Phytochemistry, Vol. 28, No. 2, pp. 499–500, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00
Pergamon Press plc.

MALONATED ANTHOCYANINS IN MALVACEAE: MALONYLMALVIN FROM *MALVA SYLVESTRIS*

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(*Received* 23 *August* 1988)

Key Word Index—*Malva sylvestris*; Malvaceae; mallow; flowers; malvidin 3-(6″-malonylglucoside)-5-glucoside; malvin.

Abstract—A new anthocyanin, malvidin 3-(6″-malonylglucoside)-5-glucoside has been characterized in both wild and cultivated forms of *Malva sylvestris*. Thus the classic source of the anthocyanin, malvin, actually contains the pigment in the flowers in malonated form. Malonated anthocyanins were also detected in *Althaea rosea*, *Lavatera olbia* and a *Sphaeralcea* sp. but they were not present in five other species in the family.

INTRODUCTION

During a recent electrophoretic survey of angiosperms for zwitterionic anthocyanins, these pigments were detected in over 24 families, one of which was the Malvaceae [1]. Although most zwitterionic anthocyanins are malonated, other organic dibasic acids have been encountered on occasion, including malic, oxalic and succinic acids [2]. Since it is not possible by electrophoresis to determine which organic acid is present, it is necessary to carry out more detailed studies of the zwitterionic pigments of a given family to determine what organic acids are present and their point of attachment. Moreover, in almost all studies prior to 1985, solvents containing hydrochloric acid were used for extraction purposes, conditions which are now known to degrade any anthocyanins acylated with aliphatic acids; milder conditions are needed to obtain the pigment in unchanged form.

We have therefore reinvestigated the anthocyanin of the common mallow *Malva sylvestris* L., which was positive for acylation in our survey. The petals of this plant are the classic source of the pigment malvin (malvidin 3,5-diglucoside), following its isolation by Willstatter and Mieg in 1915 [3]. This report of malvin in *M. sylvestris* was confirmed in 1969 by Ueno *et al.* [4]. We here describe the results of this reinvestigation, together with a further survey of the family Malvaceae for zwitterionic pigments.

RESULTS

The anthocyanin of the reddish purple petals of the garden form *Malva sylvestris* L. var. *mauritiana* was isolated and purified using water–methanol–acetic acid mixtures in our now standard procedure [5]. It was then characterized as malvidin 3-(6″-malonylglucoside)-5-glucoside by methods that have already been described [5]. Thus, it gave malvin and malonic acid on saponification, while on hydrogen peroxide oxidation, it provided 6-malonylglucose, identified by comparison with an authentic sample. This structure was confirmed by FABMS, when there was a strong molecular ion at 741, and this lost malonate (*m/z* at 655), glucose (*m/z* at 579) and malonylglucose (*m/z* at 493). Similar isolation and characterisation of the anthocyanin of the flowers of wild mallow *M. sylvestris* showed that it was the same.

This is the first report of malvidin 3-(6″-malonylglucoside)-5-glucoside in nature. Its structure corresponds to the related pelargonidin, cyanidin and delphinidin analogues that have recently been characterized [2] and since malvin is fairly widespread, it is likely to be found in this malonated form in other plants. A limited survey of other available malvaceous species showed that malonated pigments are probably present in the hollyhock *Alcea rosea* L., in the tree mallow *Lavatera olbia* L. and in an unidentified *Sphaeralcea* sp. Since Kohlmunzer *et al.* [6] have reported the 3-glucosides and 3,5-diglucosides of delphinidin, petunidin and malvidin in *Alcea rosea* var *nigra*, it is likely that some or all of these pigments are actually present in these flowers as malonyl derivatives. However, by no means all species of the Malvaceae contain such pigments. An electrophoretic survey of petal extracts of the marshmallow *Althaea officinalis* L., *Abutilon hybridum* Hort., *Anisodontea* × *hypomandarum* (Sprague) D. M. Bates, *Hibiscus rosa-sinensis* L. and *Sidalcea malviflora* (DC.) Gray showed that only unacylated pigments were present.

EXPERIMENTAL

Plant material. Reddish-purple petals of *Malva sylvestris* L. var *mauritiana* were collected from plants in the Botanic Garden of Tokyo Gakugei University. Purple petals of wild mallow *M. sylvestris* L. were collected from plants growing on the edge of a cornfield near Shottisbrooke, Berkshire. The other plants surveyed were from the Botanic Garden of the University of Reading. Work on the identification of the *Sphaeralcea* sp. under cultivation is in progress; the other spp. were identified by the curator, Mr R. Rutherford.

Isolation and identification. The pigment was extracted from fresh petals with EtOH–HOAc–H_2O (10:1:9), the solution

evapd and the residue in 5% HOAc passed through an Amberlite X AD-7 column. After washing with H_2O, the pigment was eluted with EtOH–HOAc–H_2O (10:1:9) and the solution evapd. This anthocyanin was then purified via Sephadex LH20 and PC as previously described [5]. Final purification was achieved by HPLC on an ODS (10–20 μm) column (7 × 300 mm) eluted isocratically with HCO_2H–MeCN–H_2O (2:2:21).

Acid hydrolysis gave malvidin and glucose, while saponification with 2 M NaOH in N_2 gave malonic acid and malvin, identified as previously described [5]. Oxidative degradation with H_2O_2 gave 6-malonylglucose, identified by direct comparison with an authentic sample prepared from cyanidin 3-(6″-malonylglucoside). R_f (× 100) values of the new pigment, compared with malvin in parentheses, were 44 (40) in BAW (4:1:5), 18 (05) in *n*-BuOH–HCl (1:1), 20 (08) in 1% HCl and 59 (37) in HOAc–HCl–H_2O (15:3:82). HPLC was carried out on a C_8 column with gradient elution using 20% solvent B (MeOH–HOAc–H_2O, 18:1:1) in solvent A (HOAc–H_2O, 1:1) and increasing the proportion of B by 2% per min. with a flow rate of 1 ml/min at a temp. of 25°. R_ts were 11.43 min for the malonate ester and 8.49 min. for malvin. FABMS, in the positive ion mode, gave a molecular cation at *m/z* 741, an [M − 86] at *m/z* 655, an [M − 162] at *m/z* 579, an [M − 248] at *m/z* 493 and an aglycone ion for malvidin at *m/z* 331.

Acknowledgements—We thank Dr C. A. Williams for running the HPLC analysis.

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