Phytochemistry, Vol. 26, No. 8, pp. 2417–2418, 1987.
Printed in Great Britain.

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

MALONATED CYANIDIN 3-GLUCOSIDES IN *ZEA MAYS* AND OTHER GRASSES

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(*Received* 22 *December* 1986)

Key Word Index—*Zea mays*; Gramineae; maize; pigmentation; cyanidin 3-(6″-malonylglucoside); cyanidin 3-dimalonylglucoside.

Abstract—Re-examination of the anthocyanin pigments of *Zea mays* leaf showed the presence of cyanidin 3-(6″-malonylglucoside) and cyanidin 3-dimalonylglucoside. The same two pigments are probably present in the seed coat. This represents the first complete identification of malonated anthocyanins in the Gramineae.

INTRODUCTION

More is known of the inheritance of anthocyanin pigmentation in *Zea mays* than in any other plant [1]. The molecular biology of the process is under active investigation and the gene c for anthocyanin regulation in the seed coat has recently been cloned by transposon tagging [2]. Yet curiously the exact structures of the maize anthocyanins have still not been clarified. In 1969, we examined in this laboratory the seed coat pigments of PrPr genotypes and identified cyanidin 3-glucoside and two unstable acylated derivatives of this anthocyanin; likewise prpr genotypes contained pelargonidin 3-glucoside and two unstable derivatives [3]. Since then, Lawanson and Osude [4] have reported cyanidin 3-galactoside and 3-glucoside in shoot tissues, Styles and Ceska [5] glycosides of pelargonidin, cyanidin and peonidin variously throughout the plant and Nakatani *et al.* [6] cyanidin 3-glucoside in the seed coat.

Our recent discovery that anthocyanins acylated with aliphatic dicarboxylic acids are widespread in plants [7] led to an extended survey of angiosperms for these zwitterionic pigments [8]. Two of two grass species examined, *Phragmites australis* and *Zea mays*, proved positive. This suggested that the pigments of *Zea mays* should be re-examined in detail. We here report the identification of two malonated pigments in this plant.

RESULTS

Cyanidin 3-glucoside, together with two acylated derivatives were obtained, following mild extraction of coloured leaves of *Zea mays* with methanol–acetic acid–water. After purification, the two pigments were identified as cyanidin 3-(6″-malonylglucoside) and cyanidin 3-dimalonylglucoside by direct TLC and HPLC comparison with authentic samples previously obtained from plants of the Compositae [9]. Identification of the dimalonate was confirmed by fast atom bombardment mass spectrometry (FAB-MS) in the positive ion mode, when a molecular ion at *m/z* 621, an $[M-86]^+$ ion at *m/z* 535 and an aglycone ion at *m/z* 287 were recorded. Cyanidin 3-(6″-malonylglucoside) was first described in *Cichorium intybus* [10] and has been provisionally detected in several other members of the Compositae [9]. The dimalonate, however, has only previously been recorded in stems of *Coleostephus myconis* [9]. Dimalonates still appear to be fairly rare in comparison with monomalonates [8].

Comparison of R_f values with our earlier data on the seed coat pigments [3] strongly suggests that the two previously recorded unstable acylated derivatives of cyanidin are indeed the mono and dimalonate of cyanidin. Indeed, the pattern in leaf and seed coat is known to be the same [3].

A previous survey of grass anthocyanins [11] indicated that cyanidin 3-glucoside was commonly present as a major pigment of inflorescences and leaves. Since methanolic-HCl was then used as the extractant, the presence of any malonated pigments would have been missed. Three common grass species, *Alopecurus pratensis*, *Dactylis glomerata* and a *Bromus* sp., were therefore re-examined by paper electrophoresis for zwitterionic pigments and all three proved to be positive. It is likely, therefore, that the cyanidin 3-(6″-malonylglucoside) here reported in *Zea mays* is widespread in grasses. Further species are being actively investigated to see if this is so. There are at least three plant families, the Compositae, Labiatae and Orchidaceae [8] where zwitterionic anthocyanins are the rule rather than the exception and it is possible that the Gramineae can be included in this category.

EXPERIMENTAL

Pigment was extracted from reddened maize leaves, collected from mature plants in October 1986, with MeOH–HOAc–H_2O (8:1:1). The two acylated pigments were purified by PC in *n*-BuOH–HOAc–H_2O (4:1:5) and 15% aq. HOAc, the purification being monitored by electrophoresis in acetate buffer pH 4.4 for 2 hr at 40 V/cm. Anionic mobilities were 1.7 cm (Cy 3-malonylglucoside 1.7 cm) and 4.5 cm (Cy 3-dimalonylglucoside, 4.5 cm). Their spectral properties were those of a cyanidin 3-glycoside. On acid hydrolysis, they gave cyanidin and glucose and on saponification, they gave malonic acid. TLC comparison with authentic malonates was carried out in the standard solvents [9]. HPLC was conducted 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, 1:19) and increasing the proportion of B by 2% per min., with a flow rate of 1 ml/min and a constant temp. of 25°. R_fs for *Zea mays* pigments and authentic markers in parentheses: Cy 3-glucoside 10.09 (10.05), Cy 3-malonylglucoside 14.41 (14.39) and Cy 3-dimalonylglucoside 15.69 min. (15.70 min.). For details of other techniques used, see ref. [9].

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Phytochemistry, Vol. 26, No. 8, pp. 2418–2420, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

ACYLATED LUTEOLIN GLUCOSIDES FROM *SALIX GILGIANA*

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(*Revised received* 20 *January* 1987)

Key Word Index—*Salix gilgiana*; Salicaceae; luteolin 7-(6″-*trans*-cinnamoyl)glucoside; luteolin 7-(6″-feruloyl)glucoside; luteolin 7-(6″-acetyl)glucoside; luteolin 7-(6″-*p*-coumaroyl)glucoside; apigenin 7-glucoside.

Abstract—Besides apigenin and luteolin 7-glucoside, four novel luteolin glucosides acylated with acetic, *trans*-cinnamic, *p*-coumaric and ferulic acids, respectively were isolated from the leaves of *Salix gilgiana*. The position of the acyl groups was determined to be at C-6″ by the ^{13}C NMR spectral data.

INTRODUCTION

About 40 species of *Salix* are native to Japan. They are all dioecious and produce hybrids easily by cross pollination. Many are deciduous and have no leaves in the flowering period, which sometimes makes differentiation difficult. In continuation of a chemotaxonomic study of the genus, the leaf flavonoids of *S. gilgiana*, which is natival to Japan, Korea and N.W. China [1], were investigated. Seven flavonoids including three new acylated flavone glucosides were isolated and characterised.

RESULTS AND DISCUSSION

From the ethyl acetate soluble portion of a methanolic extract of the leaves of *S. gilgiana*, seven compounds **1**–**7** were isolated, which all gave a positive Shinoda and Molish–Udransky reactions except for **1**. The compounds are numbered in their order of polarity on thin layer chromatography.

Compounds **1**, mp 350–352°, **2**, mp 265–266°, and **3**, mp 239–240° were identified as luteolin, luteolin 7-glucoside [2] and apigenin 7-glucoside, respectively, from their spectroscopic properties.

In the EIMS spectrum of **4**, a fragment at *m/z* 286 suggested the presence of four hydroxy groups in the aglycone. Further fragments at *m/z* 153, 152 and 134 indicated that two hydroxy groups were attached to both the A and B rings. The UV spectrum in MeOH showed an absorption at 269 (band II) and 350 nm (band I). A bathochromic shift with increasing intensity of band I with sodium methoxide indicated a hydroxy group at C-4′. Furthermore, a shift with sodium acetate/boric acid (24 nm) showed an *ortho*-diphenol system in the B ring. A bathochromic shift of band I (38 nm) with aluminium trichloride/hydrochloric acid indicated a hydroxy group at C-5. The absence of a sodium acetate shift indicated that the 7-hydroxyl was not free. In the ^{13}C NMR spectrum, the chemical shifts of the sugar moiety showed the presence of β-D-glucopyranose. Compound **4** was, therefore, considered to be a derivative of luteolin 7-glucoside **2**. In the ^{1}H NMR spectrum (Table 1), in addition to the protons of **2**, a double doublet at 7.32 and two doublets at 7.31 and