Phytochemistry, Vol. 26, No. 10, pp. 2759–2760, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

DIMALONATED ANTHOCYANINS FROM THE FLOWERS OF *SALVIA SPLENDENS* AND *S. COCCINEA*

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(*Received* 31 *March* 1987)

Key Word Index—*Salvia splendens*; *S. coccinea*; Labiatae; malonated anthocyanins; pelargonidin 3-caffeoylglucoside-5-dimalonylglucoside.

Abstract—The pigments of *Salvia splendens* flowers (scarlet cvs) have been identified as pelargonidin 3-caffeoylglucoside-5-dimalonylglucoside and pelargonidin 3-*p*-coumaroylglucoside-5-dimalonylglucoside. The flowers of *S. coccinea* contain the same pigments in the corolla but the calyx contains, in addition, the cyanidin analogues.

INTRODUCTION

Salvia splendens and *S. coccinea* are garden plants which produce abundant scarlet or purple flowers, depending on the variety. The anthocyanins from *S. splendens* flowers have been studied several times in the past, and the presence of pelargonidin, cyanidin and delphinidin pigments has been reported [1, 2]. Thus, from *S. splendens* (purple cvs) flowers, the 3,5-diglucosides of pelargonidin, cyanidin and delphinidin, the malonyl 3,5-diglucoside of delphinidin, and the 3-*p*-coumaroylglucoside-5-glucosides of pelargonidin, cyanidin and delphinidin have been reported [1, 3, 4]. In addition, cyanidin 3-caffeoylglucoside-5-glucoside was also detected in these flowers [2].

In a recent survey on the natural distribution in angiosperms of anthocyanins acylated with aliphatic dicarboxylic acids, zwitterionic anthocyanins were universally detected in the Labiatae [5]. These compounds were not discovered in the past, since the use of mineral acids in the solvents of extraction led to the hydrolysis of the diacid moiety. These facts and the earlier indications of malonyl substitution in pelargonidin glycosides of *Salvia* and *Monarda* [6], prompted us to reinvestigate the pigments of *S. splendens*, and to extend this study to the related species *S. coccinea*.

We here report the characterization of dimalonated anthocyanins in *S. splendens* (scarlet cvs) and *S. coccinea*.

RESULTS AND DISCUSSION

The anthocyanins of *S. splendens* flowers were isolated from fresh plant material by extraction into methanol–acetic acid–water, solvents containing mineral acid were avoided at all stages. The purification was carried out by PC in solvents BAW and 15% HOAc as described previously [7, 8] and two main pigments were isolated (**1** and **2**). The purified pigments were monitored for homogeneity by electrophoresis, HPLC and TLC before analysis. These two pigments showed an electrophoretic mobility as dimalonated anthocyanins [7, 8] but the presence of monomalonated and unmalonated pigments also was detected although in smaller amounts.

Pigment **1** showed the typical colour of a pelargonidin glycoside and its UV values supported this suggestion (λ_{max} 510 nm) as well as the substitution of the hydroxyls at 3- and 5-positions [2]. An additional maximum at 325 nm suggested the occurrence of a caffeoyl residue in the molecule. After saponification pelargonidin 3,5-diglucoside, caffeic acid and malonic acid were detected. Acid hydrolysis yielded pelargonidin, glucose, caffeic acid and malonic acid that were identified by standard procedures [2, 7, 8]. Its electrophoretic mobility suggested that it was a dimalonated anthocyanin, this being readily demonstrated by the FAB-MS spectrum. The molecular ion observed in the FAB-MS analysis (*m/z* 929) indicated the presence of pelargonidin, two glucosyl, two malonyl and one caffeoyl residue. The FAB-MS fragmentation (*m/z* 843 [M − 86] loss of malonic acid; *m/z* 757 [M − 86 − 86] loss of two malonic acid residues; *m/z* 605 [M − 324] loss of caffeoylglucose; *m/z* 595 [M − 86 − 86 − 16] loss of dimalonylglucose; *m/z* 519 [M − 324 − 86] loss of caffeoylglucose and of malonic acid; *m/z* 271 aglycone) indicated clearly that the two malonyl residues were linked to one of the glucoses and that the caffeoyl residue was linked to the other glucose. The detection of pelargonidin 3-caffeoylglucoside-5-glucoside in this plant previously, shows that the naturally occurring pigment is pelargonidin 3-caffeoylglucoside-5-dimalonylglucoside, a novel natural compound.

Pigment **2** showed the same colour and UV features as pigment **1** with the slight difference of an absorption band at 315 nm instead of that at 325 nm of compound **1**, suggesting the presence of a *p*-coumaroyl residue. Acidic hydrolysis produced pelargonidin, glucose, *p*-coumaric acid and malonic acid. After saponification, pelargonidin 3,5-diglucoside, *p*-coumaric and malonic acid were detected. The electrophoretic mobility suggested that this pigment also is a dimalonated anthocyanin, this being

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demonstrated by FAB-MS. The molecular ion (*m/z* 913) indicated the presence of pelargonidin, two glucosyl, two malonyl and one *p*-coumaroyl residues. The fragmentation (*m/z* 827 [M − 86] loss of malonic acid; *m/z* 741 [M − 86 − 86] loss of two malonic acid residues; *m/z* 579 [M − 86 − 86 − 162] loss of dimalonylglucose; *m/z* 519 [M − 308 − 86] loss of *p*-coumaroylglucose and malonic acid; *m/z* 271 aglycone) indicated a similar substitution pattern to that of pigment **1** with the only difference being of caffeic acid substituted by *p*-coumaric acid in pigment **2**, this being pelargonidin 3-*p*-coumaroylglucoside-5-dimalonylglucoside. This compound coincides with monardeain that has been recently fully characterized in *Monarda didyma* [9].

In addition, *Salvia splendens* contains in smaller amounts pelargonidin 3-caffeoylglucoside-5-malonylglucoside, pelargonidin 3-caffeoylglucoside-5-glucoside, pelargonidin 3-*p*-coumaroylglucoside-5-malonylglucoside and pelargonidin 3-*p*-coumaroylglucoside-5-glucoside.

The study of the pigments of *S. coccinea* showed that this plant contained a different pigment pattern in the corolla than in the calyx. The pigments identified in the corolla were identical to those found in *S. splendens* but the calyx contained in addition the cyanidin related compounds, namely cyanidin 3-caffeoylglucoside-5-dimalonylglucoside, cyanidin 3-*p*-coumaroylglucoside-5-dimalonylglucoside and the monomalonated and demalonated compounds in smaller amounts. These compounds were identified by comparisons with pigments **1** and **2**.

EXPERIMENTAL

Plant material. Plants of *S. splendens* and *S. coccinea* were grown from seeds in the Botanical Gardens of the Plant Science Laboratories at Reading University.

Extraction and isolation of anthocyanins. Fresh flowers were extracted with MeOH–HOAc–H_2O (19:2:19) and the pigments were purified by PC in BAW and 15% HOAc and on Sephadex LH-20 as described previously [7, 8]. Methods of identifying the products of saponification and acid hydrolysis have already been described elsewhere [2, 7]. HPLC was carried out on a Waters 600 using reversed phase Partisil 5 CSS C-8 column (25 cm × 4 nm) at 30° eluted with A, 5% aq. HOAc and B, MeOH–H_2O–HOAc (18:1:1), initial concn 20% B in A, increasing 2% B per min, flow rate 1.5 ml/min, detection at 546 nm. FAB-MS was carried out in glycerol on a Kratos MS 9/50 TC apparatus.

Acknowledgement—F.A.T.B. is grateful to the Spanish C.S.I.C. for the fellowship.

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