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LIGHT ABSORPTION OF ANTHOCYANIN-CONTAINING TISSUE OF FRESH FLOWERS BY THE USE OF THE OPAL GLASS TRANSMISSION METHOD

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Abstract—Spectrophotometric studies were made on fresh flower petals of forty-one plant species by the opal glass transmission method. The absorption spectra were classified into four groups according to the number and position of maxima in the visible region. One new type of absorption curve was observed. The type of curve obtained is related to pigment (anthocyanin) composition. Such analysis may give a clue to the chemical basis of colour variation in flowers.

INTRODUCTION

FLOWER colour variation due to anthocyanins has been extensively studied for many years, and several hypotheses[1–6] have been proposed to explain it. In recent years, Hayashi *et al.* have prepared several pigments from flower petals in a crystalline state, without using mineral acid: blue pigment complexes from blue flowers of commelina[7,8] and cornflower[9–11], red pigments from the deep-red flowers of cornflower, dahlia, and rose,[12,13] and a violet pigment from the deep-violet flowers of pansy.[14,15] According to analytical studies, the blue pigments were shown to be metallo-anthocyanins, and the red and violet pigments were formed to have a composition corresponding to [anthocyanin]-OH.

It is still not clear whether such structures represent the real state of anthocyanin in the cell sap of red and violet petals. A new approach to the problem of flower colour variation has been made here by directly measuring the absorption spectra of the pigments in the cell sap. The opal glass transmission method[16] was used for this purpose.

RESULTS AND DISCUSSION

Until now, experiments on the light absorption responsible for flower colour variation have been made exclusively with solutions of crystalline pigments or with crude petal extracts. It seems of basic importance to examine the actual light absorption of anthocyanins *in situ* in

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fresh flower petals. Therefore absorption spectra were measured with fresh petals of forty-one plant species by the use of the opal glass transmission method, and the results are given in Table 1. As shown in this table, the light absorption of flowers can be classified into the following four groups according to their maxima. Typical absorption curves of each group are shown in Figs. 1–4.

TABLE 1. ABSORPTION SPECTRA OF FLOWER PETALS

Plant species	Absorption (nm) (max.)	Flower colours	Main anthocyanidin types
	GROUP A		
Centaurea cyanus L.	550 660	Pale purple	Cyanidin
	542 664	Pink	Pelargonidin
	530 668	Red	Pelargonidin
	522 666	Red	Pelargonidin
Chrysanthemum morifolium Ramat. var. *sinense* Makino	542 675	Maroon	Cyanidin
Callistephus chinensis Nees	544 672	Purplish-red	Pelargonidin
Antirrhinum majus L.	554 664	Red	Cyanidin
Primula sieboldi E. Morren	560 664	Pale pink	Pelargonidin
	543 664	Pinkish-red	Pelargonidin
Primula polyanthus Hort.	544 668	Pinkish-red	Pelargonidin
	543 668	Red	Pelargonidin
Pelargonium inquinans Ait.	500 674	Scarlet	Pelargonidin
Rosa "hybrid tea"	542 664	Pink	Pelargonidin
	540 665	Pink	Pelargonidin
	530 662	Red	Cyanidin
	528 666	Red	Cyanidin + Pelargonidin
Chaenomeles japonica Lindl.	512 663	Orange red	Pelargonidin
Matthiola incana R. Br.	540 666	Dark red	Pelargonidin
	GROUP B		
Centaurea cyanus L.	535	Pink	Pelargonidin
	530	Dark red	Cyanidin
Dahlia variabilis Desf.	570–580	Purplish-red	Cyanidin
Sinningia speciosa Benth. et Hook. fil.	560	Dark purple	Delphinidin (?)
Pharbitis nil Choisy	570–580	Blue	Peonidin
Primula obconica Hance.	535	Red	Pelargonidin
Primula malacoides Franch	510–530	Pale red	Pelargonidin
Pelargonium inquinans Ait.	520	Orange red	Pelargonidin
Viola tricolor L.	560	Violet	Delphinidin
	535	Violet	Delphinidin
	510–520	Reddish-purple	Delphinidin
	490	Reddish-purple	Delphinidin
Lathyrus odoratus L.	540	Pale pink	Pelargonidin
	530	Scarlet	Cyanidin
Rosa "hybrid tea"	520	Pinkish-orange	Pelargonidin
Hydrangea macrophylla Seringe var. Otaksa Makino	570–580	Blue	Delphinidin
	545	Purplish-red	Delphinidin
Dianthus chinensis L.	560	Purplish-red	Cyanidin
Tulipa gesneriana L.	505	Scarlet	Pelargonidin
Iris hollandica Hort.	560	Deep bluish-purple	Malvidin + Delphinidin
Iris ensata Thunb. var. *hortensis* purple Makino et Nemoto	534	Purple	Malvidin

TABLE 1—*continued*

Plant species	Absorption (nm) (max.)			Flower colours	Main anthocyanidin types
	GROUP C				
Senecio cruentus D. C.	543	574	622	Purplish-blue	Delphinidin
	520	549	585	Purplish-blue	Cyanidin
Stokesia laevis Greene	(545)	565	605	Bluish-purple	Cyanidin
Platycodon grandiflorum A. D. C.	540	570	620	Bluish-purple	Delphinidin
Campanula lasiocarpa Cham.	540	570	615	Bluish-purple	Delphinidin
Lobelia erinus L.	573	615	660	Deep blue	Delphinidin
Saintpaulia ionantha Wendl.	(545)	565	605	Bluish-purple	Cyanidin
Gentiana scabra Bunge var. *baergeri* Maxin.	547	577	614	Blue	Delphinidin
Eustoma russellianum Griser	(545)	565	605	Reddish-purple	Delphinidin
Primula polyanthus Hort.	549	579	658	Purple	Delphinidin
Clematis florida Thunb.	545	570	615	Purple	Delphinidin (?)
Aconitum metajaponicum Nakai	538	566	610	Purplish-blue	Delphinidin
Aconitum japonicum Thunb.	538	566	610	Purplish-blue	Delphinidin
Iris ensata Thunb. var. *hortensis* Makino et Nemoto	545	582	636	Bluish-purple	Malvidin
Tradescantia reflexa Rafin	545	580	620	Bluish-purple	Delphinidin
	GROUP D				
Centaurea cyanus L.	575	660		Blue	Cyanidin
Lithospermum zollingeri A. D. C.	580	(664)		Blue	Delphinidin
Myosotis scorpioides L.	592	(670)		Blue	Delphinidin
Nemophila insignis Benth.	590	614		Blue	Delphinidin
Lupinus hirsutus L.	556	640		Purplish-blue	Delphinidin
Iris ensata Thunb. var. *hortensis* Makino et Nemoto	530	640		Maroon	Malvidin
Iris xiphium L.	556	670		Reddish-blue	Delphinidin
Commelina commiunis L.	592	672		Blue	Delphinidin

Group A. Petal colours belonging to this group exhibit an intense absorption maximum at 500–540 nm, and a smaller maximum at 660–670 nm. Although the main absorption maxima correspond to those observed for isolated pigments, the minor peaks at 660–670 nm are not present in pigments such as cyanin-OH and pelargonin-OH. It is likely that the petal colours in this group are due not only to [anthocyanin]-OH but also to some other factors.[13]

Group B. Flowers in this group are orange-red to reddish-purple. Compared to flowers of A group, they have only one absorption maximum at 490–540 nm, and none at 660–670 nm. The absorption curves are generally the same as those of isolated anthocyanins. For example, the absorption curve of *Viola* petals measured by the opal glass method is identical to that of a buffer solution of its anthocyanin, violanin-OH. Therefore, it may be assumed that violanin in the cell sap of the pansy is mostly as violanin-OH or as its potassium salt.[15] Some pansy flowers in Group B have an absorption peak below 540 nm, the maximum for violanin chloride; the reason for this hypsochromic shift is still not known. An unusual colour in this group is the deep blue of *Pharbitis*. This is apparently not due to metal complexing (see Group D spectra below) but to some other factor.

Group C. The flowers of this group are purple to blue and three absorption peaks are found in a visible region. From blue flowers of *Platycodon grandiflorum*, a bluish powder has recently been isolated, containing a single anthocyanin, which also gave three absorption peaks in aqueous solution. Details of this work will be published later.

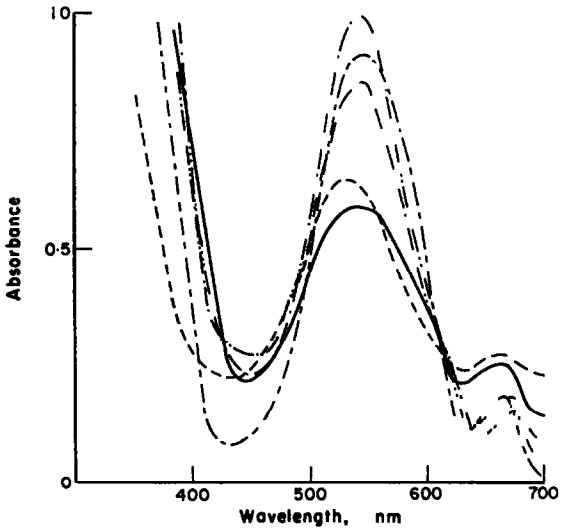

FIG. 1. ABSORPTION SPECTRA OF TYPICAL FRESH PETALS (GROUP A).

———— *Rosa* "hybrid tea", pink; — — — — *Primula sieboldi* E. Morren, pinkish-red; - - - - - *Centaurea cyanus* L., red; —··—··— *Callistephus chinensis* Nees, purplish-red; —·—·— *Chrysanthemum morifolium* Ramat. var. *sinense* Makino, maroon.

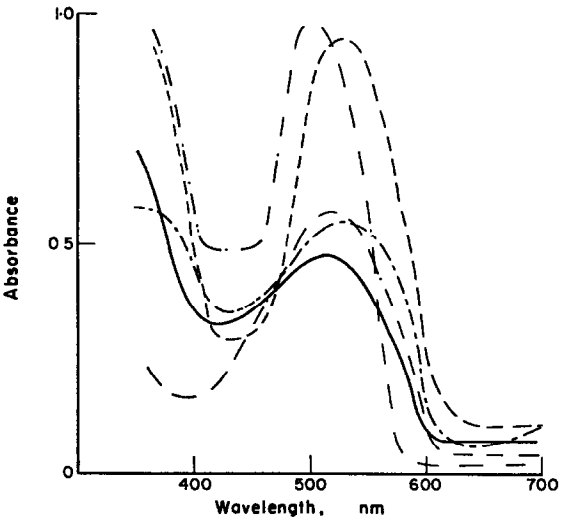

FIG. 2. ABSORPTION SPECTRA OF TYPICAL FRESH PETALS (GROUP B).

———— *Rosa* "hybrid tea", pinkish-organge; — — — — *Lathyrus odoratus* L., scarlet; —·—·— *Primula malacoides* Franch, pale red; - - - - - *Viola tricolor* L., violet; —···—··· — *Tulipa gesneriana* L., scarlet.

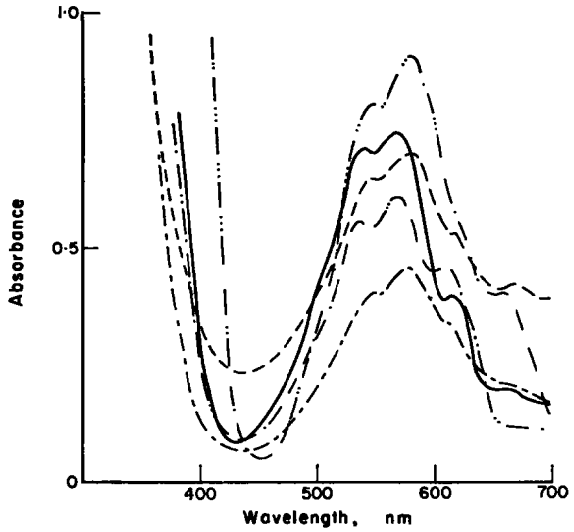

FIG. 3. ABSORPTION SPECTRA OF TYPICAL FRESH PETALS (GROUP C).

———— *Campanula lasiocarpa* Cham., bluish-purple; – – – – – – *Gentiana scabra* Bunge var. *Baergeri* Maxin., blue; —··—··— *Primula polyanthus* Hort., purple; —·—·— *Aconitum japonicum* Thunb., purplish-blue; – – – – – – *Tradescantia reflexa* Rafin., bluish-purple.

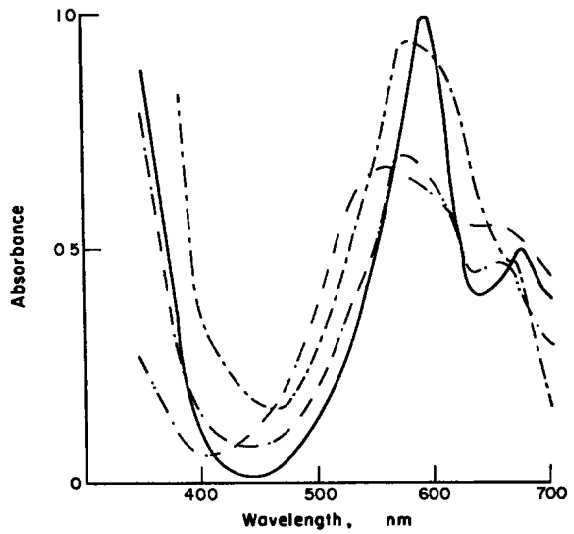

FIG. 4. ABSORPTION SPECTRA OF TYPICAL FRESH PETALS (GROUP D).

———— *Commelina communis* L., blue; – – – – – – *Lithospermum zollingeri* A. D. C., blue; —·—·— *Centaurea cyanus* L., blue; —···—··— *Lupinus hirsutus* L., purplish-blue.

Group D. Flowers belonging to this group seem to contain metallo-anthocyanins, such as commelinin[8] and protocyanin.[11] It has already been shown that blue metallo-anthocyanins exhibit two absorption peaks in the visible region. Here, it may be observed that absorption spectra of both petals and isolated pigments are identical over the entire range of the spectrum.

MATERIALS AND METHODS

Spectral methods. The flower petal, or anthocyanin-containing epidermal tissue carefully peeled off from the petal, was stuck on a glass slide, mounted with water, and covered with a thin opal glass specially prepared for this purpose. This was set in the sample position, with the opal glass as control (Fujimoto-Rikagaku Co. Ltd., Tokyo) and light absorption was measured with the automatic spectrophotometer (Model ORD/UV-5 Japan Spectroscopic Co. Ltd.). Measurement was made with a single plate of opal glass in the case of thin petals, while two to three plates were necessary for measurement of thick or deeply coloured petals. Here, it may be noted that semi-transparent paraffin paper can be used in place of opal glass. For petals having small hairs or rough epidermal protrusions, absorption was measured after dipping them into 50% aqueous glycerol containing a trace of tween 80, or sucrose solution (*ca.* 0·8%), in order to avoid scattering of light. Throughout the whole course of measurement, plant material did not undergo any colour change.

Chromatographic methods. Freshly collected plant material was extracted by maceration with methanol–conc. HCl (98:2, v/v) at room temperature. The extract was centrifuged, and filtered through Celite, and concentrated to a small volume *in vacuo* at 30–40° and refiltered. The concentrated extract (1 ml) was boiled with 4 ml of 20% HCl for 3–5 min, cooled, and after addition of water, the resultant aglycone was extracted with a small quantity of *iso*-amyl alcohol. The *iso*-amyl alcohol extract of anthocyanidin was chromatographed, with authentic anthocyanidins, in the following solvents: the Forestal mixture, *n*-butanol/acetic acid/water (4:1:5, v/v) and water/acetic acid/HCl (82:15:3, v/v).

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