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PSEUDOBASE OF MALVIDIN 3-RHAMNOSIDO-5-GLUCOSIDE IN *am* MUTANTS OF *PISUM SATIVUM*

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Key Word Index—*Pisum sativum*; Fabaceae; malvidin 3-rhamnosido-5-glucoside; pseudobase.

Abstract—*am* Mutants of *Pisum sativum* with white petals have been shown to contain the pigment malvidin 3-rhamnosido-5-glucoside in colourless pseudobase form.

de Haan [1] and Wellensiek [2] each have reported a mutant gene for flower colour in *Pisum sativum* (*am* and *aw*, respectively) in which apparently white-coloured flowers redden on exposure to acid. Lamprecht [3], using genetic analysis, was later to confirm the identity of *am* and *aw*. Unfortunately, however, Lamprecht did not conduct chemical tests to resolve an apparent anomaly in the reports of de Haan and Wellensiek, regarding the reaction with acid. Thus, de Haan found: A, *am* genotypes → red; and *a*, Am genotypes → white, whereas Wellensiek found: A, *aw* genotypes → white; and *a*, Aw genotypes → red.

I have recently confirmed [4] the correctness of de Haan's observation, from a study of 26 pure-breeding lines of *P. sativum* available in the collections of Dr. S. Blixt (Weibullsholm, Sweden) and Dr. I. C. Murfet (Hobart, Tasmania). In A, *am* plants, margins of the wing petals frequently show weak colouration in early stages of anthesis, but the main body of the petal is white. After treatment with acid, red colouration appears throughout the petal (the reaction is given only by wing petals) [7]. In other pale-coloured lines, e.g. *ce* genotypes, the whole petal is pigmented, the colour becoming intensified by acid.

Some further observations relevant to the probable nature of the 'reddening' factor in A, *am* flowers are now reported.

(1) The absorption spectrum of the supernatant liquid obtained from wing petals (Weibullsholm line No. 1088) macerated in deionized water is shown in Fig. 1 curve (a), together with subsequent spectra determined after making sequential pH adjustments, curves (b–e). Curve (e) is the normal absorption spectrum for a flavylium cation in aqueous solution (λ_{max} 525 nm). The transition shown in these curves is consistent with conversion of a pseudobase to a corresponding anthocyanin.

(2) Wing petals were steeped in cold 50% hydrochloric acid 1 min by which time they had turned uniformly pink. After washing with deionized water to remove surplus acid, the petals were extracted with methanol and the extract examined by PC and spectroscopy using authentic anthocyanins as reference. The presence of malvidin 3-rhamnosido-5-glucoside in the extract was confirmed.

Pseudobases have been reported as natural products in flowers of other plant species [5, 6]. In *Pisum* they appear to accumulate only in colourless (white) areas of wing petals in A, *am* genotypes. Gene *am* has been reported as a quantitative gene affecting anthocyanin biosynthesis in *Pisum* [7]. It would appear to operate very late in the biosynthetic sequence, i.e. after 5′-hydroxylation (B gene) and *O*-methylation (Cr gene) since the anthocyanidin obtained from WL 1088 flowers is malvidin. It is conceivable that pseudobase formation represents the

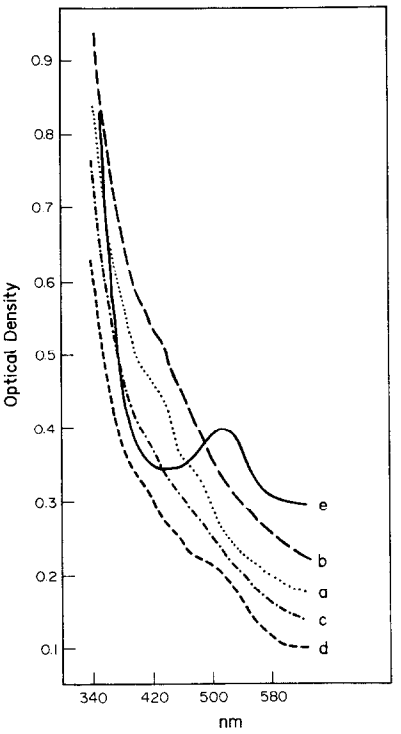

Fig. 1. Absorption spectra in the visible range of an aqueous extract of WL 1088 wing petals at various acidities: (a) pH = 6.0; (b) 3.2; (c) 2.7; (d) 2.2; and (e) 1.9.

penultimate step in normal anthocyanin bisoynthesis in *Pisum*, where it can be recognized by failure to convert to anthocyanin in *am* genotypes.

EXPERIMENTAL

The acid test. Single wing petals were removed from individual flowers and submerged in 50% HCl. A positive reaction appeared within 1–2 min.

pH measurements. Made directly using an Orion specific ion meter fitted with a combined glass electrode. pH changes were made using microlitre additions of conc. HCl.

Visible spectra. Recorded using a Pye–Unicam SP8-100 spectrophotometer. Absorption curves in H_2O (Fig. 1) show some inconsistencies in absorbance values in the 420–500 nm region due to turbidity, which could not be eliminated by centrifugation. Repeat expts in MeOH confirmed that there was a consistent increase in colour with reducing pH from 6.0 to 1.9.

Paper chromatography. Conducted using Whatman No. 1 paper and the solvent systems BAW, 5% HOAc and 1% HCl.

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THE FLAVONOIDS OF *TRICHOPHORUM CESPITOSUM*

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Key Word Index—*Trichophorum cespitosum*; Cyperaceae; *C*-glycosylflavones; flavones; flavonols; 6-*C*-glucosyl-8-*C*-arabinosylchrysoeriol; 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol.

Abstract—Fifteen flavonoids were isolated from *Trichophorum cespitosum*, including two new di-*C*-glycosylflavones, 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol and its Wessely–Moser rearrangement isomer. The known compounds are isorhamnetin 3, 7-dimethyl ether, kaempferol 3, 7-dimethyl ether, chrysoeriol 7-methyl ether, chrysoeriol, sudachitin, tricin, isorhamnetin 3-*O*-galactoside, 6,8-di-*C*-glucosylchrysoeriol, isoschaftoside, schaftoside, vicenin-2, isoscoparin and neoisoschaftoside.

INTRODUCTION

Trichophorum (Cyperaceae), a genus of three species, is also often included in the genus *Scirpus*. These sedges grow mostly in damp peaty areas in boreal coniferous forests in Europe, Asia and North America. Flavonoid chemistry has been used previously in taxonomic studies on sedges [1, 2] but this is the first detailed report of flavonoids isolated and identified from a member of this genus. In this paper we report the flavonoids of *Trichophorum cespitosum* (L.) Hartman (*Scripus cespitosus* L.) including two new di-*C*-glycosylflavones, 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol and its Wessely–Moser isomer.

RESULTS AND DISCUSSION

Stems of *Trichophorum cespitosum* were analysed for their flavonoid content using standard procedures [3]. The dichloromethane fraction contained isorhamnetin 3,7-dimethyl ether, kaempferol 3,7-dimethyl ether, chrysoeriol 7-methyl ether, sudachitin, tricin, isorhamnetin 3-*O*-galactoside and chrysoeriol. Tricin (*ca* 40 mg) was the major flavonoid component of this fraction. This compound, although relatively rare in the dicotyledons, is a common aglycone in the Cyperaceae [4]. The remaining compounds occurred in trace amounts (*ca* 1 mg each).

The water fraction contained a mixture of *C*-gly-