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ANTHOCHLOR PIGMENTS FROM THE PETALS OF *MUSSAENDA HIRSUTISSIMA* AND *ZINNIA LINEARIS*

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Key Word Index—*Mussaenda hirsutissima*; Rubiaceae; *Zinnia linearis*; Compositae; petals; anthochlors; aureusidin 4,6-diglucoside; aureusin; maritimein; marein; sulphurein.

Abstract—Aureusin, cernuoside and the new 4,6-diglucoside of aureusidin have been characterized in the orange flowers of *Mussaenda hirsutissima*. The yellow flower colour of *Zinnia linearis* has been shown to be based on the aurones, sulphurein and maritimein, and the related chalcone marein.

INTRODUCTION

Phenolic yellow pigments are still relatively uncommon in angiosperm flowers, by comparison with the much more frequently encountered carotenoids. Anthochlor pigments, i.e. chalcones and aurones, which are particularly characteristic of certain Compositae, are still only known as flower pigments in some ten plant families [1, 2]. The discovery of anthochlors in a new family is therefore of some general interest and the present paper records the identification of three derivatives of aureusidin (4,6,3′,4′-tetrahydroxyaurone) in the orange petals of *Mussaenda hirsutissima* (Hook F.) Hutch., a member of the Rubiaceae. We also record the identification of three anthochlors in a member of a previously uninvestigated genus of the Compositae—in the golden yellow flowers of *Zinnia linearis* Benth. (tribe Heliantheae).

RESULTS

The major yellow flower pigment of *Mussaenda hirsutissima* was readily identified as aureusin (aureusidin 6-glucoside) by standard procedures (see Experimental and Table 1). This is a well-known anthochlor, first isolated from flowers of *Antirrhinum majus* (Scrophulariaceae) [3]. Trace amounts of the isomeric 4-glucoside, cernuoside [4], were also detected, as was a third glucoside with distinctly higher R_f values in aqueous solvents than either monoglucoside, which therefore appeared to be a diglucoside. Its glucosidic nature was established when it gave only aureusidin and glucose on acid hydrolysis. It was also rapidly hydrolysed by β-glucosidase, which suggested that the two glucose moieties were attached to different phenolic hydroxyl groups of the aurone chromophore rather than being present as a disaccharide. Since the

Table 1. Spectral and chromatographic properties of aureusidin derivatives

	Spectral maxima (nm)			
	EtOH	+ alkali	+ $AlCl_3$	+ NaOAc–H_3BO_3
Aureusidin	254, 269, 335*, 398	Decomp.	405, 458	426
4-glucoside	255, 272, 325*, 404	460	325*, 404	430
6-glucoside	250, 273, 325, 407	482	410, 472	436
4,6-diglucoside	255, 275, 325, 411	518	325, 411	442
3′,4′-dimethyl ether	255, 268, —, 396	340, 406	397, 444	396
4,3′,4′-trimethyl ether	—, 268, —, 395	403	395	395

	R_f (× 100) in†				Colour in	
	BAW	15% HOAc	50% HOAc	PhOH	CAW	UV ± NH_3
Aureusidin	53	01	26	15	20	Y O
4-glucoside	41	08	46	31	11	Y O
6-glucoside	20	05	29	18	09	Y OR
4,6-diglucoside	29	24	57	26	06	Y OR
3′,4′-dimethyl ether	76	01	55	98	86	YG O
4,3′,4′-trimethyl either	84	01	68	98	97	YG O

Colour key: Y, yellow; O, orange; R, red; G, green.
*Inflection.
†On microcrystalline cellulose TLC.

original diglucoside gave a positive spectral shift in the presence of acetate–borate, the pigment could only be formulated as the as yet undescribed 4,6-diglucoside of aureusidin.

The above structure was confirmed by partial acid hydrolysis, which yielded aureusin and cernuoside in approximately equal amounts as hydrolysis intermediates. In addition, complete methylation and acid hydrolysis gave a pigment with all the expected properties of aureusidin 3',4'-dimethyl ether (Table 1). This 4,6-diglucoside of aureusidin has not been recorded before. The bathochromic shift of 13 nm in the neutral spectrum caused by 4,6-disubstitution of aureusidin is close to the predicted value of 15 nm derived from the shifts shown by the 4- and 6-monoglucosides separately (Table 1). This identification of aureusin, cernuoside and aureusidin 4, 6-diglucoside in *Mussaenda* constitutes the first characterization of anthochlor pigments in a member of the family Rubiaceae. There are no records of these compounds in any nearly related family [1, 2]. The closest record is the Compositae, but this is not generally regarded as having much affinity with Rubiaceae. Interestingly, the flowers of *M. hirsutissima* are regularly eaten by ants soon after emergence, so much so that there was some difficulty in collecting sufficient material for this investigation. It is now apparent that these aureusidin derivatives in the flowers have no anti-herbivore function, at least as far as ants are concerned.

In the course of the present work, anthochlor pigments were also recognized for the first time in flower extracts of *Zinnia linearis* (Compositae). The two major pigments were readily identified as the aurone maritimein (6,7,3',4'-tetrahydroxyaurone 6-glucoside) and the related chalcone marein. These two pigments were first found to co-occur in *Coreopsis maritima* [5]. They have been found in one or two related sources [1, 2] but are still relatively rare. Minor amounts of the much more common aurone sulphurein were also detected in the *Zinnia* flowers. Within the tribe Heliantheae to which *Zinnia* belongs, anthochlor flower pigments have been recorded in members of the subtribes Coreopsidinae and Helianthinae [1, 2]; this is the first report of anthochlors in a member of the subtribe Zinniinae.

EXPERIMENTAL

Plant material. Mussaenda hirsutissima was collected from the Botanical Gardens, Department of Botany, Andhra University, Waltair. *Zinnia linearis* was collected from Belhanoor, Chickmagalur District, Karnataka by Dr. A. Santha Ram and also from Siripuram, Waltair. Herbarium specimens have been deposited and they were verified by the Botanical Survey of India, Calcutta, India.

Anthochlors of Mussaenda. The conc. flower extract was separated on 3 MM paper in 15% aq. HOAc. The major lower running band (R_f 05) was rerun in 15% HOAc, when small amounts of cernuoside separated. The major component was then purified by rechromatography in BAW. It was identified as aureusin (aureusidin 6-glucoside) on the basis of colour, spectral and chromatographic comparison (Table 1) with lit. data [3]. On acid or β-glucosidase hydrolysis, it gave glucose and aureusidin, identified by direct comparison with an authentic marker. The position of sugar substitution at the 6-hydroxyl was confirmed by complete methylation (Me_2SO_4, K_2CO_3, Me_2CO, 6 hr) followed by acid hydrolysis to yield 6-hydroxy-4,3',4'-trimethoxyaurone (see Table 1). Cernuoside (aureusidin 4-glucoside) was identified in a similar way. The faster moving band (R_f 18) in the original separation (see above) was purified by rechromatography in BAW. It was identified as aureusidin 4, 6-diglucoside from R_f and spectral characteristics, hydrolytic studies (see Results) and by methylation and hydrolysis to yield aureusidin 3',4'-dimethyl ether (see Table 1).

Anthochlors of Zinnia. The three pigments, maritimein, marein and sulphurein, were readily separated by PC in BAW and 15% aq. HOAc from the flower extract. Maritimein was identified in a similar manner as for aureusin (see above), the nature of the aglucone maritimetin being determined by chromatographic and spectral comparison with an authentic specimen. Marein was identified as a chalcone from its colour reactions and spectral properties; the nature of the substitution pattern was established by its oxidative conversion to maritimein. The marein was also hydrolysed by β-glucosidase to okanin, which was identified by co-chromatography and spectral comparison with an authentic marker. Sulphurein (6,3',4'-trihydroxyaurone 6-glucoside) was identified by similar procedures as for maritimein. Trace amounts of coreopsin were apparently present, as indicated by 2D chromatography of the *Zinnia* floral extract, but it was not in sufficient amount for full characterization. A fifth yellow phenolic pigment was also detected; it was different from any known anthochlor but could not be further identified.

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