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Unusual anthocyanin reaction with acetone leading to pyranoanthocyanin formation

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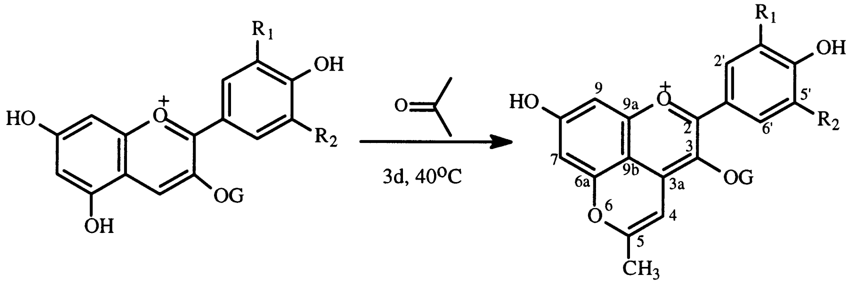
Abstract—Anthocyanins undergo unusual facile reactions with acetone to give rise to pyranoanthocyanins, thus showing that the new class of pigments isolated from blackcurrant seeds were a result of solvent participation. This oxidative addition was extended to the successful synthesis of pyranomalvidin or 2-(3,5-dimethoxy-4-hydroxyphenyl)-8-hydroxy-5-methyl-3-*O*-β-D-glucopyranosyloxypyrano[4,3,2-*de*]-1-benzopyrylium by the reaction of the principal grape anthocyanin malvidin 3-*O*-glucoside with acetone. The chemical structures of pyranomalvidin, together with pyranocyanin B and pyranodelphinin B, were fully characterised by 2D NMR. © 2001 Elsevier Science Ltd. All rights reserved.

In our previous communication,[1] we reported the isolation and structural identification of four new pyranoanthocyanins, namely pyranocyanin A (**6**) and B (**8**), and pyranodelphinin A (**7**) and B (**9**) (Fig. 1 for chemical structures), from blackcurrant (*Ribes nigrum*) seed residue. These pyranoanthocyanins are structurally related to the malvidin derived wine pigments which were formed by the reaction of grape anthocyanins and pyruvic acid or vinyl phenol[2] and appeared to be cycloaddition products between acetone, which is used in the extraction, and the known blackcurrant anthocyanins, the rutinosides and glucosides of cyanidin and delphinidin (**1**–**4**).[3] The extended conjugation confers stability to the chromophore and similar cycloadducts of anthocyanins with olefins have been prepared as a method of stabilising the colour.[4] Flavylium compounds are known to readily undergo addition at C-4 with a variety of nucleophilic reagents including phenols and enolic 1,3-diketone compounds,[5] but similar reactions with acetone are not known and until recently acetone was still recommended as the solvent of choice for the quantitative extraction of plant anthocyanins.[6] This report provides the first evidence for the oxidative addition of acetone to anthocyanins leading to the formation of pyranoanthocyanins.

Evidence for the reaction of blackcurrant anthocyanins with acetone was first detected during the course of the HPLC analysis of the respective acetone and alcohol extracts of the seed. It was noted that the pyranoanthocyanin peaks were present in the acetone but absent in the alcohol extract and the intensity of the peaks would increase gradually with the duration of the extraction. In a separate experiment to confirm these observations, the isolated blackcurrant anthocyanins were dissolved in 70% aqueous acetone, the solvent



	R1	R2	G
1, 6	OH	H	rutinose
2, 7	OH	OH	rutinose
3, 8	OH	H	glucose
4, 9	OH	OH	glucose
5, 10	OCH_3	OCH_3	glucose

Figure 1. The formation of pyranoanthocyanins **6**–**10** from anthocyanins **1**–**5** and acetone.

Keywords: pyranoanthocyanins; pyranocyanin B; pyranodelphinin B, pyranomalvidin; oxidative cycloaddition; acetone; 2D NMR.

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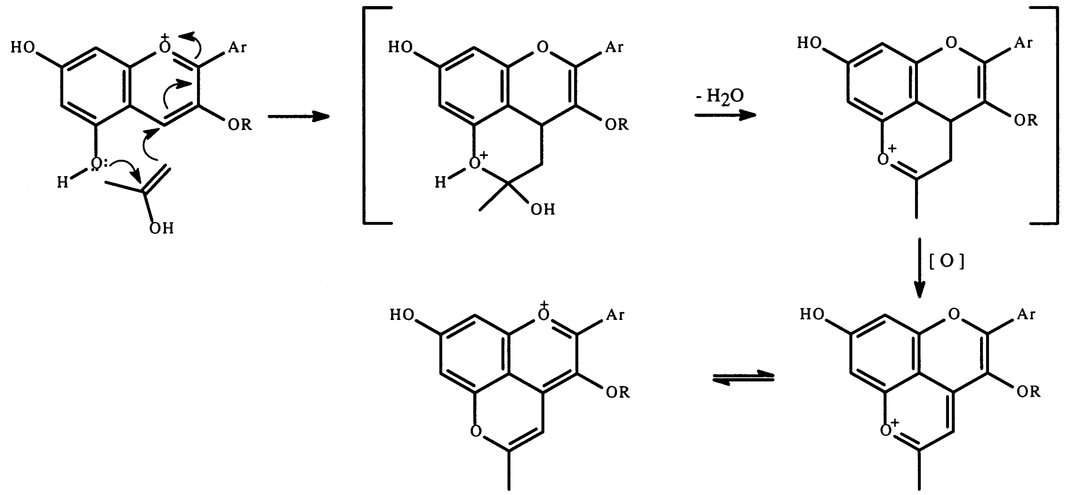

Scheme 1.

used in the extraction. The formation of pyranoanthocyanins was monitored by HPLC, which showed that the production of the conjugated pigments was slow at room temperature, but increased significantly when the temperature was raised. After three days at 40°C, the usual blackcurrant anthocyanin peaks had almost completely disappeared while the pyranoanthocyanin peaks had increased significantly in intensity. The proposed mechanism for the reaction apparently involves the nucleophilic addition of the electron-rich enol form of acetone onto the C-4 of the pyrylium salt followed by intramolecular hemi-acetal formation as shown in Scheme 1. Subsequent in situ oxidation and dehydration reactions led to the pyranoanthocyanins with the extended conjugation allowing for the delocalisation of the charge.

After 3 days at 40°C, pyranocyanin B (**7**) and pyranodelphinin B (**9**) were produced in yields of 20 and 15%, respectively. The ^{13}C NMR spectra of **7** and **9** (Table 1) showed a set of six carbon signals consistent with a β-D-glucopyranose moiety as in delphinidin 3-*O*-glucoside (**4**),[7] which was also isolated from blackcurrant seed. The carbon signals for the aglycone in **7** and **9** were almost identical to those of pyranocyanin A (**6**) and pyranodelphinin A (**8**), respectively, confirming that **7** and **9** were 2-(3,4-dihydroxyphenyl)-8-hydroxy-5-methyl-3-*O*-β-D-glucopyranosyloxypyrano[4,3,2-*de*]-1-benzopyrylium and 2-(3,4,5-trihydroxyphenyl)-8-hydroxy-5-methyl-3-*O*-β-D-glucopyranosyloxypyrano[4,3,2-*de*]-1-benzopyrylium, respectively.

This facile reaction of acetone was further demonstrated with malvidin 3-*O*-β-D-glucopyranoside (**5**), the major anthocyanin present in grapes and wines.[8] In this case, pyranomalvidin (**10**) was produced and was isolated by semi-preparative HPLC in a 10% yield. Its on-line UV spectrum with $\lambda_{\max}$ at 480 nm was similar to those of pyranodelphinin A and B indicating that they all shared the same fused aromatic ring system. The ^{1}H NMR spectrum of **10** showed a characteristic six-proton singlet at δ 3.98 attributable to the two magnetically equivalent methoxyls in the molecule. Other additional signals observed were a three-proton singlet at δ 2.67 accounting for the methyl group, a doublet at 4.68 ($J=7.6$) for the sugar β-anomeric proton, a *meta*-coupled doublet at 7.09 ($J=1.8$ Hz) for H-7, a broad singlet at 7.23 for both H-4 and H-9 and a sharp singlet at 7.65 for the H-2′ and H-6′, which were all fully consistent with the proposed structure. The assignment of the broad peak at δ 7.23 was made by ^{1}H–^{13}C COSY, which showed the signal to be correlated to the

Table 1. ^{13}C NMR spectral data of pyranoanthocyanins **7**, **9** and **10** in CD_3OD/TFA (9:1)

No.	**7**	**9**	**10**	No.	**7**	**9**	**10**
2	164.75	165.10	164.98	1′	121.93	120.87	120.36
3	134.88	135.03	135.14	2′	118.35	111.87	109.91
3a	150.64	150.40	151.09	3′	147.12	147.35	149.69
4	103.36	103.19	103.41	4′	153.69	142.27	143.73
5	174.03	173.97	174.38	5′	117.07	147.35	149.69
6a	155.50	155.53	155.63	6′	126.45	111.87	109.91
7	101.62	101.56	101.62	1″	105.66	105.69	105.34
8	168.78	168.73	169.06	2″	75.79	75.80	75.95
9	101.35	101.24	101.62	3″	78.09	78.12	78.17
9a	154.32	154.42	154.53	4″	71.68	71.64	71.80
9b	109.43	109.32	109.42	5″	79.08	79.11	79.31
CH_3	21.97	21.93	21.53	6″	63.09	63.01	62.99
OCH_3			57.53				

two carbon signals at δ 101.6 and 103.4 for C-9 and C-4, respectively.

Except for the characteristic methoxy carbon resonance at δ 57.5, the ^{13}C NMR spectrum of **10** (Table 1) was similar to that of **9** in all other aspects, confirming that the chemical structure of pyranomalvidin (**10**) to be 2-(3,5-dimethoxy-4-hydroxyphenyl)-8-hydroxy-5-methyl-3-*O*-β-D-glucopyranosyloxypyrano[4,3,2-*de*]-1-benzopyrylium. The chemical structure of pyranomalvidin was further corroborated by electron spray mass spectroscopy (ESMS) operated in the positive mode which gave the $[M]^+$ peak at m/z 531, which was consistent with the molecular formula of $C_{26}H_{27}O_{12}$ (found: 531.1509; calcd: 531.1497). The facile oxidative addition reaction with acetone may suggest that grape pigments could react in a similar way with other carbonyl compounds present in the wine to form the respective pyranoanthocyanins as part of wine colour evolution during maturation and ageing of wine.

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