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Colour Change in Flowers of *Lathyrus hirsutus* during Senescence

ALTHOUGH there has been some work on the nature of changes in flower colour during development[1-3], little is known about changes during senescence. The anthocyanin-pigmented flowers of *Lathyrus hirsutus* L. exhibit as they age a well marked colour change immediately before the petals show obvious loss of turgor. The mature standard petal is bluish red and it fades through blue to greenish blue; the wing petals are pale blue and fade to greenish blue. It was suggested in earlier work that blueness in mature flowers of *Lathyrus* species is promoted by flavonol glycoside co-pigments[3-5]. Thus it was found that the blue wing petals of *L. hirsutus* contain greater quantities of co-pigment relative to anthocyanin than the standard petals[4]. In this species the *p*H of the expressed sap of the wings was found to be rather higher than that of the standard petal, and it was suggested that this *p*H difference might contribute to the bluer appearance of the wing petal.

Three anthocyanins were found in the standard petal—namely, glycosides of delphinidin, petunidin and malvidin[4]. In the wing petals only the malvidin glycoside was detected, and this behaved chromatographically like the 3-rhamnoside, 5-glucoside identified in *Lathyrus* by Harborne[6]. It should be noted that the malvidin 3-rhamnoside attributed by Harborne[2] to *L. hirsutus* was, in fact, from the species *L. sativus* L.[7].

The present work was carried out to determine whether the factors believed to be involved in the determination of the colour of the mature petals are also involved in determining the colour fade.

Hydrolysed extracts of mature and faded flowers were prepared and then examined by filter-paper chromatography[4]. Determinations of the *p*H of expressed sap from other samples of petals were made with a micro-electrode[4]. Small but consistent differences of *p*H were found.

Table 1. CONSTITUENTS OF HYDROLYSED EXTRACTS AND *p*H OF EXPRESSED SAP IN RELATION TO PETAL COLOUR

Petal	Colour	*p*H	Anthocyanidins*			Flavonols	
			D	*P*	*M*	*Q*	*K*
Mature standard	Bluish red	5·6	+	+	+ + †	+	+
Faded standard	Greenish blue	6·1	−	+	+ +	+	+ + +
Mature wings	Pale blue	6·1	−	−	+	+ +	+ +
Faded wings	Greenish blue	6·7	−	−	+	+ + +	+ + +

* *D*, Delphinidin; *P*, petunidin; *M*, malvidin; *Q*, quercetin; *K*, kaempferol.
† + + +, + +, +, Relative quantities of constituents; −, not detected on chromatograms at loading used.

The results (Table 1) confirm that the mature wing petals differ from the mature standard petals in having a higher *p*H and a greater quantity of flavonol glycoside relative to anthocyanin. The data also show that as the standard and wing petals age, the *p*H of the expressed sap increases. The differences are not large but are likely to be important[8]. The chromatographic data (Table 1) clearly indicate that the faded petals, standard and wing alike, contain greater quantities of flavonol glycosides relative to anthocyanin than the mature petals. These changes could arise from a synthesis of flavonol glycoside, from a destruction of anthocyanin, or from a combination of both processes. To determine which of these processes is involved, quantitative estimations of total anthocyanin and flavonol glycoside were carried out. Samples of ten mature and ten faded petals were extracted with 1 per cent methanolic hydrochloric acid. The optical density of the extracts was determined at 535 mμ as an estimate of anthocyanin and at 260 mμ as an estimate of flavonol glycoside[3]. The results indicated that there is no significant change in the quantity of flavonol glycoside during senescence of either the standard or wing petals. The anthrocyanin content of the standard petals decreases by about 37 per cent as the tissue fades, however, and that of the wings falls by about 23 per cent.

It seems very probable that the fading of the petals of *L. hirsutus* results from an increase in the *p*H of the cell sap and to a greater influence of co-pigment, which results from a decrease in the quantity of anthocyanin present. Thus the same factors, co-pigmentation and *p*H, which were suggested as being responsible for the control of the colour of the mature flowers, appear also to operate in the control of the colour change during senescence.

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Effect of Cycocel and its Analogues on Growth and Soluble Protein Content of Young Barley Seedlings

CYCOCEL, (2-chloroethyl)trimethylammonium chloride, and its analogues have a profound effect on plant growth[1-3]. The same cycocel analogues which retard stem growth[1,2,4] also inhibit gibberellin biosynthesis in *Fusarium moniliforme*[4], reverse auxin[5,6] and coumarin[5,7] inhibition of lettuce seed germination and root growth and accelerate abscission of petiole in cotton[8]. This communication describes an attempt to find a metabolic basis to explain the action of this class of compounds.

Barley (*Hordeum vulgare*, var. Besbar, 1965 crop) seeds were sterilized for 15 min with clorox (1 per cent) and washed thoroughly with running water. Twenty seeds were germinated in 9 cm deep Petri plates lined with one layer of Whatman No. 1 filter paper and moistened with 8 ml. of test solutions. Seeds were incubated in the dark at 25° C for 4 days. After this, coleoptile (with primary leaves) and roots (all seminal roots) were severed from each seed with a sharp razor blade, weighed and their length measured. In the case of root the length of the longest seminal root was recorded. Dry weight was obtained by drying the tissues at 65° C for 24 h. Coleoptile and root tissues, each weighing 250 mg, were homogenized in cold phosphate buffer[9] (*p*H 6·1) and centrifuged in the cold at 5,000 r.p.m. for 10 min. The supernatants were used for assay. Soluble protein was determined by the method of Lowry *et al.*[10]. Cycocel and choline were obtained from Eastman Organic Chemicals and Merck and Co., Inc., respectively. Other analogues used in this experiment were synthesized by Dr. N. E. Tolbert[1] and were kindly supplied by him.

The effects of various cycocel analogues on growth of coleoptile and soluble protein content at two concentrations are given in Table 1. Cycocel and (2-bromoethyl)-trimethylammonium bromide decreased coleoptile growth