Phytochemistry, Vol. 25, No. 12, pp. 2906–2907, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

^{1}H NMR SPECTRAL ANALYSIS OF THE MALYLATED ANTHOCYANINS FROM *DIANTHUS*

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(*Received* 7 *April* 1986)

Key Word Index—*Dianthus caryophyllus*; Caryophyllaceae; carnation; FT-NMR; pelargonidin 3-*O*-(6-*O*-malyl-β-D-glucopyranoside); cyanidin 3-*O*-(6-*O*-malyl-β-D-glucopyranoside).

Abstract—The structures of malylated anthocyanins from carnation *Dianthus caryophyllus* flowers were confirmed as the 3-*O*-(6-*O*-malyl-β-D-glucopyranosides) of pelargonidin and cyanidin by 400 MHz FT-NMR.

INTRODUCTION

In previous work, it was found that several *Dianthus* species contain zwitterionic anthocyanins, which are anionic on electrophoresis at pH 4.4 [1, 2]. Two such pigments have been identified as the 3-malylglucosides of pelargonidin (**1**) and cyanidin (**2**) from a red carnation cultivar, *Dianthus caryophyllus*, and from *D. deltoides* [2] or a purplish-red carnation cultivar [3], respectively, by means of HPLC, H_2O_2 oxidation, IR and fast atom bombardment mass spectrometry (FAB-MS). We report here the detailed structure elucidation of these two malylated anthocyanins through proton FT-NMR techniques.

RESULTS AND DISCUSSION

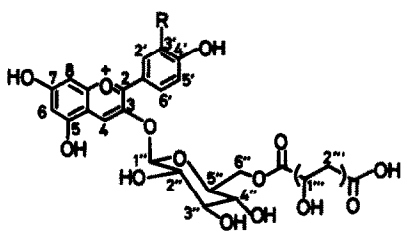
In addition to FAB-MS, high resolution FT-NMR spectroscopy has recently been applied to the analysis of anthocyanin structures [4, 5]. A mixture of DMSO-d_6 and CF_3COOD has also proved to be an excellent NMR solvent for acylated anthocyanins with aliphatic dicarboxylic acids [4–6]. Using such a solvent, pigments **1** and **2** isolated respectively from red and purplish-red flowers of carnation were measured on a 400 MHz ^{1}H NMR spectrometer. All signals in the spectra could be completely assigned by a two-dimensional analysis, ^{1}H–^{1}H shift correlated spectroscopy (COSY). As shown in Table 1, the NMR features of **1** and **2** resemble each other closely except for the signals corresponding to the B-ring protons in pelargonidin (AA'XX' type) or cyanidin (AA'X type) in the lower magnetic field.

The two sets of double doublets near δ2.7 and 2.8 ppm coupling with a double-doublet signal at δ4.35 ppm in both spectra apparently indicate the presence of a malic acid residue. This is because the non-equivalent methylene protons at C-2‴ in the malate moiety couple geminally (J = *ca* 16 Hz) and each has different couplings (*ca* 5 and 7.5 Hz) against the vicinal methine proton binding to the asymmetric C-1‴.

Glucose C-6″ methylene protons appear non-equivalently at lower fields (δ4.18 and 4.50 ppm in **1** or δ4.20 and 4.51 ppm in **2**) than non-acylated glucose C-6″ protons (over δ4.1 ppm) [4, 7]. These deshielding shifts demonstrate that the glucose C-6″ hydroxyl of each carnation anthocyanin is substituted by malic acid due to the electron-withdrawing effect of the adjacent ester carbonyl group. Related C-6″ hydroxyl acylation has been found commonly in some other acylated anthocyanins [1, 4–6, 8]. However, in the malic acid moiety of **1** or **2**, it has not been possible to determine which carboxyl is linked to glucose, nor has the absolute configuration been determined.

Among the sugar signals, the one with the lowest shift at δ5.41 ppm in **1** (δ5.42 ppm in **2**) shows anomeric proton absorption and is split into a doublet (7.5 Hz in **1** or 8.0 Hz in **2**) by H-2″. Other glucose protons also have large coupling constants (9.0–9.5 Hz) owing to their *trans*-diaxial interactions (Table 1). These findings show that the glucose is present in the β-pyranoside configuration with the chair conformation.

Further analysis by 2D nuclear Overhauser effect spectroscopy (NOESY) reveals a strong NOE interaction between H-1″ and H-4 in both pigments (Table 1), indicating that the glucose is attached to the 3-position of each aglycone, as already indicated by the results of H_2O_2 oxidation [2]. Strong NOEs are also observed among H-1″, H-3″ and H-5″. These axial protons are shown to be orientated on the same side on the sugar ring, and therefore the sugar moiety in **1** and **2** can be ascertained as the β-anomer with a chair form.



1 R=H
2 R=OH

Table 1. ^{1}H NMR spectral data of malylated anthocyanins **1** and **2** in *D. caryophyllus* [400 MHz, in DMSO-d_6–CF_3COOD (5:2), TMS as internal standard, δ-values in ppm from TMS]

Anthocyanidin moiety			Glucose moiety			Malic acid moiety		
H	1	2	H	1	2	H	1	2
4	8.95 *s*	8.90 *s*	1″	5.41 *d*	5.42 *d*	1‴	4.35 *dd*	4.35 *dd*
6	6.99 *d*	6.93 *d*	2″	3.58 *dd*	3.66 *dd*	2‴a	2.80 *dd*	2.81 *dd*
8	6.84 *d*	6.80 *d*	3″	3.49 *t*	3.51 *t*	2‴b	2.68 *dd*	2.69 *dd*
2′	8.61 *d*	8.12 *d*	4″	3.33 *dd*	3.36 *dd*			
6′		8.25 *dd*	5″	3.86 *ddd*	3.87 *ddd*			
3′	7.11 *d*	—	6″a	4.50 *dd*	4.51 *dd*			
5′		7.10 *d*	6″b	4.18 *dd*	4.20 *dd*			

J (Hz): **1**: 6,8 = 2.0; 2′ + 6′, 3′ + 5′ = 9.0; 1″,2″ = 7.5; 2″,3″ = 3″,4″ = 9.0; 4″,5″ = 9.5; 5″,6″a = 1.6; 5″,6″b = 7.5; 6″a,6″b = 11.5; 1‴,2‴a = 5.0; 1‴,2‴b = 7.5; 2‴a,2‴b = 16.0. **2**: 6,8 = 2′,6′ = 2.0; 5′,6′ = 8.5; 1″,2″ = 8.0; 2″,3″ = 3″,4″ = 9.0; 4″,5″ = 9.5; 5″,6″a = 1.8; 5″,6″b = 7.5; 6″a,6″b = 11.5; 1‴,2‴a = 4.8; 1‴,2‴b = 7.5; 2‴a,2‴b = 15.8.

NOE observed in **1** and **2**: 1″,4; 1″,3″; 1″,5″; 3″,5″; 4″,6″a; 4″,6″b.

EXPERIMENTAL

The major flower anthocyanins were isolated respectively from the red cv. Scania and the purplish-red cv. Nina of carnation *Dianthus caryophyllus* by the procedure previously reported [2]. The purified pigments were measured on a 400 MHz ^{1}H FT-NMR spectrometer, JNM GX-400 (Jeol), in DMSO-d_6–CF_3COOD (5:2) with TMS as internal standard.

Acknowledgements—We are grateful to Mr. J. Kuwahara, Kui-Shinkaen, Hiroshima for providing the plant material. Thanks are due to Prof. M. F. Petersen for his critical reading of the manuscript.

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