



Isolation of the first C-2 addition products of anthocyanins

Yinrong Lu,* L. Yeap Foo and Herbert Wong

Industrial Research Limited, PO Box 31310 Lower Hutt, New Zealand

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Abstract—Addition of anthocyanins with nucleophilic agents normally occurs at the C-4 centre and the isolation of C-2 adducts between acetone and cyanidin-3- and delphinidin-3-rutinosides is the first definitive demonstration of the reactivity of this carbon site. These C-2 addition products were isolated as diastereoisomers and their chemical structures elucidated by 1D and 2D NMR spectroscopy. © 2002 Published by Elsevier Science Ltd.

Anthocyanins are natural pigments widely distributed in the plant kingdom and are responsible for a variety of colors in flowers and fruits.¹ While they are stable in intact flowers and fruits due to a copigmentation mechanism,² they show a propensity to color degradation when they are extracted or when tissues are disrupted. The bleaching of the anthocyanin color is understood to be a result of hydration of the flavylium chromophore. In aqueous media the anthocyanins exist in equilibrium with their colorless water adducts, the hemiacetals and their ring-opened chalcones. The color is determined by the ratio of each species and is pH-dependent.^{3,4} The presence of the colorless hemiacetals in equilibrium with the anthocyanins has been demonstrated by ¹H NMR spectroscopy,^{5,6} but such adducts have never been isolated. In contrast anthocyanins have been shown to readily undergo nucleophilic addition at

C-4 leading to the formation of more stable condensation products^{7,8} including the recently identified pyranoanthocyanins which have been found in red wines.^{9,10}

In recent communications, we reported the isolation and structural elucidation of pyranocyanin A and B and pyranodelphinin A and B from blackcurrant (*Ribes nigrum*) seed¹¹ and demonstrated¹² that these were formed by the addition of acetone and subsequent cyclisation at C-4 of cyanidin- and delphinidin-3-rutinosides (**1** and **2** in Fig. 1) and the corresponding glucosides.^{13,14} Further investigation of the blackcurrant seed extract has led to the isolation of additional colorless adducts of anthocyanins with acetone. This report is devoted to the structural elucidation by NMR spectroscopy of these novel colorless anthocyanin adducts which have been isolated for the first time.

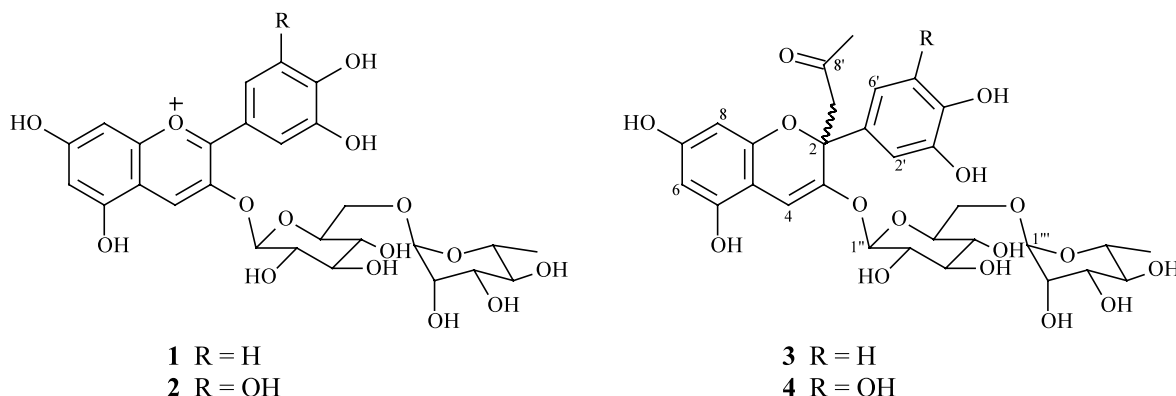


Figure 1. Chemical structures of anthocyanins **1** and **2** and their C-2 acetone adducts **3** and **4**.

Keywords: anthocyanins; cyanidin-3-rutinoside; delphinidin-3-rutinoside; acetone; colorless C-2 adducts; NMR.

* Corresponding author. Fax: +64-4-5690055; e-mail: y.lu@irl.cri.nz

Evidence for the presence of two additional colorless components in the extract of blackcurrant seed residue from acetone/water (7:3) was detected by HPLC analysis which showed two peaks with near identical UV absorption characteristics (λ_{max} : 282, 234 nm). Their isolation was achieved by HPLC monitoring of fractions from column chromatography of the extract on MCI-HP20 eluting with water with increasing methanol

Table 1. ^{13}C NMR data (75 MHz, CD_3OD) of anthocyanin C-2 acetone adducts **3** and **4**

C	3		4	
	Isomer 1	Isomer 2	Isomer 1	Isomer 2
2	81.64	81.79	81.63	81.80
3	150.11	150.65	150.13	150.51
4	96.25	96.09	96.36	96.27
5	153.34	153.60	153.39	153.64
6	97.63	97.87	97.60	97.86
7	158.33	158.33	158.31	158.31
8	96.99	97.31	97.05	97.43
9	154.71	154.54	154.71	154.53
10	104.52	104.77	104.54	104.82
1'	136.42	136.52	134.11	134.09
2'	114.83	114.58	106.82	106.55
3'	146.13	146.13	146.83	146.83
4'	146.37	146.13	135.88	135.89
5'	116.30	116.30	146.83	146.83
6'	119.04	118.90	106.82	106.55
7'	54.19	54.46	54.23	54.43
8'	210.40	210.22	210.42	210.26
9'	31.80	31.61	31.76	31.57
1''	102.91	102.06	103.13	101.98
2''	75.33	75.11	75.33	75.14
3''	78.46	78.46	78.46	78.46
4''	72.36	72.16	72.35	72.15
5''	77.74	77.59	77.75	77.62
6''	68.30	67.98	68.28	67.97
1'''	102.72	102.57	102.73	102.58
2'''	72.04	72.04	72.10	72.02
3'''	72.85	72.85	72.83	72.91
4'''	74.56	74.56	74.59	74.56
5'''	70.10	70.17	70.16	70.15
6'''	18.40	18.40	18.36	18.38

content (up to 20%) followed by semi-preparative HPLC (acetonitrile/water 15:85 containing 2% acetic acid).

The ^{13}C NMR spectrum of compound **3** (Table 1) contained 30 pairs of carbon signals with each pair of signals having a relative peak size in a ratio of ca 3:2. This pattern of signals suggested that **3** consisted of a mixture of two diastereomers. The carbon signals showed a similarity to cyanidin-3-rutinoside,^{14,15} suggesting that each diastereoisomer contained a phloroglucinol A-ring, a catechol B-ring and a rutinose (α -L-rhamnosyl-(1 \rightarrow 6)- β -D-glucose). This deduction was corroborated by the ^1H NMR spectrum (Table 2), which, in addition to showing duplication of proton signals, also displayed an ABX resonance system for the catechol B-ring, the two *meta*-coupled doublets characteristic of the phloroglucinol A-ring, two doublets (J 7.6 or 1.3 Hz) for the β -glucose and α -rhamnose anomeric protons and a three-proton doublet characteristic for the rhamnose methyl group. These data, together with the absence of the characteristic singlet at ca δ 9.0 for the anthocyanin H-4,¹⁶ indicated that **3** was likely to be derived from cyanidin-3-rutinoside by an addition reaction to the pyrylium C-ring of the cyanin moiety.

The remaining unaccounted carbon signals for the major isomer consisted of three quaternary carbons (δ 210.4, 150.1, 81.6), a CH (δ 96.3), a CH_2 (δ 54.2) and a CH_3 (δ 31.8), which were established by using DEPT. Heteronuclear 2D correlation experiments (HMQC) clearly showed the correlation of the CH carbon (C-4) with the proton singlet at δ 6.31, the CH_2 carbon (C-7') with the two proton doublets at δ 3.12 and 3.35 (J 15.5 Hz), and the CH_3 carbon (C-9') with the proton singlet at δ 2.21. The olefinic proton (δ 6.31) was assigned to H-4 by long range heteronuclear 2D correlation experiments (HMBC) (Fig. 2) from its three-bond correlations with the quaternary C-9 (δ 154.7), C-5 (δ 153.3) and C-2 (δ 81.6). The signal at δ 81.6 was assigned to C-2 due to it being long range coupled to H-2' (δ 7.02) and H-6' (δ 6.90), indicating that the C-2 of cyanidin-3-

Table 2. Selected ^1H NMR data (300 MHz, CD_3OD) of anthocyanin C-2 acetone adducts **3** and **4**^a

H	3		4	
	Isomer 1	Isomer 2	Isomer 1	Isomer 2
4	6.31 s	6.23 s	6.30 s	6.22 s
6	5.88 d (2.2)	5.90 d (2.0)	5.87 d (2.2)	5.88 d (2.1)
8	5.89 d (2.2)	5.95 d (2.0)	5.89 d (2.2)	5.93 d (2.1)
2'	7.02 d (2.2)	6.99 d (2.2)	6.59 s	6.55 s
5'	6.69 d (8.5)	6.66 d (8.5)	—	—
6'	6.90 dd (8.3, 2.2)	6.92 dd (8.4, 2.2)	6.59 s	6.55 s
7'a	3.12 d (15.3)	3.02 d (15.5)	3.09 d (15.4)	3.00 d (15.3)
7'b	3.35 d (15.7)	3.38 d (15.7)	3.33 d (15.3)	3.35 d (15.5)
9'	2.21 s	2.25 s	2.18 s	2.23 s
1''	4.87 obscured	4.98 d (7.6)	4.85 obscured	4.97 d (7.6)
1'''	4.73 d (1.3)	4.60 d (1.4)	4.72 br s	4.60 br s
6'''	1.27 d (6.2)	1.24 d (6.2)	1.26 d (6.2)	1.23 d (6.2)

^a Coupling constants (J) in parentheses.

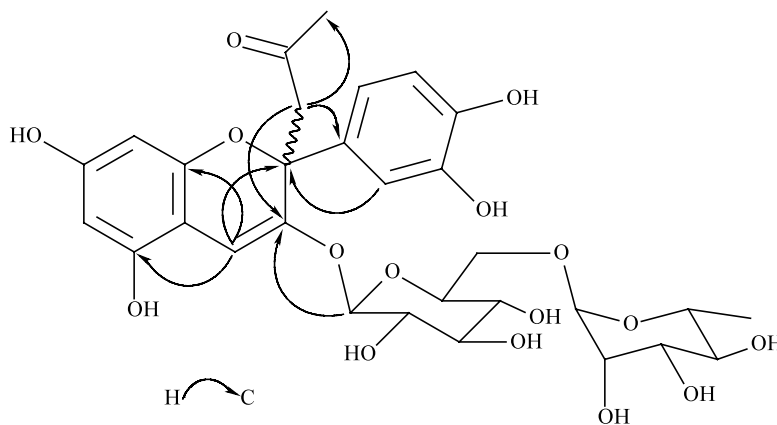


Figure 2. Key long range correlations observed in the HMBC spectrum of compound **3**.

rutinoside was the point of addition in **3**. This deduction was supported by the similarity of the chemical shift of H-4 (δ 6.31) with that of the H-4 (δ 6.29) of the reported C-2 water adduct of malvidin-3-glucoside.⁵

The attachment of the CH₂ (C-7') group to the newly formed asymmetric carbon C-2 was evidenced by the observation of its two magnetically non-equivalent proton signals appearing as two mutually coupled doublets. This deduction was elaborated by HMBC which showed long range couplings between the CH₂ protons and C-1' (δ 136.4) or C-3 (δ 150.1), the latter being in turn coupled with the β -glucose anomeric proton (δ 4.87, H-1''). The CH₂ (C-7') chemical shift was also long range coupled with the CH₃ (C-9') group and also the ketone carbon (δ 210.4, C-8'), the extraneous three-carbon unit was therefore confirmed as the 2-oxopropyl or acetyl group. Hence **3** is a mixture of two diastereomers of 2-acetyl-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxybenzo[2H]pyran-3-O-rutinoside. The full assignment of their ¹H and ¹³C signals established by intensive 2D NMR experiments including HMBC is shown in Tables 1 and 2. The identity of the adducts was also confirmed by electron spray mass spectroscopy (ESMS) operated in negative mode, which gave a [M-H]⁻ peak at *m/z* 651 consistent with the molecular formula of C₃₀H₃₆O₁₆ (found: 651.1919; calcd: 651.1931).

The identity of compound **4** followed from its NMR spectral similarities to those of **3** (Tables 1 and 2). The only striking difference in the NMR data was due to a pyrogallol B-ring system in **4** instead of the catechol B-ring in **3**, thus suggesting that **4** were diastereomers corresponding to the C-2 acetone adducts of delphinidin-3-rutinoside, which was the other major anthocyanin found in blackcurrant. The duplication of NMR signals observed in **4** was also indicative of the presence of two diastereomers, whose ¹H and ¹³C signals were assigned by analogy to those of **3**. ESMS also confirmed the identity of **4** by giving an [M-H]⁻ peak at *m/z* 667 consistent with the molecular formula of C₃₀H₃₆O₁₇ (found: 667.1920; calcd: 667.1880).

The corresponding C-2 acetone adducts of cyanidin-3-glucoside or delphinidin-3-glucoside, these being two known minor anthocyanins of blackcurrant, were apparently also formed in a similar way. Evidence of their presence was shown by HPLC analysis which showed two additional peaks at slightly longer retention times than those of **3** or **4** and with identical UV spectra. However, their presence in small amounts prohibited their isolation for confirmation by NMR.

The formation of two diastereomers of each of **3** or **4** showed that the addition of acetone can take place from both sides of the planar pyrylium unit. Such a phenomenon was also observed in the C-2 water adducts of the anthocyanins^{5,6} and in the C-4 bisulfite adducts of malvidin-3-glucoside, the latter reaction gave rise to diastereomers in equal amounts.^{17,18} The C-2 acetone adducts of the cyanin and delphinin described in the report were quite stable in contrast to the corresponding water addition products. Compounds **3** and **4** represent the first reported stable colorless C-2 anthocyanin adducts and demonstrate the unexpected reactivity of acetone as a solvent.

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