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IDENTIFICATION OF MALONATED ANTHOCYANINS IN THE LILIACEAE AND LABIATAE

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Key Word Index—*Hyacinthoides non-scripta*; Liliaceae; bluebell; *Stachys*; *Salvia*; Labiatae; malonated anthocyanins; cyanidin 3-*p*-coumarylglucoside-5-malonylglucoside.

Abstract—The pigment of bluebells, previously reported as delphinidin 3-*p*-coumarylglucoside-5-glucoside, has been shown to be malonated and it has been identified as malonylawobanin. A cyanidin derivative present in several members of the Labiatae, has been characterized as the related 3-*p*-coumarylglucoside-5-malonylglucoside.

INTRODUCTION

It has recently been recognized [1, 2] that anthocyanins occur widely in the angiosperms in zwitterionic form, through acylation with aliphatic dicarboxylic acids. These acyl groups are labile and are lost during extraction with methanolic hydrochloric acid. This has meant that a number of previous structural assignments among known anthocyanins have had to be revised [e.g. 3–5].

As a result of electrophoretic survey [1, 2], it was found that members of both the Liliaceae and the Labiatae contained zwitterionic pigments. In the Liliaceae, three species of 11 surveyed contained such pigments and in particular the bluebell, *Hyacinthoides non-scripta* (L.) Chouard ex Rothm., previously reported as containing delphinidin 3-*p*-coumarylglucoside-5-glucoside [6]. In the Labiatae, every species of 17 taxa surveyed proved to be zwitterionic, confirming earlier indications of malonyl substitution in pelargonidin glycosides of *Monarda* and *Salvia* [7]. It would appear from this survey that nearly all pigments previously reported in this family [6–8] need reinvestigation.

We here report the characterization of malonated anthocyanins in representatives of these two families and the identification of a new cyanidin derivative.

RESULTS AND DISCUSSION

The pigment **1** of the bluebell, after purification, had the R_f values and electrophoretic properties as shown in Table 1. Its structure as delphinidin 3-(6-*p*-coumarylglucoside)-5-(6-malonylglucoside) was established from the results of saponification, which yielded delphinidin 3,5-diglucoside, *p*-coumaric acid and malonic acid and of H_2O_2 oxidation which gave *p*-coumarylglucose and 6-malonylglucose. On FAB-MS, **1** gave the required molecular ion and all the expected fragmentations (Table 2). This same structure has recently been assigned to the pigment of the flower of *Commelina communis* (Commelinaceae) [3], which has been named malonylawobanin. Direct comparison (HPLC and TLC in four solvents) with an authentic specimen of the *Commelina* pigment confirmed our identification of the bluebell pigment as **1**. Compound **1** has also been provisionally identified (by co-chromatography in four solvents) in the blue flowers of *Scilla pensylvanica* and may occur in other related Liliaceae.

The labiate pigment **2** was isolated from the flowers of a *Stachys* species and is probably widely occurring in the family (see below). Its properties (Table 1) confirmed its acylated nature. Its identity as cyanidin 3-(*p*-coumarylglucoside)-5-malonylglucoside followed from the results of saponification when it yielded cyanidin 3,5-diglucoside, *p*-coumaric and malonic acid and of H_2O_2 oxidation which gave *p*-coumarylglucose and 6-malonylglucose. This structure was amply confirmed by the fragmentation pattern derived from FAB-MS (Table 2).

The pigment **2** is probably widespread in members of the Labiatae. Thus cyanidin 3-(*p*-coumarylglucoside)-5-glucoside has been reported previously in flowers of *Salvia horminum* and *S. splendens* [9, 10] and in leaves of *Perilla nankinense* and *P. ocimoides* var. *crispa* [11, 12] and it is now almost certain that this pigment occurs in these plants as the malonate **2**. In the present survey [2], *Salvia nemorosa*, *S. verticillata*, *S. virgata* and *S. viridis* flowers all gave evidence of malonated pigments, but no *Perilla* species has so far been re-examined. It is also likely that pigments previously described in labiates as the corresponding pelargonidin and delphinidin analogues are also malonated at the 5-glucose, i.e. that monardein from *Monarda didyma* is pelargonidin 3-(*p*-coumaryl glucoside)-5-malonylglucoside and that the delphinidin derivative of *Salvia splendens* is identical to **1**. Indeed, the flower pigment of *Monarda didyma* has just been reported as having not one but two malonyl groups attached to the 5-glucose [13]. A malonyl di-*p*-coumaryl ester of peonidin 3,5-diglucoside has already been characterized in leaves of *Plectranthus argentatus* [1] so that the related methyl ethers also probably await characterization among the pigments of this family.

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Table 1. R_f values and electrophoretic mobilities of malonated anthocyanins **1** and **2**

Pigment	R_f (× 100) in BAW	BuHCl	1% HCl	HOAc–HCl	Electrophoretic mobility* (cm)
DELPHINIDIN DERIVATIVES					
3-*p*-Coumarylglucoside-5-malonylglucoside (**1**)	37	33	07	29	0.9
3-*p*-Coumarylglucoside-5-glucoside	40	31	06	25	0.0
3-Malonylglucoside-5-malonylglucoside†	22	31	22	46	7.6
3,5-Diglucoside	10	02	07	20	0.0
CYANIDIN DERIVATIVES					
3-*p*-Coumarylglucoside-5-malonylglucoside (**2**)	35	37	11	40	2.7
3-Malonylglucoside-5-malonylglucoside†	27	24	26	53	7.0
3,5-Diglucoside	23	06	13	34	0.0

*Anionic mobility after electrophoresis at 40 V/cm in acetate buffer pH 4.4 for 1.5 hr.
†Pigments isolated from *Dahlia* [5] included for comparative purposes.

Table 2. FAB-mass spectral fragmentation patterns of pigments **1** and **2**

	1	**2**
Molecular ion $[M]^+$	859	843
Loss of malonic acid $[M-86]^+$	773	757
Loss of *p*-coumaric acid $[M-146]^+$	713	697
Loss of malonylglucose $[M-248]^+$	611	553
Loss of *p*-coumarylglucose $[M-308]^+$	511	535
Ratio of intensities $[M-248]^+$: $[M-308]^+$	1:2.40	1:2.43
Aglycone	303	287

Two general points about the identification of acylated anthocyanins emerge from the above study. First, it is apparent from the R_f data of Table 1, and from R_fs earlier published on malonated anthocyanins (in ref. [5]) that whereas acylation with hydroxycinnamic acid increases the R_f in butanolic solvents and lowers it in aqueous solvents, acylation with malonic acid tends to increase R_f in all solvents. Second, it is now apparent that H_2O_2 oxidation, which originally appeared to specifically identify the 3-substituent in anthocyanins [14], is capable of also yielding the 5-substituent if an acylated sugar is present. In such cases then, H_2O_2 oxidation will not provide the means of determining which acylated sugar is present at the 3- or the 5-position and other evidence must be sought. In the case of pigments **1** and **2**, the structural assignments are clear, because in the demalonated pigments, the *p*-coumaric acid residue has been located at the 3-position; the malonic acid must therefore, by elimination, be attached to the glucose in the 5-position. Confirmation of these assignments in pigment **1** has been provided by ^1H NMR spectral measurements on malonylawobanin [3]. That **1** and **2** are closely similar in structure is apparent from the identical fragmentation patterns in the FAB-mass spectra (Table 2). In particular, the ratio of the intensities of the fragments formed by the intact loss of *p*-coumarylglucose from the 3-hydroxyl and of malonylglucose from the 5-hydroxyl is the same (over 2:1) in both pigments. It may be possible to assign substituents to the 3- and 5-positions from such measurements but further examples need to be studied to be sure that such regularities exist.

EXPERIMENTAL

The sources of the plant materials used have been given elsewhere [2]. Fresh tissues were extracted with MeOH–HOAc–H_2O (19:2:19) and the pigments were purified on Sephadex LH20, by PC in BAW and 15% HOAc, and then again on Sephadex LH20. The R_f values (TLC on microcrystalline cellulose) and electrophoretic mobilities are given in Table 1. Methods of identifying the products of saponification, acid hydrolysis and H_2O_2 oxidation have already been described in detail elsewhere [5]. HPLC was carried out on a reversed phase Spherisorb-hexyl column at 35°, with gradient elution involving increasing amounts of MeOH in 0.6% $HClO_4$ [5]. R_ts were: cyanidin 3-glucoside 4.4 min, **2** 22.3 min, awobanin 25.2 min, bluebell pigment 27.9 min, malonylawobanin 27.9 min. FAB-MS was carried out in glycerol on a Kratos MS 9/50 TC apparatus.

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