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Structure of rosacyanin B, a novel pigment from the petals of *Rosa hybrida*

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Abstract—A novel violet pigment, rosacyanin B, was isolated from the petals of *Rosa hybrida* cv. 'M'me Violet', and its structure was elucidated by spectroscopic methods. rosacyanin B is the first C-4 substituted anthocyanidin isolated from intact plants.

Many rose breeders have made efforts to breed a blue rose for more than two hundred years. There are many investigations on rose pigments, for example, cyanidin 3,5-diglucoside is known as the major red pigment and many kinds of carotenoids exist in roses.[1] However, there are no reports about blue pigments from roses to date. Several mauve roses, which are called 'blue roses' such as 'M'me Violet' and 'Lavande', are known to have the colors from reddish purple to bluish silver gray, but the breeders have not succeeded in making truly blue roses. We speculated that these mauve roses might contain novel blue pigments, and tried to extract blue pigments from the mauve roses. As a result, we have found a small amount of a novel violet pigment in the petals of *Rosa hybrida* cv. 'M'me Violet', and the novel pigment was named rosacyanin B (**1**).

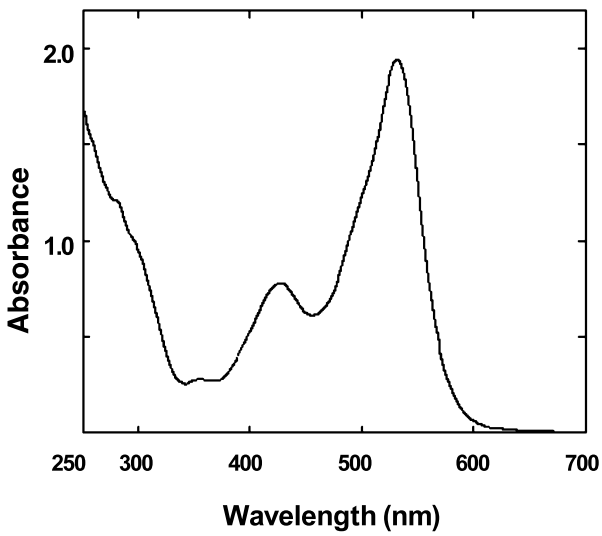

Figure 1. Absorption spectrum of **1**.

In this paper, we report the isolation and structural elucidation of **1**. The novel C–C covalent bond of cyanidin nucleus with gallic acid was formed as shown in Fig. 1. As the figure indicates, the C–C bond formation at C-4 with C″-1 of gallate further involves two cyclizations between the flavylium and gallate moieties. One ring formation occurred between the hydroxyl group at C-5 of the flavylium moiety and C″-6 of gallate by creating an ether linkage, and another forming an α-pyrone ring occurred between the hydroxyl group at C-3 of the flavylium moiety and the carboxylic acid at C-2″ of gallate by esterification.

Pigments were extracted from 7.9 kg of flower petals of *Rosa hybrida* cv. 'M'me Violet' with 80% aqueous acetonitrile containing 0.1% TFA and the extract was allowed to stand overnight at room temperature. The exudation was roughly fractionated by chromatography on a Sephadex LH-20 (Amersham Pharmacia Biotech) column. The 80% aqueous acetone fraction containing **1** was further purified by a HP-20 (Mitsubishi Chemical Co. Ltd, Japan) column. After washing with water, elution was carried out stepwise with aqueous acetoni-

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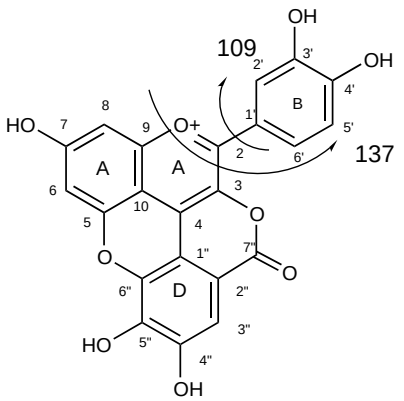

Figure 2. Structure of **1** and FAB-MS fragmentation.

trile containing 0.1% TFA (15, 20 and 30% acetonitorile). The 30% aqueous acetonitorile fraction was collected and lyophilized. This fraction was purified by preparative reverse phase HPLC using an ODS-UG-15/30 (5ϕ×50 cm, Nomura Chemical) column while monitoring at A520 nm at a flow rate of 32 ml/min. The solvent systems used were as follows: a linear gradient elution for 80 min from 20 to 100% solvent B (50% MeCN, 0.5% TFA in H_2O) in solvent A (0.5% TFA in H_2O). The fraction containing **1** was further purified by HPLC using a D-ODS-5 (2ϕ×30 cm, Nomura Chemical) column while monitoring at A260 nm at a flow rate of 6 ml/min. The solvents used for this HPLC purification were prepared as solvent A (0.1% HCl in H_2O) and solvent B (50% MeCN, 0.1% HCl in H_2O). The isocratic elution by a mixture of 70% B with 30% A was carried out for 30 min, and then a linear gradient elution was carried out for 20 min from 70 to 100% B in solvent A. Crude **1** was dissolved in a small amount of ethanol then water was added to the ethanol solution. This solution was allowed to stand at 4°C to give 2 mg of **1** as a violet precipitate.

The molecular formula of **1** is $C_{22}H_{11}O_9$ as revealed by high-resolution fast-atom bombardment mass spectrometry (HRFAB-MS) [m/z 419.0409 $[M]^+$, Δ+0.6 mmu]. The FT-IR absorption spectrum of **1** revealed the presence of the phenolic hydroxyl (3320∼3080 and 1168 cm^{-1}) and α-pyrone (1725 cm^{-1}) functional groups.[2] Compound **1** exhibited an absorption maximum in the visible region (λ_{max} 531 nm, log ε=4.204), suggesting that it was similar to cyanidin[3] (Fig. 2), and the λ_{max} was changed in pH 3; 526, 6; 525 and 7; 574 nm. Compound **1** was very stable in acidic alcohol solution, however, under neutral or weakly acidic aqueous condition, it precipitated before forming the colourless pseudobase. In the ^1H NMR spectrum of **1**, all six proton signals appeared in the aromatic region (Table 1). Five of the six aromatic protons were assigned to H-6 (δ 7.33, d, J=1.0) and H-8 (δ 7.41, d, J=1.0) in the A-ring and H-2′ (δ 7.98, d, J=1.5), H-5′ (δ 7.17, d, J=8.5) and H-6′ (δ 8.05, dd, J=8.5, 1.5) in the B ring of the flavylium form of the cyanidin nucleus. FAB-MS fragment ions at m/z 109 and 137 generated by cleavages as shown in Fig. 1 were the same as those observed with anthocyanidin.[4]

In addition, NOE was observed between H-8 and H-2′/H-6′ as well as in anthocyanin.[5] Therefore, from the above observations, the anthocyanidin nucleus was ascertained to be cyanidin. The remaining proton signal (δ 7.92 singlet) was assigned as follows. The low-field H-4 singlet (δ 8.60∼9.10) characteristic for anthocyanins was missing in the spectrum of **1**. This suggested that the C-4 position of **1** was substituted. The $^1H\{^{13}C\}$-HSQC spectrum of **1** showed that the signal of H-3″(δ 7.92) correlated with the ^{13}C signal of C-3″(δ 113.73). In the $^1H\{^{13}C\}$-HMBC spectra of **1**, the signal for the H-3″ proton in the D ring correlated with the ^{13}C-signal of C-1″(δ 109.07), C-2″(δ113.43), and the carbonyl carbon (C-7″, δ 156.67) attached to C-2″, C-4″(δ 131.62), C-5″ (δ 139.78) and C-6″ (δ 140.48), as shown in Fig. 3. The chemical shift of C-7″(δ 156.67)

Table 1. ^1H and ^{13}C NMR spectral data of **1**

	^1H		^{13}C
	Chem. shift (δ)	J (Hz)	Chem. shift (δ)
A-2			155.60
A-3			156.05
A-4			133.75
A-5			151.58
A-6	7.33	d 1	101.75
A-7			167.98
A-8	7.41	d 1	98.88
A-9			152.70
A-10			102.74
B-1			118.85
B-2	7.98	d 1.5	116.59
B-3			146.36
B-4			154.00
B-5	7.17	d 8.5	116.99
B-6	8.05	dd 8.5, 1.5	125.10
D-1			109.07
D-2			113.43
D-3	7.92	s	113.73
D-4			131.62
D-5			139.78
D-6			140.48
D-7	C=O		156.67

Solvent: 1% DCl/DMSO-d_6.

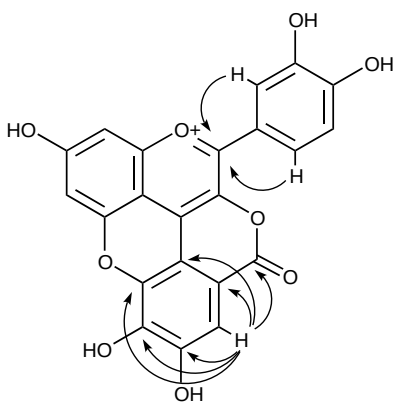

Figure 3. The significant $^1H\{^{13}C\}$-HMBC correlation to elucidate the structure of **1**.

corresponds very well to those found for similar carbonyl carbons present in α-pyrone ring systems such as coumarins[6] and ellagitannins.[7]

Thus, taking the chemical shifts for these carbons into account, the connectivity of these carbons was determined, and its structure was identified to be a galloyl group. Methylation of **1** with diazomethane gave a penta-*O*-methyl ether derivative (**2**) [FAB-MS m/z 489 $[M]^+$], indicating the presence of five phenolic hydroxyl groups in molecule **1**.

Based on these NMR and MS spectral data in addition to the above-mentioned IR data, the structure of rosacyanin B (**1**) was proposed to be that shown in Fig. 2, and the IUPAC name of this structure is 11-(3,4-dihydroxyphenyl)-4,5,8-trihydroxy-2-oxo-2*H*-1,6-dioxa-10-oxoniabenzo[*cd*]-pyrene.

Recently, it was reported that some 4-substituted anthocyanins were detected in red wines made from *Vitis vinifera* and then isolated from these wines. However, these anthocyanins were produced during fermentation or storage, and were not detected in any of the skins of fresh grapes.[8,9] The 4-substituted anthocyanins are known to be more stable to oxidation and pH analysis compared to the unsubstituted anthocyanins.[10] To the best of our knowledge, none of the pigments that have been isolated from intact plants contains a substituent at C-4 of the anthocyanidin nucleus. Therefore, we have accomplished the first isolation of a new anthocyanidin with a substituent at the 4-position of the anthocyanidin molecule from petals of *Rosa hybrida*. Studies on the stability of rosacyanin B towards oxidation and effects of pH on this molecule are now in progress.

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