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A MALONYLATED ANTHOCYANIN AND FLAVONOLS IN THE BLUE FLOWERS OF *MECONOPSIS*

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Key Word Index—*Meconopsis horridula*; *M. grandis*; *M. betonicifolia*; Papaveraceae; blue Himalayan poppy; malonylated anthocyanin; cyanidin 3-malonylsambubioside 7-glucoside; kaempferol 3-gentiobioside; kaempferol 3-xylosylgentiobioside.

Abstract—The blue Himalayan poppies, *Meconopsis horridula*, *M. grandis* and *M. betonicifolia*, contain cyanidin 3-malonylsambubioside 7-glucoside as the anthocyanin. They also contain large amounts of kaempferol 3-gentiobioside and very small amounts of kaempferol 3-xylosylgentiobioside. The ratio of flavonol to anthocyanin was found to be 5.6:1, suggesting that the flavonol plays a role as a co-pigment in the blueing of *Meconopsis* flowers.

INTRODUCTION

Though the blue flower colour of *Meconopsis* is known to be produced by cyanidin derivatives and co-pigment [1], little is known of the basis of this blue colour. It is already known that blue flower colour is usually caused by delphinidin derivatives, as in *Commelina communis* [2–5], *Hydrangea macrophylla* [6–8], *Salvia patens* [9] and *Lupinus* [10, 11], and only very rarely by cyanidin pigments, as in *Centaurea cyanus*, where the metals Mg and Fe are involved, together with the co-pigment [12–14]. Therefore, we investigated the pigment of the blue *Meconopsis* flower to clarify its nature and establish the basis of the true blue colour.

RESULTS AND DISCUSSION

Preliminary tests by TLC suggests that the blue *Meconopsis* flowers contain one major anthocyanin and one main flavonol, together with minor flavonoids. Furthermore, the anthocyanin shows anionic mobility on electrophoresis (+12 mm, pH 4.4, 400 V, 0.75 mA cm^{-1}, 1 hr), suggesting the pigment is zwitterionic [15]. In the present study, we used mainly blue flowers of *M. horridula*, and also petals of *M. grandis* and *M. betonicifolia*. TLC analysis showed that pigment components were practically the same in all these flowers. Dried or frozen petals of *Meconopsis* were extracted with MAW (methanol–acetic acid–water) or MFW (methanol–formic acid–water) and the major pigments were purified by paper chromatography and HPLC. Purified anthocyanin yielded cyanidin, glucose, xylose and malonic acid, which were identified by TLC. The presence of malonic acid was further confirmed by TLC after alkaline hydrolysis, which gave also the deacylated anthocyanin. On controlled acid hydrolysis, the deacylated anthocyanin yielded cyanidin 3-monoglucoside, 3,7-glucoside and cyanidin 3-sambubioside, but neither 3,5-diglucoside nor 5-glucoside was detected. In addition, H_2O_2 degradation of the deacyl anthocyanin gave sambubiose (2-β-xylosylglucose). On the other hand, oxidation of the original anthocyanin with H_2O_2 yielded malonylsambubiose (Table 1), which was positive to the colour reagent for an aliphatic acid on TLC, and gave rise to malonic acid and sambubiose by alkaline hydrolysis. Thus, the *Meconopsis* anthocyanin was revealed to be cyanidin 3-malonylsambubioside 7-glucoside. This was further confirmed by FAB mass spectrometry, which showed the presence of a molecular ion $[M]^+$ at *m/z* 829 ($C_{35}H_{41}O_{23}$ requires 829). Additionally, there was a fragment at *m/z* 754 corresponding to [M − malonic acid (86)]$^+$.

In addition to the anthocyanin, we also purified the flavonols, the main compound **1** and the minor compound **2**. By acid hydrolysis, both yielded kaempferol as the aglycone, while **1** gave glucose and **2** gave glucose and xylose. By controlled acid hydrolysis, **1** yielded only kaempferol without any intermediates, but **2** gave **1** and kaempferol. Furthermore, in the hydrolysate of **1**, gentiobiose (6-β-glucosylglucoside) was detected by TLC, while geniobiose and xylose were found in that of **2**. The attachment of the sugar at position 3 of kaempferol was determined by H_2O_2

Table 1. R_f values for acylated sugar and sugars obtained by H_2O_2 oxidation of *Meconopsis* anthocyanin, its deacylated anthocyanin and flavonols **1** and **2**

Sugar	R_f (×100) in*		
	BTPW	BAW	BEW
Acylated sugar from anthocyanin (malonylsambubiose)	15†	11†	12†
Sugar from deacylated anthocyanin	20	11	12
Sugar from compound **1**	5	4	4
Sugar from compound **2** (xylosylgentiobiose)	4	3	0
Sambubiose	20	11	12
Gentiobiose	5	4	4
Glucose	24	13	17
Xylose	27	20	24

*R_fs were measured on microcrystalline cellulose after ascending TLC in *n*-butanol–toluene–pyridine–H_2O (5:1:3:3), *n*-butanol–acetic acid–H_2O (4:1:5) and *n*-butanol–ethanol–H_2O (4:1:2.2).

†Detected with glucose–aniline reagent.

degradation, whereby **1** yielded geniobiose, and **2** gave xylosylgentiobiose, which was further confirmed by degradation to gentiobiose and xylose on acid hydrolysis (Table 1). This assignment for the binding position of sugar was also confirmed by UV spectral analysis (Table 2). The spectral data showed that hydroxyl groups in 5-, 7- and 4′-positions of both **1** and **2** are free, while 3-hydroxyls of the flavonols were occupied [16]. The FAB mass spectral measurement gave $[M+H]^+$ at *m/z* 611 ($C_{27}H_{30}O_{16}$ requires 610), $[M+Na]^+$ at *m/z* 633 and $[M+K]^+$ at *m/z* 649 for **1**, and $[M+H]^+$ at *m/z* 743 ($C_{32}H_{38}O_{20}$ requires 742) and $[M+Na]^+$ at *m/z* 765 for **2**. Thus, **1** and **2** were identified as kaempferol 3-gentiobioside and kaempferol 3-xylosylgentiobioside, respectively.

Thus, the blue colour of *Meconopsis* is based on the presence of cyanidin 3-malonylsambubioside 7-glucoside as the chromophore and kaempferol 3-gentiobioside as the main co-pigment. To obtain further information on the blueing of *Meconopsis* flowers, the molar ratio of **1** to anthocyanin was analysed by HPLC. The blue petals of *M. horridula* were then found to contain cyanidin 3-malonylsambubioside 7-glucoside and kaempferol 3-gentiobioside in the ratio of 1:5.6. The proportion of flavonol could be enough to form a complex with the anthocyanin, suggesting that the flavonol plays an important role as co-pigment. However, as the *Meconopsis* anthocyanin is a cyanidin derivative, metals such as Mg and Fe, together with the co-pigment, may be involved in the blueing of *Meconopsis* flowers, as in the case of *C. cyanus* [12–14].

EXPERIMENTAL

Plant materials. Dried or frozen blue petals of *M. horridula*, *M. grandis* and *M. betonicifolia* were used.

Isolation of anthocyanin and flavonols. Pigments were extracted from flowers with MAW (10:1:9) or MFW (10:1:9) and the filtered extracts were evapd dryness *in vacuo* at 30°. The residue, after dissolution in MFW, was passed through a Sephadex LH 20 column (1.0 × 2.0 cm) in the same solvent. The pigment frs were evapd to dryness and further purified by PC in the solvents *n*-BuOH–HOAc–H_2O (BAW) (4:1:5) and 15% HOAc. Finally, anthocyanin and flavonols were purified by HPLC on an ODS column (30 × 1.6 cm) with MFW (22:5:73, 37:5:58 and 10:1:9) and also with HCO_2H–MeCN–H_2O (4:5:41 for anthocyanin; 2:5:18 for flavonol).

Identification of anthocyanin and flavonols. Anthocyanin was identified by standard procedures [17, 18]. Analysis included acid and alkaline hydrolysis followed by identification of the aglycone, sugar, organic acid and deacylated anthocyanin, H_2O_2 oxidation and FAB-MS. Malonic acid was detected with glucose–aniline reagent. R_f values (×100) of the original anthocyanin, deacylated anthocyanin, cyanidin 3-sambubioside, cyanidin 3,5-diglucoside and cyanidin 3-glucoside on TLC using cellulose plate were 75, 71, 45, 31 and 18 (HOAc–HCl–H_2O, 15:3:82) and 3, 4, 24, 6 and 16 (*n*-BuOH–2 N HCl, 1:1). The flavonols were identified on the basis of complete acid hydrolysis, controlled acid hydrolysis, UV spectral shifts [16] and FAB-MS. R_f values (×100) of **1**, **2** and kaempferol on TLC using cellulose plate were 40, 37 and 85 (BAW, 4:1:5), 44, 70 and 0 (15% HOAc).

Contents of anthocyanin and flavonol. Pigment was thoroughly extracted with MFW at 4°. Using an aliquot of the pigment extracts, anthocyanin was determined in 1% MEOH–HCl as its chloride by measuring the absorbance at 530 nm. Flavonol was determined by HPLC analysis as described previously [9].

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Table 2. UV spectral shifts of **1** and **2**

Compound	λ_{max} (nm)* in					
	MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+NaOAc/H_3BO_3
1	347 (I) 268 (II)	404 (I)	395 (I)	396 (I)	275 (II)	352 (I)
2	345 (I) 267 (II)	392 (I)	391 (I)	397 (I)	275 (II)	348 (I)

*(I) Band I; (II) band II.

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