

Figure 1. Chemical structures of pyranoanthocyanins.

Keywords: pyranoanthocyanins; pyranocyanin C and D; pyranodelphinin C and D; NMR; blackcurrant (*Ribes nigrum*); Grossulariaceae; synthesis.

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Table 1. ^{1}H NMR spectroscopic data of pyranoanthocyanins **5**–**8** in CD_3OD/TFA (9:1)[a]

H	**5**	**6**	**7**	**8**
4	7.98 s	8.04 s	7.94 s	7.88 s
7	7.14 s	7.16 d (1.8)	7.15 s	6.99 d (1.9)
9	7.08 s	7.08 d (1.8)	7.10 s	7.07 d (1.9)
2′	7.84 d (1.9)	7.51 s	7.84 d (2.1)	7.40 s
5′	6.94 d (8.5)	–	6.96 d (8.5)	–
6′	7.87 dd (8.5, 2.0)	7.51 s	7.88 dd (8.5, 2.0)	7.40 s
8′/12′	8.09 d (8.6)	8.13 d (8.9)	8.12 d (8.5)	8.04 d (8.9)
9′/11′	7.01 d (8.6)	7.03 d (8.8)	7.02 d (8.5)	6.93 d (8.8)
1″	4.72 d (7.7)	4.75 d (7.7)	4.79 d (7.7)	4.71 d (7.8)
1‴	4.32 s	4.36 br s	–	–

[a] Coupling constants (J) in parentheses.

blackcurrant pigments and phenolic acids, after standing at room temperature for several months, revealed the presence of an additional set of four peaks with their λ_{max} between 500 and 506 nm that resembled the malvidin-derived pigment A (λ_{max} 507 nm) reported by Fulcrand et al.[4] Initial column chromatography of this mixture on polyamide afforded an orange–red fraction which on further chromatography on a MCI-HP20 column and purification by semi-preparative HPLC led to the isolation of compounds **5** and **6**. The NMR spectra of **5** and **6** showed the presence of the pyranoflavylium core similar to those of pyranocyanin A **1** or pyranodelphinin A **2** and the sugar rutinose. The only difference was the absence of the methyl signals found in **1** or **2** which instead were replaced with the resonances of a *p*-hydroxyphenyl group in **5** or **6**. The latter signals in **5** or **6** were evident from two mutually coupled doublets at δ 7.0 and 8.1 ($J = 8.6$ Hz) in the ^{1}H NMR and six carbon resonances appearing at δ 118.2 (×2), 122.7, 131.9 (×2) and 165.8 in the ^{13}C NMR spectra. Thus, compound **5** was identified as pyranocyanin C or 2-(3,4-dihydroxyphenyl)-8-hydroxy-5-(4-hydroxyphenyl)-3-(6-*O*-α-rhamnopyranosyl-β-D-glucopyranosyloxy)pyrano[4,3,2-*de*]-1-benzopyrylium and 6 as pyranodelphinin C or 2-(3,4,5-trihydroxyphenyl)-8-hydroxy-5-(4-hydroxyphenyl)-3-(6-*O*-α-rhamnopyranosyl-β-D-glucopyranosyloxy)pyrano[4,3,2-*de*]-1-benzopyrylium. The identity of compound **5** or **6** was further corroborated by electrospray mass spectroscopic analysis operated in positive mode, which showed the *m*/*z* peaks at 711.1947 and 727.1856 consistent with $C_{35}H_{35}O_{16}$ (calcd 711.1920) and $C_{35}H_{35}O_{17}$ (calcd 727.1869) for **5** and **6**, respectively.

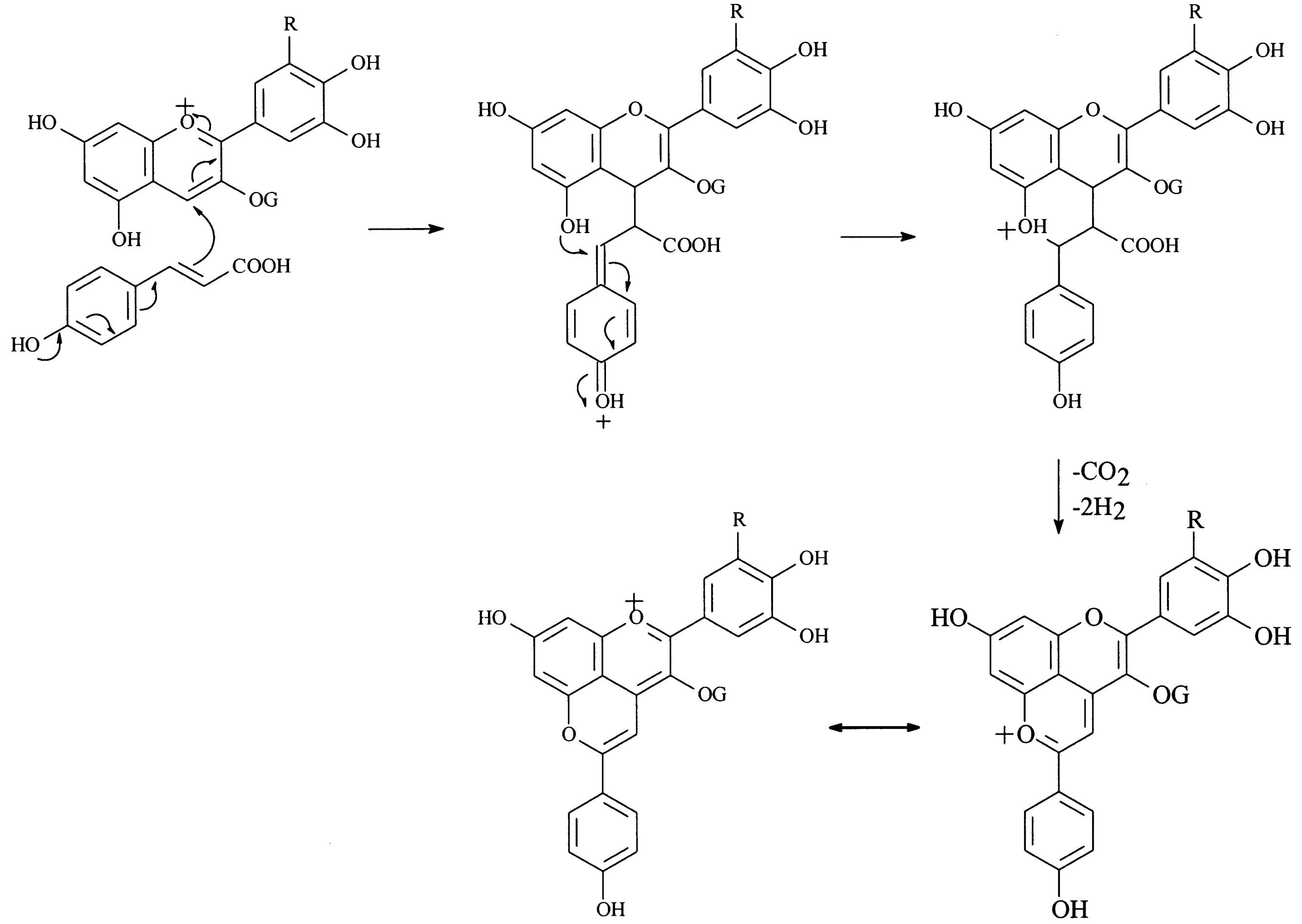
Full assignment of the NMR data of **5** or **6** (Tables 1 and 2) was established by 2D NMR including HMBC experiments and further supported by comparison with those of the malvidin-derived pigment A. It is noteworthy that the carbon signal at δ 149 which showed weak two-bond coupling with H-4 (δ 8.0) in HMBC was assigned to C-3a in agreement with our previous findings for other pyranoanthocyanins. However, this chemical shift value was in contrast to that (δ 108) for the corresponding carbon reported for the malvidin-derived pigment A.

Table 2. ^{13}C NMR spectroscopic data of pyranoanthocyanins **5** and **6** in CD_3OD/TFA (9:1)

C	**5**	**6**	C	**5**	**6**
2	163.82	164.08	7′	122.66	122.75
3	135.19	135.38	8′/12′	131.87	131.88
3a	149.28	149.23	9′/11′	118.19	118.19
4	98.34	98.30	10′	165.83	165.79
5	169.64	169.68	1″	106.67	106.73
6a	154.80	154.87	2″	75.85	75.86
7	101.60	101.50	3″	77.82	77.77
8	168.47	168.42	4″	72.12	72.03
9	101.72	101.68	5″	78.23	78.25
9a	154.06	154.14	6″	69.82	69.81
9b	108.83	108.91	1‴	102.88	102.97
1′	122.41	121.39	2‴	72.37	72.44
2′	118.96	112.26	3‴	72.67	72.63
3′	146.77	147.11	4‴	74.09	74.11
4′	153.22	141.78	5‴	70.07	70.10
5′	116.92	147.11	6‴	18.21	18.22
6′	126.33	112.26			

The two minor compounds **7** and **8** were also isolated and their ES MS showed the *m*/*z* peaks at 565.1355 and 581.1256 consistent with those calculated, 565.1341 for $C_{29}H_{25}O_{12}$ and 581.1289 for $C_{29}H_{25}O_{13}$, respectively. These values, as well as their ^{1}H NMR chemical shifts (Table 1) were identical to those of **5** and **6** with the exception of the sugar signals, suggesting that these compounds were the corresponding pyranoanthocyanins with a hexose instead of a rutinose unit. The hexose was deduced as glucose based on the known parent blackcurrant cyanidin and delphinidin-3-*O*-glucosides and this was confirmed by NMR spectral comparison with that of delphinidin 3-*O*-glucoside. Thus, compound **7** was identified as 2-(3,4-dihydroxyphenyl)-3-β-D-glucopyranosyloxy-8-hydroxy-5-(4-hydroxyphenyl)pyrano[4,3,2-*de*]-1-benzopyrylium or pyranocyanin D and **8** as 2-(3,4,5-trihydroxyphenyl)-3-β-D-glucopyranosyloxy-8-hydroxy-5-(4-hydroxyphenyl)-pyrano[4,3,2-*de*]-1-benzopyrylium or pyranodelphinin D. The identity of these pigments was confirmed by the chemical synthesis of **7** (see below).

These pyranoanthocyanins were absent in fresh blackcurrant seed extract and their levels in the extract



Scheme 1. The proposed formation of pyranoanthocyanins from anthocyanins and *p*-coumaric acid.

increased gradually with time, indicating they were post enzymic products. Their formation was likely to be from the reaction of the anthocyanins and *p*-coumaric acid, the latter possibly as a result of hydrolysis of 1-*p*-coumaroylglucoside, the major component reported in blackcurrant.[6] This reaction was confirmed when the isolated blackcurrant anthocyanins mixture was treated with *p*-coumaric acid at elevated temperature (60°C) over 2 days to afford the four pyranoanthocyanins which gave identical UV absorption and HPLC characteristics to those found in the extract. Furthermore, in a separate experiment[11] the product from the reaction between cyanidin-3-*O*-glucoside and *p*-coumaric acid gave identical ^{1}H NMR data to those of pyranocyanin.

The proposed mechanism for the reaction involves the nucleophilic addition onto C-4 of the pyrylium salt[12] by the double bond of *p*-coumaric acid followed by intramolecular cyclisation (Scheme 1). Subsequent in situ decarboxylation and dehydrogenation gave the pyranoanthocyanins.

These post enzymic reactions may provide new insights into the evolution of colour during maturation of red wine.

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11. Experimental procedure: 90 mg (0.2 mmol) of cyanidin-3-*O*-glucoside and 50 mg of *p*-coumaric acid (0.3 mmol) were dissolved in 40 ml of ethanol/water (8:2) and the resulting solution was kept in an oven at 60°C for 3 days. After removal of ethanol, the residue was chromatographed on a Polyamide column followed by semi-preparative HPLC purification. Yield: 8 mg. On-line UV λ_{max} 500 nm. ^{1}H NMR (300 MHz): see Table 1. ^{13}C NMR (75 MHz): δ 163.79 (C-2), 135.31 (C-3), 149.59 (C-3a), 98.51 (C-4), 169.75 (C-5), 154.88 (C-6a), 101.36 (C-7), 168.47 (C-8), 101.59 (C-9), 154.13 (C-9a), 108.79 (C-9b), 122.05 (C-1′), 118.82 (C-2′), 146.89 (C-3′), 153.19 (C-4′), 116.90 (C-5′), 126.02 (C-6′), 122.60 (C-7′), 131.87 (C-8′/12′), 118.28 (C-9′/11′), 165.79 (C-10′), 106.34 (C-1″), 75.88 (C-2″), 78.19 (C-3″), 71.68 (C-4″), 78.85 (C-5″), 63.11 (C-6″).
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