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ANTHOCYANINS OF *HEDYSARUM CORONARIUM* AND THEIR CONTRIBUTION TO FLOWER COLOUR VARIATION

ALI CHRIKI and JEFFREY B. HARBORNE*

Laboratoire de Genetique, Faculte des Sciences de Tunis, Campus Universitaire, Tunisie; *Department of Botany, The University, Whiteknights, Reading, RG6 2AS, U.K.

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Key Word Index—*Hedysarum coronarium*; Leguminosae; anthocyanins; flavonoids; colour variation.

Abstract—Peonidin 3-monoglucoside, peonidin 3,5-diglucoside and malvidin 3,5-diglucoside have been identified as flower pigments in *Hedysarum coronarium*. These pigments in varying admixtures appear to be responsible for the different flower colours in this species.

INTRODUCTION

Hedysarum coronarium L., a forage plant of wide distribution throughout Mediterranean regions, has been studied with regard to its reproductive biology and morphological variability [1, 2]. Previous studies on the inheritance of flower pigmentation in this taxon [3] has indicated the presence of two different white-flowered mutants and hence that the conversion of dihydroflavonols to anthocyanins is controlled by two complementary genes. In spite of the range of red, mauve and violet flower colours in this species, no work has been done up to now to identify the pigments responsible for these different colours.

RESULTS AND DISCUSSION

Anthocyanin flower pigments were separated, purified and analysed according to standard procedures [4]. The results on the different flower phenotypes are shown in Table 1. All three phenotypes have peonidin 3-monoglucoside in common, but this pigment is never abundant. Red flower colour is due to peonidin 3,5-diglucoside, while the shift to mauve and violet colours is caused by increasing amounts of malvidin 3,5-diglucoside. Mauve and violet flowers have the same qualitative composition; however, there is an important quantitative variation in the ratio of peonidin 3,5-diglucoside and malvidin 3,5-diglucoside from one phenotype to another. Mauve flowers contain small amounts of malvidin 3,5-diglucoside whilst the violet flowers produce a large quantity of this pigment.

Since there are only trace amounts of flavones or flavonols in these flowers, copigmentation of anthocyanin by other flavonoids which is commonly involved in mauve or violet flowers [5] does not seem to operate. At least, the colour variation encountered in *H. coronarium* can be accounted for simply by changes in the relative amounts of the three anthocyanins present (Table 1). The major difference between red and mauve/violet flowers is due to the presence/absence of 5′-hydroxylation in the anthocyanidin nucleus and a gene controlling this step in synthesis is presumably dominant in plants with mauve or violet flowers.

The discovery of the 3,5-diglucosides of peonidin and malvidin and of peonidin 3-glucoside in *Hedysarum coronarium* flowers is expected and accords with the known anthocyanin glycosylation patterns encountered in the Leguminosae [6]. Although several peonidin glycosides have been reported in legume flowers [7], this appears to be the first unequivocal identification of peonidin 3,5-diglucoside in this family.

EXPERIMENTAL

Plants were collected from natural populations in Tunisia and voucher specimens are deposited in our laboratory. Flowers were collected in April 1982. Anthocyanins were separated, purified and identified according to standard procedures [4]. In all cases, identifications were confirmed by co-chromatography in several solvents with authentic anthocyanin specimens.

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Table 1. Anthocyanin patterns of the colour forms of *Hedysarum coronarium*

	Percentage of pigments in*		
	red flowers	mauve flowers	violet flowers
Peonidin 3-glucoside	20	20	20
Peonidin 3,5-diglucoside	80	60	20
Malvidin 3,5-diglucoside	—	20	60

*Pigment amounts, based on visual inspection of spot intensities on chromatograms, are approximate.

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CHRYSIN 7-GENTIOBIOSIDE FROM THE FLOWERS OF *SPARTIUM JUNCEUM*

SALVATORE DE ROSA and SALVATORE DE STEFANO

Istituto per la Chimica M.I.B. del C.N.R., Via Toiano N.6, 80072 Arco Felice, Napoli, Italy

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Key Word Index—*Spartium junceum*; Leguminosae; flavonoid glycoside; chrysin 7-gentiobioside.

Abstract—Three flavonoids were isolated from the flowers of *Spartium junceum*: the known compounds, chrysin and chrysin 7-glucoside and a new glycoside, chrysin 7-gentiobioside. All three constituents were active in the root growth bioassay.

INTRODUCTION

Spartium junceum L. is known for its diuretic, vasoconstrictor, sedative and other therapeutic properties [1]. There have been several reports [2–4] of B-ring substituted flavonoids and isoflavonoids from this plant. From the flowers of *S. junceum* we now report three unusual flavones (**1**–**3**) with no B-ring substituents.

RESULTS AND DISCUSSION

The flowers of *Spartium junceum* were extracted with acetone and, after evaporation of the solvent, the remaining water was extracted with diethyl ether and then *n*-butanol. Compound **1** was recovered from the ether extract after separation by CC, while **2** and **3** were recovered from the butanol extract.

Compound **1** (mp 288–292°, ethanol) exhibited a molecular ion at *m/z* 254 (100%) in accord with a flavone structure containing two hydroxyl groups. Its UV spectrum suggested the presence of two free hydroxyl groups at C-5 (bathochromic shifts with aluminium chloride, aluminium chloride–hydrochloric acid and sodium methoxide) and at C-7 (bathochromic shifts with sodium acetate). Compound **1** was identified as chrysin (5,7-dihydroxyflavone) from the spectral data and by comparison with an authentic sample.

Compound **2** exhibited UV maxima in methanol at 269 and 306 nm, bathochromic shifts with aluminium chloride, aluminium chloride–hydrochloric acid and sodium methoxide and the absence of a shift with sodium acetate, suggested the presence of a free hydroxyl group at C-5. The ^{1}H NMR spectrum (DMSO-d_6) showed, in addition to the aromatic signals resembling those of **1**, other signals at δ 3.2–4.2, suggesting the presence of a sugar substitution on the hydroxyl group at C-7. This hypothesis was confirmed by the ^{13}C NMR spectrum (DMSO-d_6) which showed six signals at δ 60.5, 69.4, 72.9, 76.2, 77.0 and 99.7 for the sugar moiety and by acid hydrolysis which yielded D-glucose and chrysin (**1**). The β-configuration of glucose was suggested by permethylation of **2**. In fact the ^{1}H NMR spectrum ($CDCl_3$) of the permethylated derivative showed a doublet (1H) at δ 4.95 (J = 8 Hz) attributable to the anomeric proton with a β-configuration [5]. Therefore, **2** was identified as chrysin 7-β-D-glucopyranoside.

The UV spectrum of **3** was similar to that of **2**, suggesting again the presence of a free hydroxyl group at C-5. The ^{1}H NMR and ^{13}C NMR spectra showed, in the aromatic region, similar signals to those of **2** and more signals in the sugar region suggesting that the sugar moiety linked to the hydroxyl group of chrysin at C-7, was more complex than in **2**. Partial acid hydrolysis of **3** yielded **1**, **2** and D-glucose, while complete hydrolysis yielded **1** and glucose. The β-configuration of both glycosidic linkages was evident from the presence of two doublets at δ 5.32 and 5.42 (1H, each, J = 8 Hz) in the ^{1}H NMR spectrum (DMSO-d_6) of permethylated **3**. Acid