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To,

My Dear Parents

Acknowledgments

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Abstract

Chemotaxonomic studies of anthocyanin and related flavonoid pigments were conducted in seven species of Impatiens(Balsaminaceae): I. balfourii, I. balsamina, I. capensis, I. linearifolia, I. platypetala, I. schlechterii and I. sultani. In addition, pigments of 15 other taxa including I. hersogi, I.holstii, and I. pallida, were examined to some degree. Pigments were analysed qualitatively and quantitatively in vegetative and floral tissues of from two to seven replicate specimens of each species. Red pigmentation was observed in the roots and root tips of young seedlings and mature plants and appeared to be a continuing feature of root development. Localization of pigmented cells was found to be species-specific as seen in cross-sections of roots, stems, and petioles.

Major pigments in stems and leaves were invariably 3-glycosides of cyanidin, accompanied in the cases of I. balsamina, I. linearifolia, I. platypetala, and I. schlechterii by 3-glycosides of delphinidin. Major pigments in flowers of I. balsamina, I. linearifolia, I. schlechterii, and I. sultani were pelargonidin glycosides, while cyanidin glycosides were characteristic of I. capensis, and malvidin glycosides of I. balfourii and I. platypetala. Peonidin was found in place of pelargonidin in some varieties of I. sultani, and aurantinidin glycosides were present only in two cultivars (Hybrid and Sweetsue).

Pigment data obtained on the seven species were analysed by computer. Following discriminant analysis of these data, all seven species could be separated. Pigment-data keys were constructed by which the 15 additional taxa were classified. Anthocyanins appeared to be an aid in classification of species and cultivars of Impatiens.

Résumé

Des études chemiotaxonomiques des pigments flavonoïdes, plus particulièrement des anthocyanines, ont été conduites chez sept espèces d'Impatiens (Balsaminacées): I. balfourii, I. balsamina, I. capensis, I. linearifolia, I. platypetala, I. schlechterii, and I. sultani. De plus les pigments de 15 autres taxa incluant I. herzogi, I. holstii, and I. pallida ont été examinés plus ou moins en détail. Les pigments des tissus végétatifs et floraux des plantes ont été analysés qualitativement et quantitivement. Une pigmentation rouge a été observée dans les racines et à l'extrémité des racines de jeunes plantules et de plantes matures et a semblé être une cataotéristique persistante du développement de la racine. Des coupes transversales des racines, des tiges, et des pétioles ont révélé que la localisation des cellules pigmentées était spécifique aux espèces.

Les pigments majeurs des tiges et des feuilles étaient invariablement des 3-glycosides de cyanidine accompagnés de 3-glycosides de delphinidine en ce qui concerne I. balsamina, I. linearifolia, I. platypetala, and I. schlechterii. Les pigments majeurs des fleurs d' I. balsamina, I. linearifolia, I. schlechterii, and I. sultani étaient des glycosides de pélargonidine alors que des glycosides de cyanidine et malvidine étaient caracteristiques d'I. capensis et d'I. balfourii et I. platypetala, respectivement. La pélargonidine a été remplacée par la péonidine chez quelque variétés d'I.sultani et les glycosides d'aurantinidine étaient présents seulement chez les deux variétés Hybride et Sweetsue.

Toutes les données concernant les pigments des sept espèces ont été analysées par ordinateur. Suivant l'analyse du discriminant de ces données, on peut séparer les sept espèces. Des clés basées sur les données concernant les pigments ont été construites à partir desquelles on peut classifier les 15 taxa additionels. Les anthocyanines ont semble utiles a la classification des espèces et variétés d'Impatiens.

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Introduction

I. Chemotaxonomy

Chemotaxonomy is a novel field of research attracting considerable attention in recent years from many botanists and biochemists around the world (Parker and Bohm, 1979; Wallace and Markham, 1978; Harborne, 1975; Gibbs, 1974, Hegnauer, 1964; Alston and Turner, 1963 and Geissman, 1962). Biochemical characters are perhaps more constant than morphological ones, because they are presumed to be affected only quantitatively by environmental and cultural conditions whereas morphological characters are influenced qualitatively (e.g. leaf forms) by modifiers as reported in Baptisia by Alston and Turner (1963) and in Spirodela oligorhiza by McClure and Alston (1964).

Rapid progress in chromatographic and spectroscopic techniques has revealed various biochemical relationships among botanical taxa, evolutionary pathways, ecological adaptations and other features. Many samples of plant material can be examined easily for a particular class of compounds by one or other of the many chromatographic procedures. Such compounds include terpenoids, amino acids, alkaloids, quinones, fatty acids, sulphur compounds, mustard oils and phenolic compounds especially flavonoids (Harborne, 1970). Flavonoids have been preferred to the terpenoids or alkaloids in systematic studies because they are universally distributed in vascular plants, show structural diversity, are chemically stable, are easily detectable in herbarium tissues or fresh plant material and are rapidly identified

(Harborne, 1975). Flavonoids are chemically based on a C_{15} skeleton (C_6-C_3-C_6) and include anthocyanins, flavones, flavonols, chalcones, aurones, flavanones, isoflavones, biflavonyls, and leucoanthocyanidins.

Flavonoid patterns have been studied at various levels of classification. Moore et al.(1970) examined flavonoid patterns in several hybrids between closely related species. Flavonoid data have also been found useful in classification at the sectional level in the genus Pinus (Erdtman, 1963), at the tribal level in the family Plumbaginaceae (Harborne, 1967a), at the subfamily level in the family Gesneriaceae (Harborne, 1967b, 1966b) and in the taxonomy of the family Limnanthaceae (Parker and Bohm, 1979). A complex interfamilial relationship of anthocyanins and the visibly red but chemically unrelated betacyanins has been observed in plants within the order Centrospermae (Mabry, 1966). In this order plants in the family Caryophyllaceae contain purple-red anthocyanins whereas plants of all the other families contain only betacyanins (Harborne 1967).

II. Anthocyanin Pigments and Chemotaxonomy

The use of anthocyanin pigments in chemotaxonomic studies is discussed by Timberlake and Bridle,(1975)and Harborne, (1967, 1975). Stoutamire (1960) reported the relation of anthocyanins to the taxonomy of the genus Gaillardia. The species with red rays were separated into four groups on the basis of anthocyanin mixtures present in the ray and disk flowers. Acheson et al.(1956) studied the distribution of anthocyanins in the poppies, Papaver species, and found striking differences in the kinds and distribution of these pigments in various species. Harborne (1966c) related the distribution

of 3-deoxyanthocyanins to the taxonomy of the family Gesneriaceae at the subfamilial level. Since then many investigators have observed the significance of anthocyanins in plant classifications (Hegnauer, 1964; Clevenger, 1964, 1971).

Anthocyanins occur almost universally in the higher plants with the exception noted above, but have a restricted distribution in the lower plants such as ferns, mosses and gymnosperms (Timberlake and Bridle, 1975; Harborne, 1967). They are sap soluble and responsible for nearly all attractive colors, red, pink, violet, mauve and blue in the flowers, fruits, stems, leaves and roots (Harborne, 1967). Anthocyanins are glycosides in which sugars are attached to positions 3 and/or 5 and sometimes 7 of the anthocyanidin C_6-C_3-C_6 skeleton (Fig. 1). They possess very distinctive colors on chromatograms, may fluoresce when viewed in ultraviolet (UV) light, and are therefore readily identified in plant extracts (Harborne, 1967). On acid hydrolysis anthocyanins produce water-insoluble anthocyanidins of which six are of common occurrence. These are pelargonidin (Pg), cyanidin (Cy), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and malvidin (Mv), (Fig. 2). The common sugars involved in the structure of anthocyanins are glucose, galactose, xylose, arabinose and rhamnose. The several known classes of anthocyanidin glycosides are 3-monoglycosides, 3-bioglycosides, 3-trioglycosides, 3,5-diglycosides, and 3,7 glycosides. Acylated anthocyanins in which an acyl group is attached to the sugar in the 3-position of the molecule are also known to occur in several plants, the acyl group of these pigments is nearly always p-coumaric acid but caffeic and ferulic acid also occur occasionally (Harborne, 1967). More than 250 anthocyanins are known at the present time

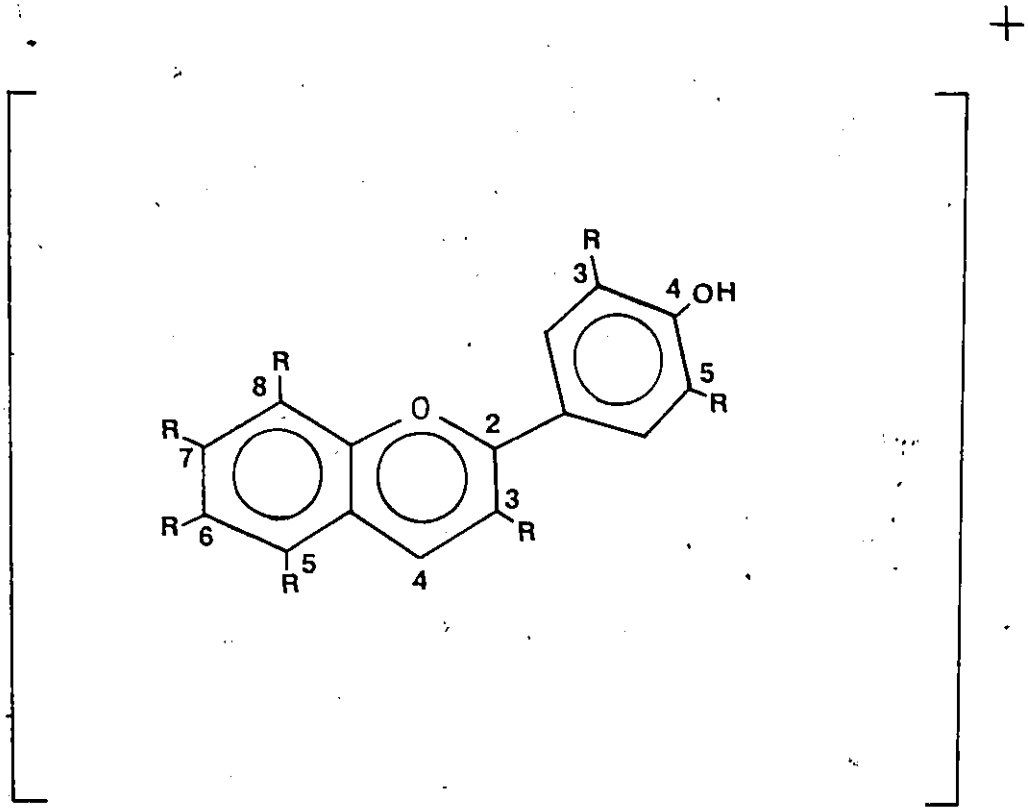
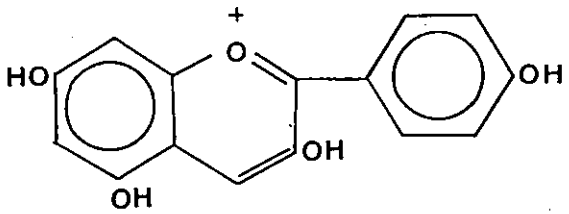
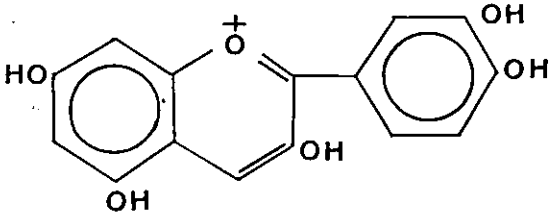


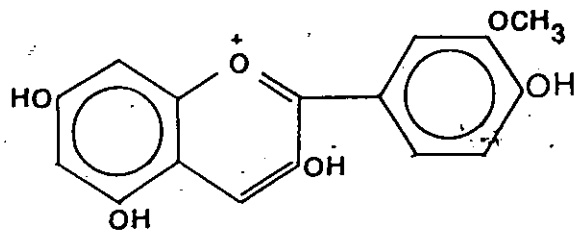
Fig. 1 The Flavylium cation (from Timberlake and Bridle, 1975)



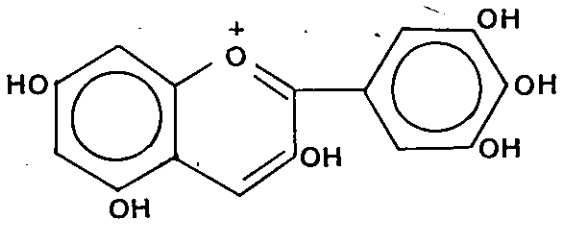
Pelargonidin



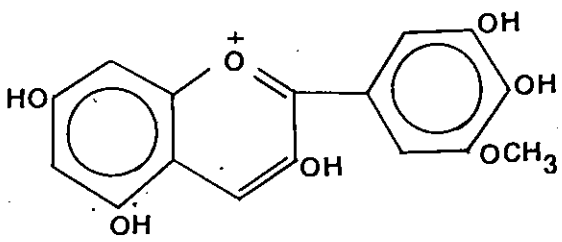
Cyanidin



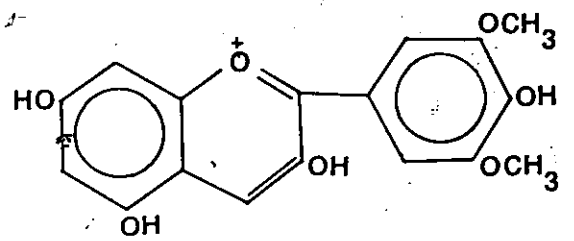
Peonidin



Delphinidin



Petunidin



Malvidin

Fig 2 Six naturally occurring common anthocyanidins. (from Walker, 1975)

(Harborne 1977, Timberlake and Bridle, 1975) as compared to the limited number of anthocyanidin types.

Various techniques, paper chromatography (Bate Smith, 1948), paper chromatography and UV spectroscopy (Harborne and Geissman, 1956), column chromatography (Nebresky 1949, and Zimmerman 1957), thin layer chromatography (Harborne and Hurst, 1966; Harborne, 1967 and Ibrahim et al., 1971), gas liquid chromatography (Albach et al, 1965 and Alshakir, 1967), high pressure liquid chromatography (Asen, 1977), infra red spectroscopy and nuclear magnetic resonance (Seikel and Mabry, 1965; Wagner, 1965 and Mabry et al., 1970) have been used for identification and purification of anthocyanins and other flavonoid pigments.

III. Leucoanthocyanidins

Leucoanthocyanidins (also termed leucoanthocyanins, condensed tannins or proanthocyanidins) yield anthocyanidins on acid treatment (Haslam, 1975). They are distributed more commonly in the tissues of woody plants than in those of herbaceous plants but they are found in plants of many predominantly herbaceous families: Balsaminaceae, Saxifragaceae, Polygonaceae, Ficoideae, Oxalidaceae and Limnanthaceae (Bate Smith and Lerner, 1954; Harborne, 1967). Chemically leucoanthocyanidins are dimers, derived from one unit of flavan-3,4 diol linked to a catechin (flavan-3-ol) and these substances do not have covalently linked sugar units in their structure (Harborne, 1967).

IV. The genus- Impatiens

The genus Impatiens belongs to the family Balsaminaceae. It comprises approximately 600 species (Wood, 1975; Chinnappa and

Gill, 1974; Jones and Smith, 1966; Khoshoo, 1957) and is primarily distributed in tropical and subtropical Asia and Africa with a few species in southeast Asia and a still lower number in Northern Asia, Europe and North America. Warberg and Reiche (1835) divided the genus Impatiens into fourteen sections on the basis of morphological characters.

Impatiens species are commonly known as balsam and are of great value to horticulture owing to their ability to flower continuously in shady and semishady areas, their decorative and brightly colored flowers., self cleaning habit (both leaves and branches abscise during prolonged dry weather), and tolerance to air pollutants (Winters, 1973).

Recently, new introductions of Impatiens from New Guinea were brought to the United States by H.F. Winters and J.J. Higgins of the Plant Introduction Service, U.S.D.A., Maryland (Winters, 1970, 1973). Grauman (1972) released 23 Impatiens introductions from U.S.D.A.* to plant breeders and nurserymen. These new introductions vary in flower size from 2.5 to 7.5 cm in diameter with spurs of equal length. Flower colors range from pure white through pastel shades of lavender and pink to magenta, and from pale orange to dark vermillion or scarlet. Stem and leaf colors vary from green to intense dark red. In addition leaves in some species are beautifully variegated with white, yellow or pink colors (Winters, 1973).

The genus has medicinal value also, for example Stopp (1963) reported that Mt. Hagen people of New Guinea rub large petals and young leaves of 'Kontip', I. mooreana schl., into burns and according

*U.S.D.A.= United States Department of Agriculture

to Marie-Victorin (1964) in 'Flore Laurentienne' plants of I. capensis can be used to treat the skin irritations following contact with poison ivy. Taylor (1940) reported that the stems of I. biflora (I. capensis Meerb.) are made into a decoction with roots of Veronica officinalis and the bark of Ulmus fulva and drunk to ease childbirth.

V. Computers and taxonomy

Recently, many taxonomists and biologists have used computers in the classification of plants (Pankhurst, 1975; Sneath and Sokal, 1973). Baum and Lefkovitch (1972) present a model for cultivar classification and identification in Oats (Avena) and Baum (1977b) used various computations in a study of the taxonomy of the tribe Triticeae (Poaceae). Duncan and Estabrook (1976) described an operational method for the evaluation of classifications. Thus it appears that computer analyses are a useful aid in biosystematic studies.

VI. Purpose of the present study

The genus Impatiens is very large (approx. 600 species) but most species share the character of possessing visible red pigments and leucocyanidins (Gibbs, 1974) which presumably are useful taxonomic characters of the genus. These pigments have been examined in detail in only a few species with little reference to chemotaxonomy (Asen, 1977; Clevenger, 1971; and Hegnauer, 1964).

The purpose of the present study was to first isolate, identify, and quantify anthocyanins, and the chemically related leucocyanidins, by chromatographic and spectroscopic methods from all parts of the plant- roots, stems, leaves, and flowers- of about 20 taxa; then to take the

pigment data so obtained and a) subject it to computer analysis and b) construct dichotomous keys. The purpose of the latter step was to evaluate if this approach might be of value in identification or separation of the taxa under study, and if not, as an indication of genetic similarity within the genus.

Materials and Methods

I. Plant Material

A. Seeds

Seeds of various species of Impatiens were obtained from several botanical institutions or purchased from commercial sources (see Appendix 2). Seeds were surface sterilized with 10% sodium hypochlorite solution, washed with distilled water several times, soaked in distilled water overnight and placed on moist filter paper in plastic petri plates (9 cm. in diameter). They were germinated under ambient conditions of light and temperature in the laboratory. Seedlings were potted in soil and grown to maturity in the greenhouse.

B. Mature Plants

Plants of some species, recently introduced by the United States Department of Agriculture (U.S.D.A.), Plant Introduction Service, and their interspecific hybrids were grown from cuttings (see Appendix 2) kindly provided by Dr. T. Arisumi, U.S.D.A., Beltsville, Maryland. Cuttings were also collected from local plantings of I. sultani and plants of I. capensis were collected in the wild. All plants were grown in soil either in plastic pots on a wire screen or in clay pots on gravel beds under normal greenhouse conditions.

II. Visual Observations of Pigmentation

A. In Intact Plants

Distribution of pigmentation was observed in roots, stems, leaves and flowers of young seedlings and mature plants. Photographs of representative plants of most species and cultivars were taken.

B. In tissues

Localisation of pigments was determined by examining water-mounted hand cut transverse sections of roots, stems and leaves, under a compound microscope. Photomicrographs were taken in color of many of these cross-sections.

III. Analysis of Pigments

A. Preparation of tissues

Fresh, dried or frozen tissues were used as a source of pigments. Flowers were collected, as they appeared and only petals were dissected out and dried over silica gel for 2-3 days. Stem, leaf and sometimes flower tissues were stored in the freezer and these frozen tissues were either extracted directly or freeze-dried before extraction.

B. Extraction of pigments

(i) From intact-tissues

(a) With methanol

Plant tissues except flowers, were finely chopped and steeped in cold methanol-1% HCl overnight in the refrigerator. The amount of tissue used (taken as volume of chopped tissue in a beaker) was based on the visual intensity of pigment in the plant: approximately 30-40 ml for pale, 20-25 ml for medium and 10-15 ml for intensly colored stem and leaf tissues. As many as 8-10 if pale-colored or only 2-4 if intensely-colored flower petals were taken. The colored solutions were decanted and the tissues re-extracted with fresh solvent by crushing in a mortar with a pestle. The supernatants were again

decanted and the process repeated until the tissues were colorless or nearly so. All the extracts were combined and centrifuged at 2500 x g to sediment tissue particles. The supernatant was kept in the fume-hood for evaporation to dryness or flash evaporated at 30-35°C *in vacuo*. The evaporated extracts were first washed with ethyl acetate and petroleum ether to remove some flavonols, phenolics and chlorophylls and then dissolved in a minimal amount of methanol-1% HCl for chromatographic analysis.

(b) From methanol extracted residual tissues

The residual tissues from the above procedure were boiled with 50% aqueous HCl for 10 min (Clevenger, 1971). The hydrolysate was filtered and an approximately equal amount of distilled water was added to the filtrate. Red pigments were then extracted with a small volume of iso-amyl alcohol, concentrated, and used for chromatography. An approximation of the amount of these pigments was made by relating the visually estimated intensity of red color of the isoamyl alcohol extract in a volume proportional to the dry weight of the tissues extracted.

(ii) From intact tissues following acid-hydrolysis

The flowers and stem tissues of some plants were extracted by direct hydrolysis with 2N-HCl at 100°C for 45-60 min. (Harborne, 1973). The acid solution was then filtered and the filtrate first extracted with ethyl acetate to separate most of the phenolics and some of the flavonoids. Traces of ethyl acetate were then removed by heating for 2-3 min. at 80°C. The anthocyanidins were then taken up with a small volume of isoamyl alcohol. Distilled water was added to

facilitate the extraction. The isoamyl alcohol was evaporated on watch glasses placed on beakers of boiling water. The dried pigment was then redissolved in a small volume of acidified methanol and chromatographed.

C. Chromatographic Separation of Extracted Pigments

(i) Anthocyanins

(a) 2-D paper chromatography

The concentrated crude pigment extracts in methanol-1% HCl were applied to Whatman no. 1 chromatography paper for two-dimensional chromatography. One quarter of a 46 x 57 cm sheet of Whatman no. 1 filter paper was used for each chromatogram. The pigments were spotted in one corner 6 cm from one edge and 2.5 cm from the other edge until a dark spot was obtained. The papers were taped along the short edge to form cylinders and chromatographed ascendingly for 4-5 h. using BAW (n-butanol-acetic acid-water, 4:1:5, top layer) solvent for the first direction. After this run the chromatograms were air-dried and viewed in visible and in UV light. The positions of pigment spots were marked with a lead pencil, then the chromatograms were run in the second direction descendingly using HOAC-HCl (acetic acid-conc. HCl-water, 15:3:82, v/v/v) solvent system (Harborne, 1967). The chromatograms after development were air-dried and viewed again in visible and UV light and the pigment spots were encircled. The solvent system showing the better resolution of pigments was then used for a unidimensional descending run on paper.

(b) 1-D paper chromatography

The pigment extracts were applied in 23 cm bands on Whatman

no. 1 strips. The amount of pigment extracts applied in bands varied depending on the visual intensity of extract: for example only 2-3 applications were sufficient for deep red extracts whereas as many as 15-20 applications were required for pale to very pale extracts. If streaking occurred with a resultant poor separation of bands, a second and if necessary, a third chromatogram was made using fewer applications in order to obtain satisfactory separation of bands. Similarly if the separated bands were insufficiently visible, additional chromatograms were made with heavier banding.

(c) Quantitation of chromatographic bands

When a satisfactory separation of bands was obtained on each chromatogram, relative intensities were determined. The approximation of quantities of pigments was made for each species by estimating visible intensity of color with ISCC-NBS (Inter-Society color council-National Bureau of Standards) color name charts illustrated with centroid colors; measuring the width of pigment bands on the paper chromatogram and correlating these two parameters (Table 1). Direct comparison from one species to another of pigment quantities estimated in this way is not valid unless the initial amounts of tissue analysed and the total pigment content are approximately equal.

(d) Elution of pigment bands from paper

Pigment bands separated on unidimensional chromatograms were cut out as paper strips and prepared for elution with a square trim at one end and an angled or pointed trim on the other. The strips were then hung downward from a petri dish containing Harborne's elution mixture (methanol-acetic acid-conc. HCl, 70:5:25, v/v/v) over a beaker

Table 1. Quantitation of chromatographic bands

Type of pigment	Width of band on chromatogram (cm)	Visible color corresponding to centroid color*	Expressed quantity (width & visible color)	
			Number	Verbal equivalent
1. Pg-glycosides	1.5	26 s.y. Pink	1	Trace
	3.0	26 s.y.Pink	2	Poor
	2.0	27 s.y. Pink	3	Fair
	2.0	between 26 s.y. Pink and 35 s.r.o.	4	Good
	2.0	35 s.r.o.	5	Very good
2. Au-glycoside	2.0	between 26 s.y. Pink and 35 s.r.o.	3	Fair
3. Cy-glycoside	1.0	249 l.p. Pk.	1	Trace
	3.5	252 p.p. Pk.	1	Trace
	1.5	250 m.p. Pk.	2	Poor
	3.5	249 l.p. Pk.	3	Fair
4. Pn-glycosides	1.0	247 s.p. Pk.	3	Fair
	1.8	between 247 s.p.Pk. and 248 deep p. Pk.	4	Good
5. Dp-glycosides	1.5	249 l.p. Pk.	1	Trace
	1.0	247 s.p. Pk.	1	Trace
6. Mv-glycosides	1.5	240 l.r. P.	1	Trace
	3.0	240 l.r. P.	2	Poor
	2.5	241 m.r. P.	3	Fair
	1.5	237 s.r. P.	4	Good

*Abbreviations: l = light, m = medium; p.Pk = purplish-pink; r.O = reddish-orange; r.P = reddish-Purple; s = slightly; y = yellow.

(Plate 1). The eluates were collected as they dripped off the paper, evaporated to dryness and redissolved in a small volume of methanol-1% HCl for further purification on paper or on TLC plates.

A preliminary test was made for sugars elutable from filter paper by cutting a blank paper strip from a chromatogram used for anthocyanin identification, and eluting with Harborne's mixture. The eluate was evaporated to dryness in the fumehood, redissolved in 10% isopropanol and tested for sugars as described in Section E, iii, b.

(e) PVP column chromatography

Polyvinylpyrrolidone (PVP) columns were prepared as follows: fine particles were removed by suspending PVP powder in water. After five minutes of settling, the water was decanted. This process was repeated 19 times to get a desirable slurry. Columns of 25X2 cm size were used. Glass fibre filter paper was first placed over the drain hole of the column. PVP slurry suspended in water was poured down the sides of column using a widemouth 10 ml. volumetric pipette to obtain a final column length of 15 cm. the column was drained until nearly all the water had run out.

The evaporated crude pigment extracts were redissolved in a small volume of acidified distilled water, the pH was adjusted to 3.5-4 with 4 N-NaOH added dropwise and the solution obtained was filtered or centrifuged to remove solid particles. This pigment extract was then carefully added to the top of the column until a band about 1 cm wide had been adsorbed. The column was washed first with distilled water to remove free salts and sugars and then eluted with 30% ethanol acidified with 2N-HCl (2 ml. in 1000 ml. of ethanol).

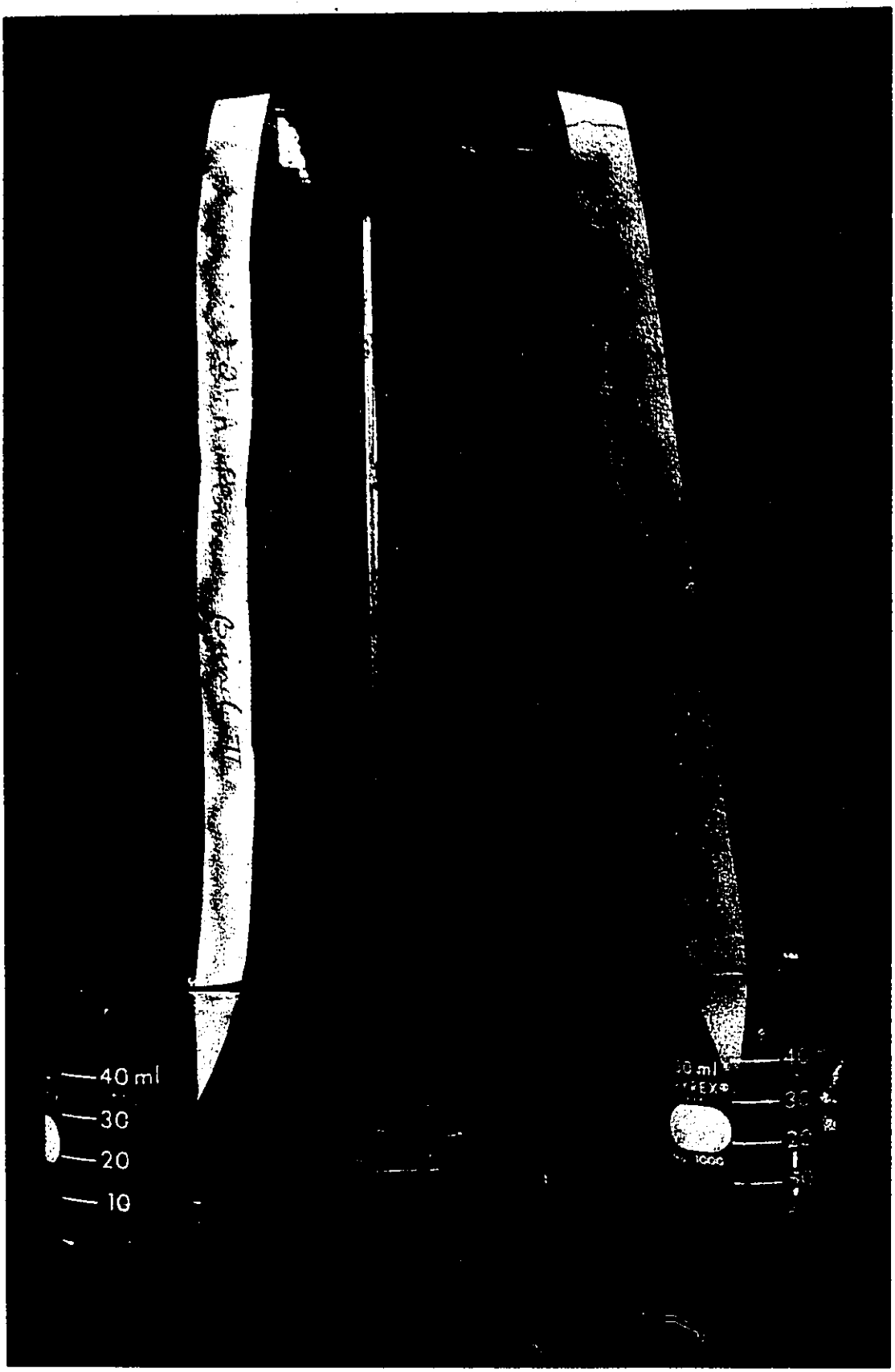


Plate 1 Apparatus showing elution method

Fractions of 10 ml. were collected until all of the anthocyanin pigments were eluted. These fractions were evaporated in a fumehood or on a sample concentrator at 40°C.

(ii) Anthocyanidins (Aglycones)

Anthocyanidin extracts obtained by direct hydrolysis of tissues with hot acid were banded in 23 cm. widths on Whatman No. 1 paper and separated by a descending run in Forestal solvent (acetic acid-conc. HCl-water, 30:3:10, v/v/v/). Authentic pelargonidin, cyanidin, delphinidin and malvidin were spotted at each end of the extract band as standards.

D. Purification of pigments

Pigment eluates collected from paper were purified by repeated paper chromatography either in BAW or HOAC-HCl solvent depending on which solvent showed better separation. Polyamide-TLC plates were used to check the purity of pigments in aqueous (n-butanol-acetone-dioane-water, 45:35:20:210, v/v/v) and organic (benzene-methanol-ethyl-methyl ketone-water, 55:25:18:3 v/v/v) solvents. The process of elution and rechromatography was repeated until the purified individual pigment showed a single spot in different solvents under visible and UV light.

Evaporated fractions obtained from the PVP column and redissolved in methanol-1% HCl, were spotted on polyamide DC-6 coated plates and run ascendingly in 0.01 N-HCl to determine which fractions could be combined. Sequential fractions showing similarity of spots in visible and UV light were combined and further purified by repeated paper chromatography as already described.

Anthocyanins purified by paper chromatography were adsorbed on a PVP column to separate them from a surprising amount of sugars present in paper eluates, and eluted with acidified 80% ethanol (2 ml. of 2N-HCl in 1000 ml. of ethanol). Eluates were evaporated to dryness and redissolved in a small volume of 50% methanol for further chromatographic and spectral tests and for hydrolysis with trifluoroacetic acid or 2N-HCl.

E. Identification of Purified Pigments

(i) From Chromatographic Behaviour

Whatman No. 1 filter papers prewashed with 1% HCl and distilled water were dried and spotted in quadruplicate with anthocyanin extracts and authentic samples of cyanin and Malvin, for development in the following solvent systems (Harborne, 1967):

1 BAW (n-butanol-acetic acid-water, 4:1:5, top layer);

2 HOAC-HCl (acetic acid-conc. HCl-water, 15:3:82, v/v/v);

3 1% HCl (conc. HCl-water, 3:97, v/v);

4 Bu:HCl (n-butanol-2N-HCl, 1:1 top layer).

After solvent irrigation, the chromatograms were dried in a fumehood and observed under visible and UV light. Presence or absence of fluorescence was recorded.

(ii) From spectra

Absorption spectra of methanol-1% HCl solutions were recorded in visible and UV regions using a Unicam SP 1800 spectrophotometer. Spectra were also taken of pigments dissolved in acid free ethanolic-$AlCl_3$ (5%) for determination of the presence or absence of free

O-dihydroxyl groups in the B-ring (Harborne, 1967).

(iii) From aglycone and sugar moieties produced by acid hydrolysis

More rigorous identification of selected anthocyanins was made by a final acid hydrolysis. Hydrolysis was carried out with addition of 2N-HCl or trifluoroacetic acid and heating to 100°C for 60-90 min. on a waterbath (Harborne, 1973).

(a) Aglycones

The aglycones (anthocyanidins) were extracted into a small volume of isoamyl alcohol which in some cases was removed by evaporation and replaced by a small volume of methanol-1% HCl before spotting and chromatography on paper along with the authentic anthocyanidins, Pg, cy, Dp and Mv. Three standard solvent Systems were used (Harborne, 1967):

1 Forestal (acetic acid-conc. HCl-water, 30:3:10, v/v/v);

2 Formic (Formic acid, conc. HCl-water, 5:2:3, v/v/v);

3 BAW (n-butanol-acetic acid-water, 4:1:5, top layer).

(b) Sugars

1 Preparation of sugar residues:

The acid solutions containing sugar residues were left to evaporate in the fumehood. Distilled water was added and evaporated 2-3 times to remove any traces of acid. The dried sugars were finally dissolved in 10% isopropanol to prevent bacterial growth (Harborne, 1973) and used for further chromatographic analysis. The standard sugars used were 0.2% solutions of D(+) glucose, D(+) galactose, L(+) Arabinose, D(+) xylose and rhamnose.

2 Chromatography of sugar residues

The sugar residues were spotted on Baker Flex cellulose microcrystalline sheets with the aid of 10 µl micropipettes along with standard sugar solutions and were developed ascendingly in EPW (ethyl acetate-pyridine-water, 10:3:2:2, v/v/v) solvent (F.W. collins, pers. commun.), air-dried and run again in the same solvent and in the same direction for better separation of the spots. The dried chromatograms were sprayed with aniline hydrogen phthalate (AHP) reagent (Partridge, 1949) made by dissolving 1.6 g phthalic acid and 0.92 ml. aniline in 100 ml. of distilled water saturated with n-butanol. The chromatograms were dried and heated in an oven at 105°C for 4-5 min. The color reactions of sugar residues were recorded.

A preliminary test was made to determine the minimal amount of sugars detectable on the chromatogram. Varying amounts of a 0.2% solution of glucose were spotted and sprayed as usual. It was found that 2 µl of 0.2% solution was the minimum amount detectable. Anthocyanin sugar residues were thus spotted to ensure that this minimum amount was present on the chromatogram. In order to do that weight of sugar had to be determined in residue. This was done as follows: absorbance of the anthocyanin was measured before hydrolysis and using the average extinction coefficient of 0.013 (Hrazdina, pers. commun.), the total number of µmoles present in the solution was determined. The number of µmoles of sugars/µmole of aglycone was estimated from chromatographic behaviour, the mobility of a monoside in acid solution, for example, is much less than that of a trioside. For example the absorbance at 540 nm of a solution of anthocyanin was 0.035 after passing through a PVP column. Since the volume was 1 ml, µmoles

total was $0.035X1.3X10^{-2}$ = 0.455 µmoles. If a monoside, the minimum number of µmoles of sugar present was also 0.455 µmoles and if the sugar was an hexose-glucose (MW-180), the weight of sugar present was 0.455X0.18=0.82 mg.

F. Flavonoids other than anthocyanins

These pigments were observed on chromatograms and characterized by colors in UV light and Rf values in alcoholic (BAW) and acidic (HOAC-HCl) solvent systems (Harborne, 1967).

IV. Analysis of Pigment Data and Preparation of Keys

A. Key construction

All the pigment data for anthocyanins, leucoanthocyanidins and other flavonoids (flavonols and phenolics) in vegetative and floral parts were first summarized (Tables 7 & 8). These were then transcribed to computer sheets and subjected to computer analysis. For this purpose plants examined were divided into two groups, one of "known" (identified) consisting of species for which one or more replicates were analysed and another of "unknowns" consisting of specimens with or without certain identity but for which only one specimen was available. Means and variances of all characters of the replicates of the known species were computed and a secondary table, in which all characters showing no intraspecific variance were listed, was drawn up. A dichotomous key was produced from this table which was useful to separate only four species.

A discriminant analysis (Seal, 1964) of pigment data was performed on the inseparable species. Following this discriminant analysis, classification function coefficients (Nie, et al.,1975) were computed and added to the

key. A key based on floral pigment characters was similarly constructed.

B. Testing the Keys

Attempts to identify unknowns with the aid of the keys based on vegetative characters were made.

C. Construction of a key based on aglycone data

A key based on characters simpler to determine than anthocyanin pigments, such as, type of aglycones, leucoanthocyanidins, presence or absence of anthocyanins and localisation of pigmented cells in cross sections of stem and leaves, was also constructed.

Results

I. Plant Material

A. Germination of Seeds

Seeds of I. glandulifera Royale; I. noli-tangere L.; I. parviflora D.C.; I. scabrida D.C.; and I. capensis Mearb. did not germinate under the laboratory or greenhouse conditions even after 5-7 weeks of a chilling treatment at 4-5°C in a refrigerator. Gibberellic acid (GA_3) treatment (Baskin and Baskin 1970 & 1971; and Jouret 1977) was also ineffective for seed germination of these species. Plants of I. balfourii Hook F.; I. balsamina L. and I. holstii Hook F. however, were grown from seeds.

B. Mature plants

Plants of I. linearifolia warb., I. platypetala Lindl.; I. schlechterii warb. and I. sultani were obtained from cuttings and under normal greenhouse conditions most began to flower within a few weeks. Some species bloomed continuously (I. platypetala and I. sultani) but most were seasonal and a few, I. linearifolia (no. 20) and some unidentified species, never bloomed in over two years.

II. Visual Observation of Pigmentation

A. In intact plants

(i) Stem

The site of pigmentation varied depending on the species, from the base only to all along the stem. Color also varied from faint red to very deep red and deep purple red.

(ii) Leaves

Leaves varied from completely green or with faint red petioles only to those with a red coloration in petioles and on the lower surface of leaf-blades. Several kinds had variegated leaves with faint red in the petioles.

(iii) Flowers

Flowers were of a wide variety of shades, ranging from pure white through pastel shades of lavender, pink, magenta, pale orange to dark vermilion or scarlet and purple.

A detailed description of visual localisation of pigments in plants of Impatiens is summarized in Table 2 and illustrated by plates 2 to 8.

B. In tissues

The distribution of pigmented cells in different organs and tissues was characteristic of a species.

(i) Roots

Roots of only 2-3 species were examined in this way and typical cross sections obtained are shown in plates 9 and 10. Pigmented cells can be seen to be scattered in the epidermis, cortex and central stele in I. balsamina (Plate 9) but only in the central stele in I. holstii (Plate 10).

(ii) Stems

Different sites of pigmentation were seen in cross sections of stems depending on the species and the stage of development and varied from the epidermal region only to all across the sections.

Table 2. Localisation of visual pigmentation in intact plants

Species	STEM		LEAVES		FLOWER	ILLUSTRATION
	Pigment site*	Color	Pigment site*	Color	Color	
1. I. balfourii Hook F.	B	1	G	-	1 & 2	
2. I. balsamina L. (llHHPrPr)	B	1	G	-	3 & 4	Plate 2
3. I. capensis Meerb.	AA and BN	1	P	1	5	
4. I. linearifolia Warb.						
no. 18	AA	2	PL	2	6 & 7	
no. 19	AA	2	V and PL	2	8	Plate 3
no. 20	AA	2	V and PL	2	-	
5. I. platypetala Lindl.						
no. 21-A	AA	3	PL	3 & 2	1 & 9	Plate 4
no. 21-B	AA	3	PL	3 & 2	1 & 9	
no. 21-D	AA	3	PL	3 & 2	1 & 9	
6. I. schlechterii Warb.						
no. 5	AA	1	G	-	Vermilion	
no. 8	AA	4	PL	4	Bright Red	
no. 10	AA	2 & 4	V and PL	2	orange-pink	
no. 11	AA	4	PL	4	dull scarlet	Plate 5
no. 12	AA	2 & 4	PL	2 & 4	bright Vermilion	
no. 23	AA	2 & 4	PL	2 & 4	bright Vermilion	

no. 12	AA	2 & 4	PL	2 & 4	bright Vermilion	
no. 23	AA	2 & 4	PL	2 & 4	bright Vermilion	
no. 34	AA	4	PL	4	3 & 10	Plate 6
7. I. sultani Hook F.						
I	AA	5 & 6	V	5 & 6	3 & 10	Plate 7
II	AA	5 & 6	U	5 & 6	11 & 12	Plate 8
III	AA	5 & 6	U	5 & 6	2 & 13	
IV	AA	5 & 6	U	5 & 6	8 & 14	
V	-	-	G	-	2	
8. I. herzogii K. Schum	N	N	N	N	3	
9. I. holstii Hook F.	AA	6	U	8	11 & 12	
10. USDA Introductions						
A'flame	N	7	N	7	-	
Aloha	N	1	P	1	4 & 15	
Arabesque	AA	4	PL	4	2 & 8	
Cheers	N	N	N	N	16	
Hybrid (TangerineX366029)	B and AA	1	P	7	4 & 5	
Sweetsue	AA	4	PL	4	3	

* Pigment Site

Stem: B = mainly at the base

AA = all over stem

BB - mainly at the base and nodes

Leaves: G = green

P = red in petioles only

V = variegated

PL = red in petioles, mid ribs, veins

Stem: B - mainly at the base

AA = all over stem

BB - mainly at the base and nodes

Leaves: G = green

P = red in petioles only

V = variegated

PL = red in petioles, mid ribs, veins and lower surface of leaf

U = Petioles and undersurface of leaf

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color of Stem and leaves

1 = Centroid 12 S Red

2 = Centroid 13 deep Red

3 = Centroid 16 d. Red

4 = Centroid 14 v. deep Red

5 = Centroid 256 deep p.R.

6 = Centroid 257 deep p.R.

7 = Centroid 15 m. Red

8 = Centroid 258 m.p.R.

Colors of flowers

1 = Centroid 237 s.r.P.

2 = Centroid 263 white

3 = Centroid 34 v.r.O.

4 = Centroid 48 v.O.

5 = Centroid 66 v.Oy

6 = Centroid 4 l. pink

7 = Centroid 5 m. pink

8 = Centroid 2 s. pink

9 = Centroid 238 deep r.P.

10 = Centroid 36 deep r.O.

11 = Centroid 254 v.p.R.

12 = Centroid 256 deep p.R.

13 = Centroid 11 v.Red

14 = Centroid 3 deep pink

15 = Centroid 50 s.O.

16 = Centroid 27 deep y.Pink

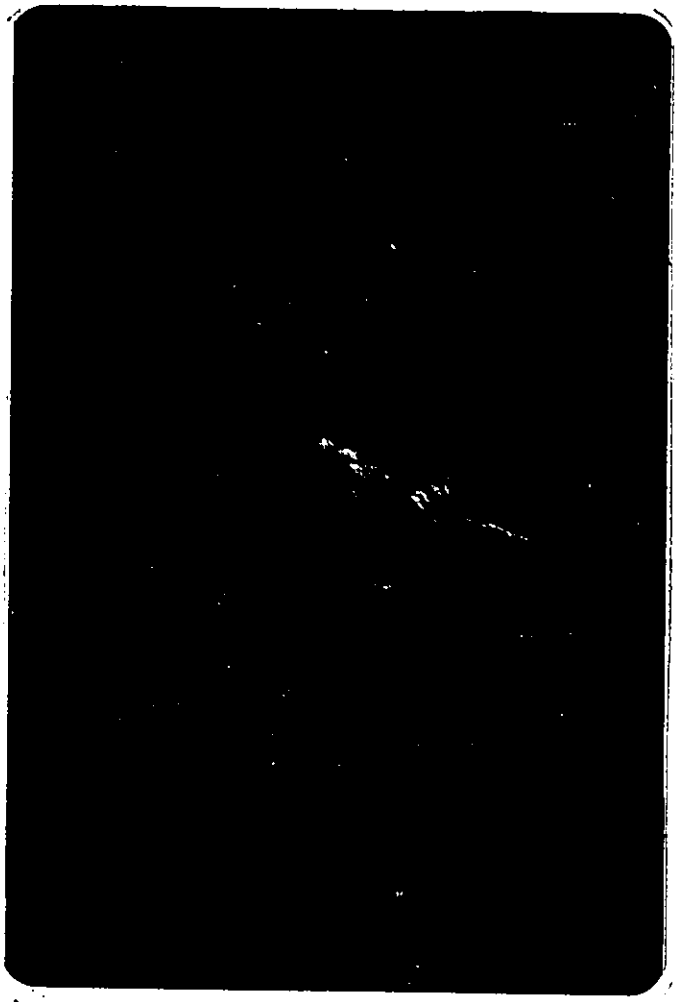


Plate 2 Vegetative and floral morphology of *I. balsamina*.

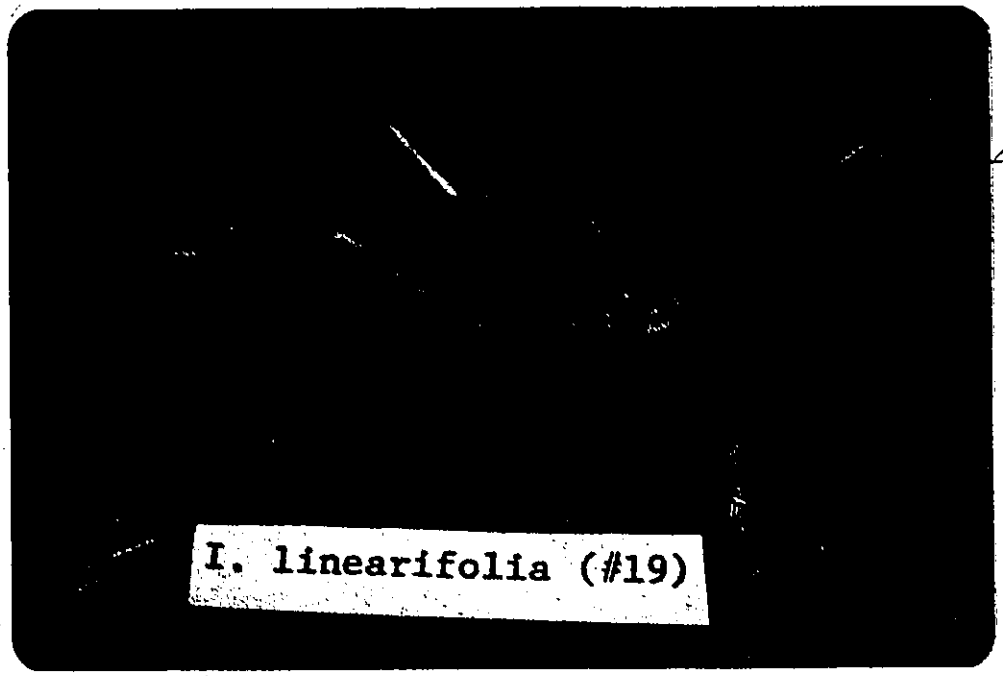


Plate 3 Vegetative and floral morphology of I. linearifolia. (#19)

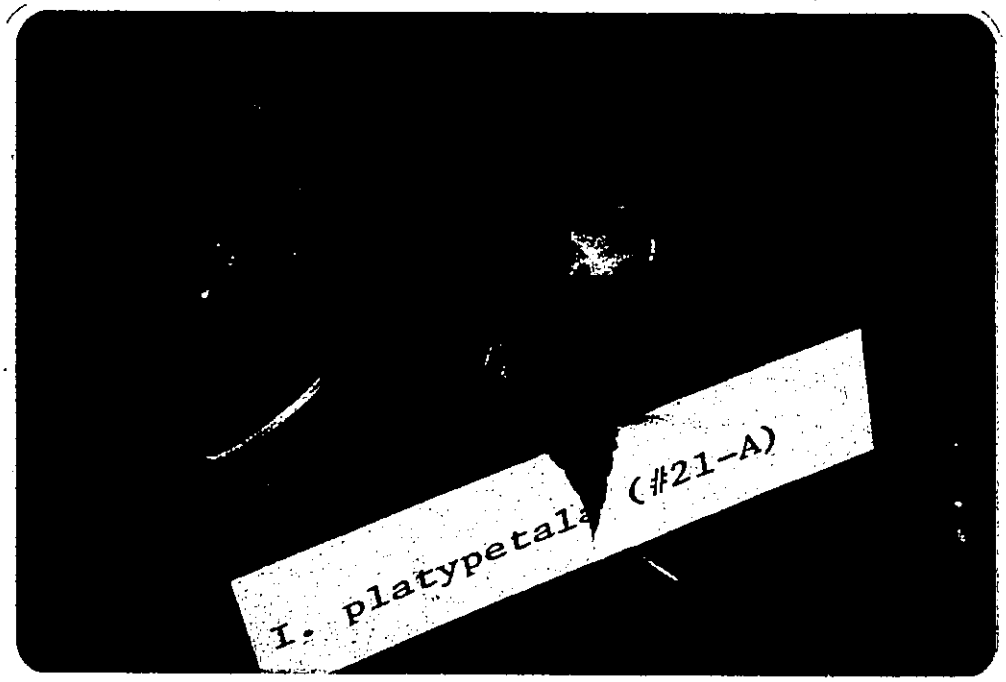


Plate 4 Vegetative and floral morphology of I. platypetala. (#21-A)

Plate 5 Vegetative and floral morphology of
I. schlechterii. (#12)

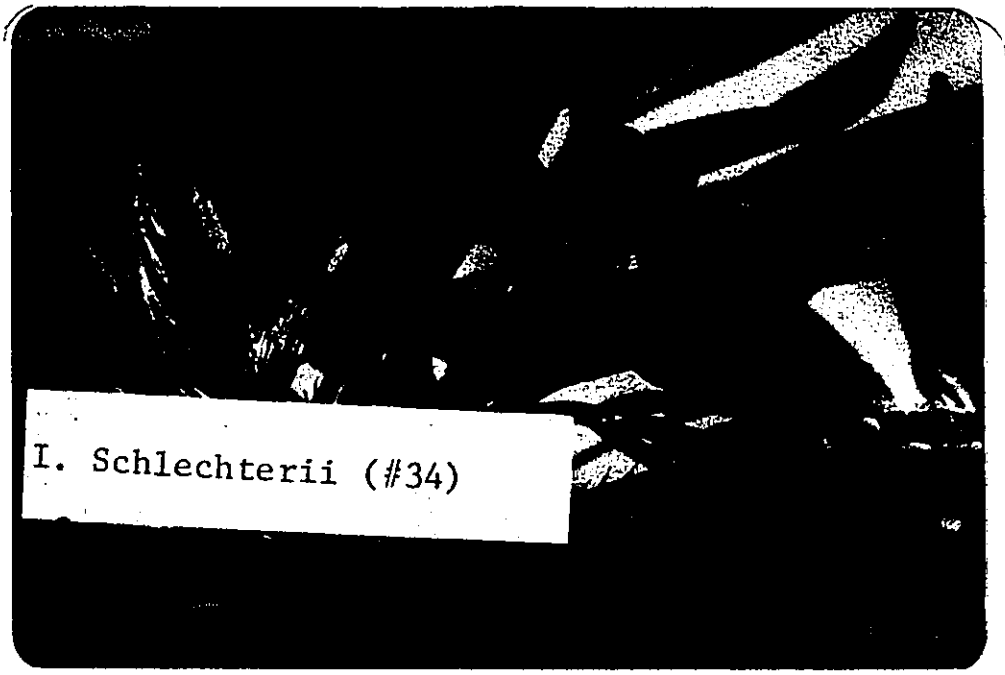


Plate 6 Vegetative and floral morphology of
I. schlechterii. (#34)

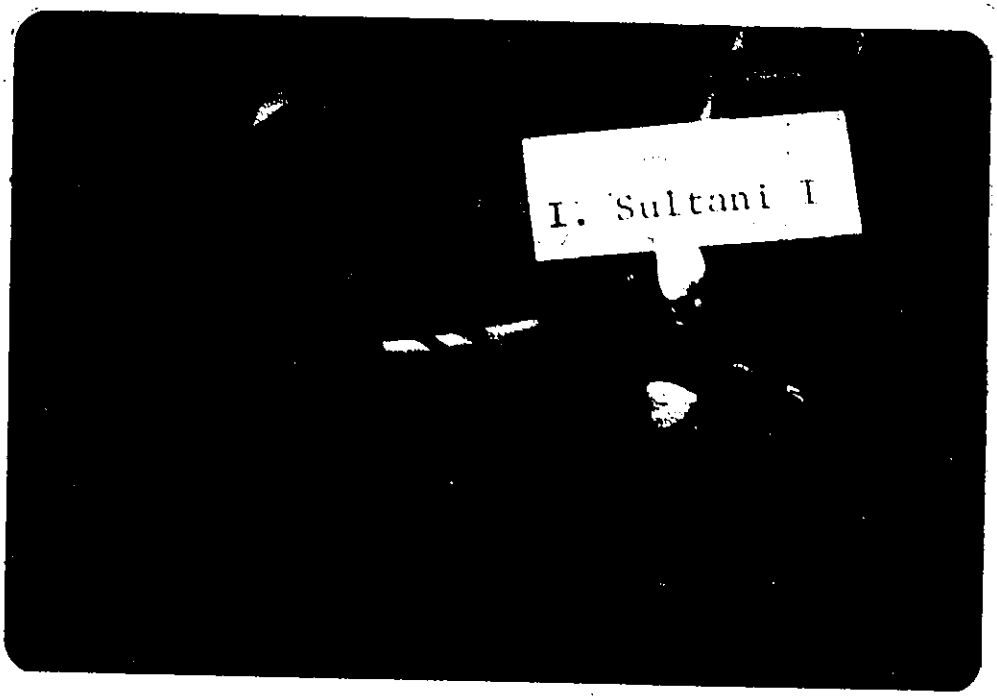


Plate 7 Vegetative and floral morphology of I. sultani (I)

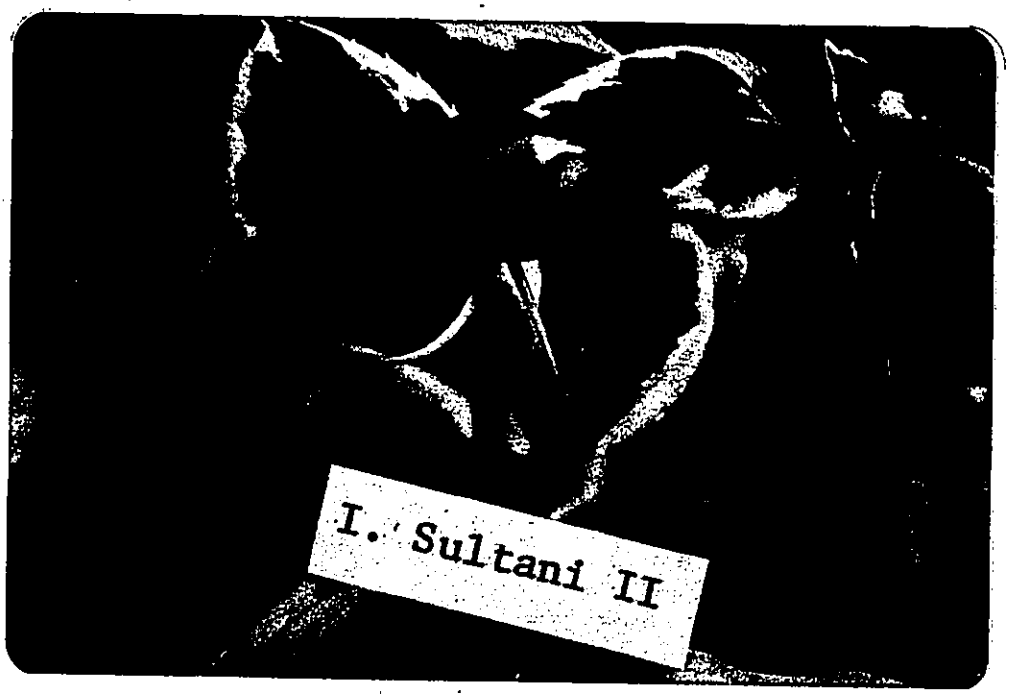


Plate 8 Vegetative and floral morphology of I. sultani (II)

Pigmented cells in stem cross sections cut at the equivalent stage of development were localised in the epidermis only as in I. balfouri (Plate 11) or epidermis and cortex as in I. balsamina and the cultivar arabesque. Pigmented cells scattered in the epidermis, cortex and near vascular bundles were typical of I. linearifolia and I. schlechterii (Plate 12 &13) whereas localisation in the epidermis, cortex and central pith region as found in I. platypetala and cultivar Sweetsue (Plate 14). Pigments were found all over in the epidermis, across the cortex, near the vascular bundles and in the central pith in I. holstii, I. sultani and cultivar hybrid (Plate 15).

(iii) Leaves

Pigmented cells in cross sections of petioles of most of the species were present in epidermis, cortex and near the central vascular bundles (Plate 16) for example, in I. platypetala, I. schlechterii and I. sultani. Pigmented cells present in the epidermis and near the vascular bundles were typical of I. holstii and their presence only in the cortex and near the vascular bundles was characteristic of the cultivar Arabesque, whereas pigmented cells could be found scattered in the epidermis and upper cortex as in I. linearifolia (Plate 17) or in the epidermis and across the cortical region as in Sweetsue.

Cross sections of the leaf blades of some species were also observed and pigmented cells were found to be localised in both the epidermis and in mesophyll tissue, for example, I. linearifolia (no. 18), I. platypetala (no. 21-A), I. schlechterii, I. sultani and cultivar Sweetsue.

Plate 9 Cross Section of I. balsamina root,
showing pigmented cells all over the section.

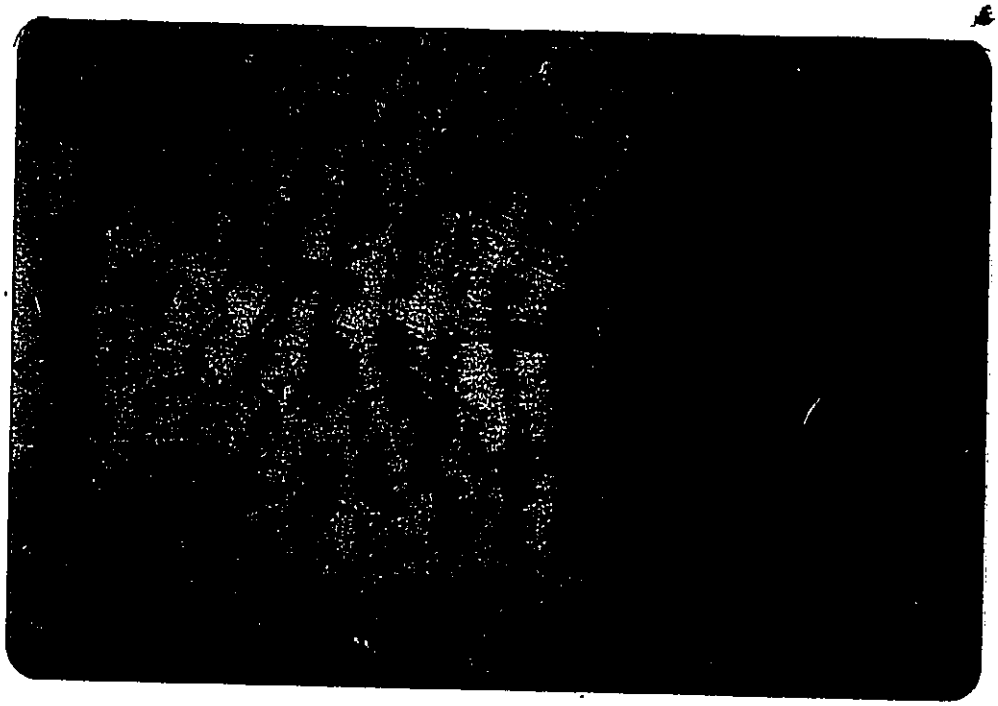


Plate 10 Cross Section of I. holstii root,
showing pigmented cells in central
stele region only.

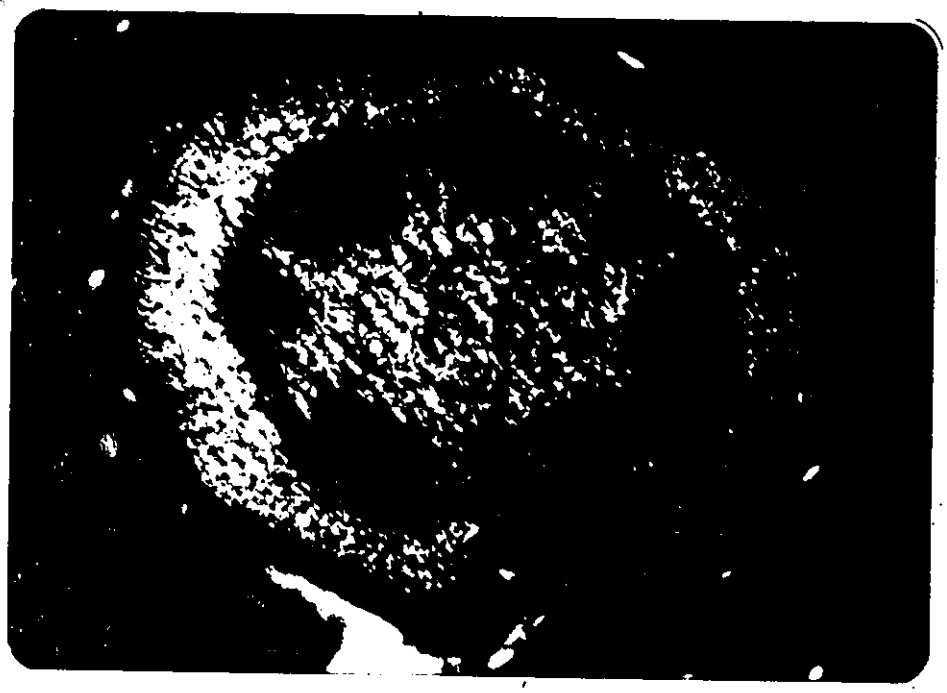


Plate 11 Cross Section of I. balfourii stem at the 5th internode showing pigment only in epidermal region.

Plate 12 Cross Section of I. linearifolia (#18) stem at the 5th internode showing pigment in epidermis, upper cortex and among vascular bundles.

Plate 13 Cross Section of I. schlechterii (#34) stem at the 5th internode showing the same localisation of pigments as I. linearifolia (#18).

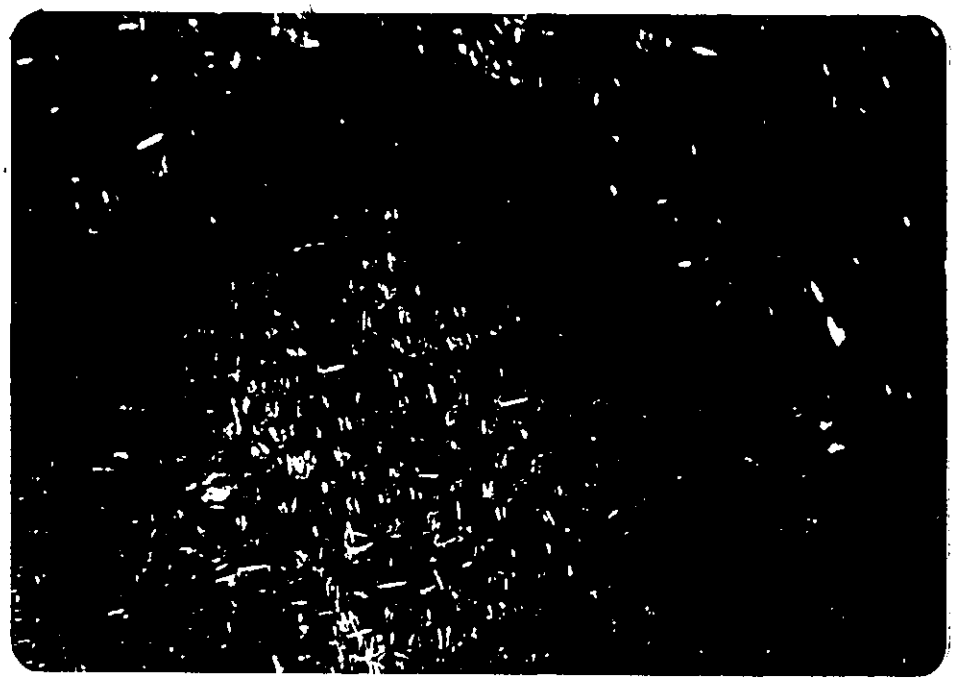


Plate 14 Cross Section of I. platypetala stem at the 5th internode showing pigmented cells in the epidermis, and scattered across both the cortex and pith.

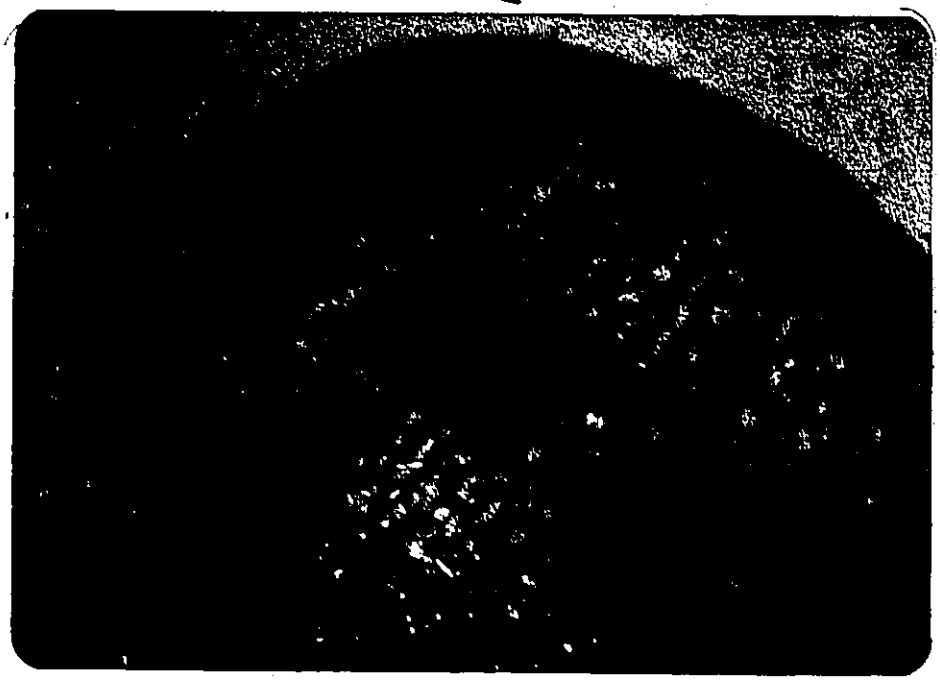


Plate 15 Cross Section of I. sultani (I) stem at the 5th internode showing pigmented cells present all over in the epidermis, in the cortex, near vascular bundles and pith.

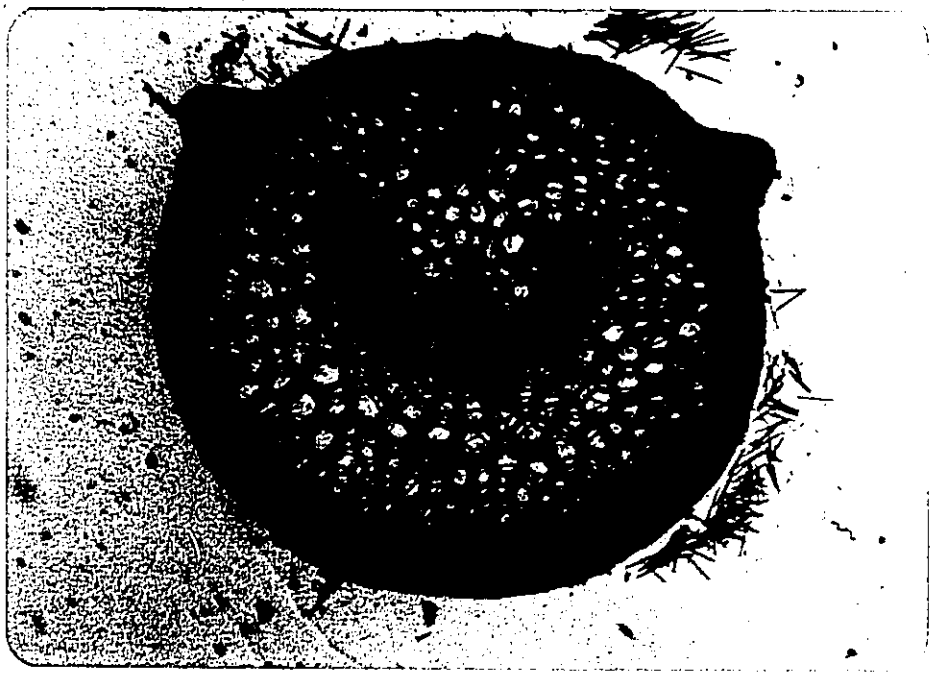


Plate 16 Cross Section of I. schlechterii (#34) leaf petiole showing pigment localised in the subepidermis, outer cortex, and near the central vascular bundle.

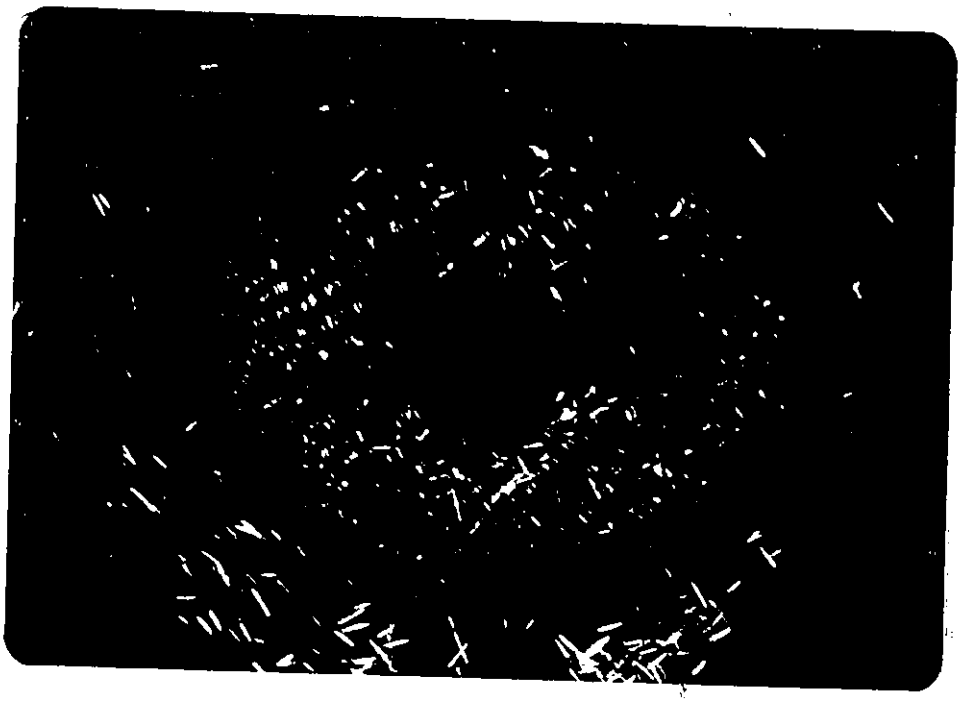


Plate 17 Cross Section of I. linearifolia (#18) leaf petiole with pigmented cells localised in the subepidermis and upper cortex.

III. Analysis of Pigments

A. Identification of pigments used as standards

Several pigments isolated from vegetative and floral tissues of seven taxa were identified more or less rigorously as glycosides of pelargonidin, aurantinidin, cyanidin, peonidin; delphinidin and malvidin (Tables 3 & 4).

(i) From chromatographic behaviour

Pigments glycosylated in the 3-position were differentiated from 3,5 diglycosides by the fluorescence of the latter in UV light (Harborne, 1967), and their relative mobilities, for example, 3,5 diglycosides have lower Rf values in alcoholic and higher Rf values in acidic solvents as compared to 3-glycosides.

(ii) From spectra

The spectra maxima of 3, and 3,5 diglycosides are similar but differ in intensity in the 400-460 nm region, 3-glycosides showing a shoulder or inflection in the 400-460 nm region. The nature of the sugar molecule has no effect on the spectra (Harborne, 1967). Acylated pigments were identified by the presence of an extra peak in the 280-335 nm region. Typical spectra of a monoglycoside of Pg, 3,5-glycosides of Pg and Mv and acylated glycosides of Pg and Mv and Au-glycosides are presented in Figures 3-8.

(iii) From aglycone and sugar moieties produced by acid-hydrolysis

(a) Aglycone

Identification of anthocyanidins is facilitated by the relationship between Rf values and number of hydroxyl or methoxyl

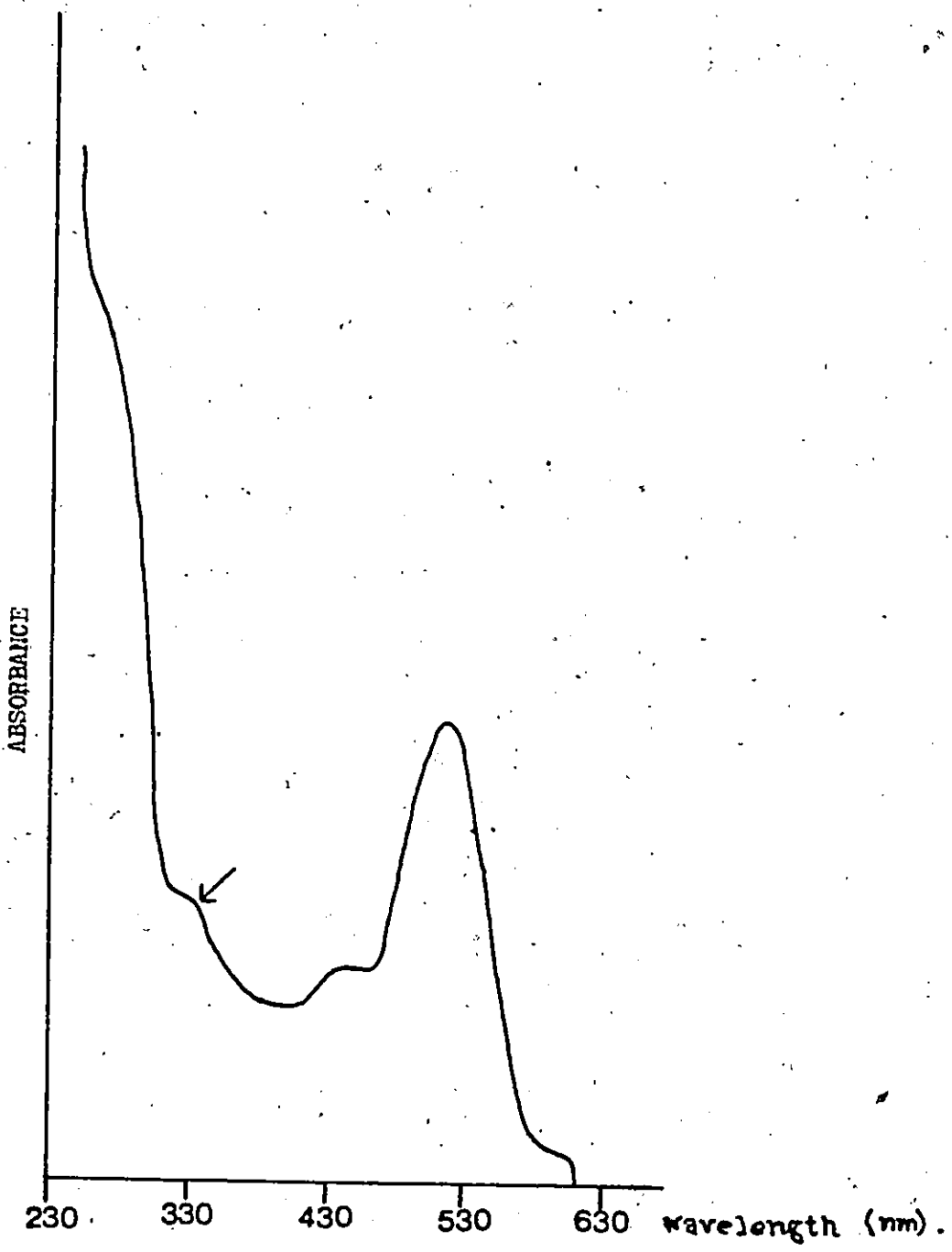


Fig 3 Characteristic spectrum of Pg-monoglycoside 'B' isolated from I. schlechterii flower extract.

→ machine error at 220 nm

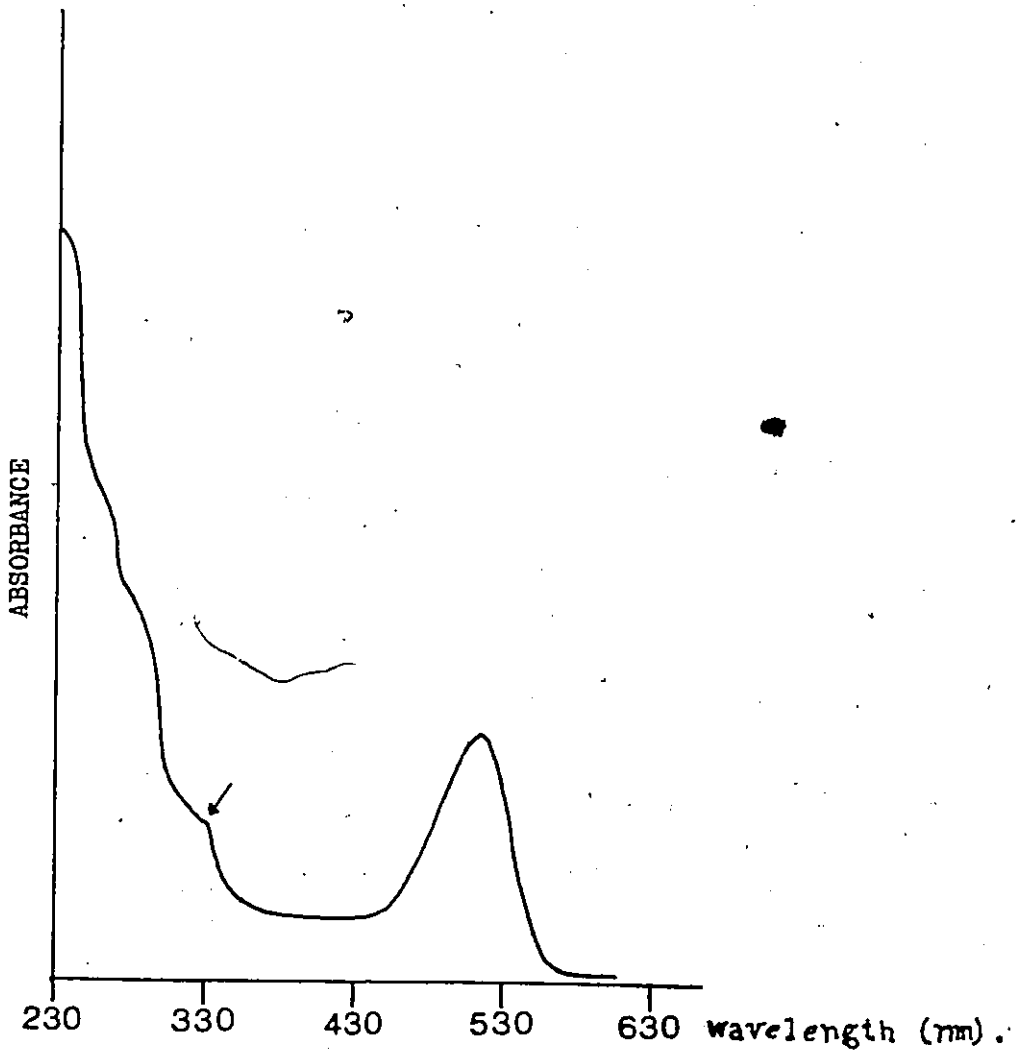


Fig 4 Characteristic spectrum of Pg-3,5 diglycoside isolated from I. schlechteri flower extract.

→ machine error at 330 nm

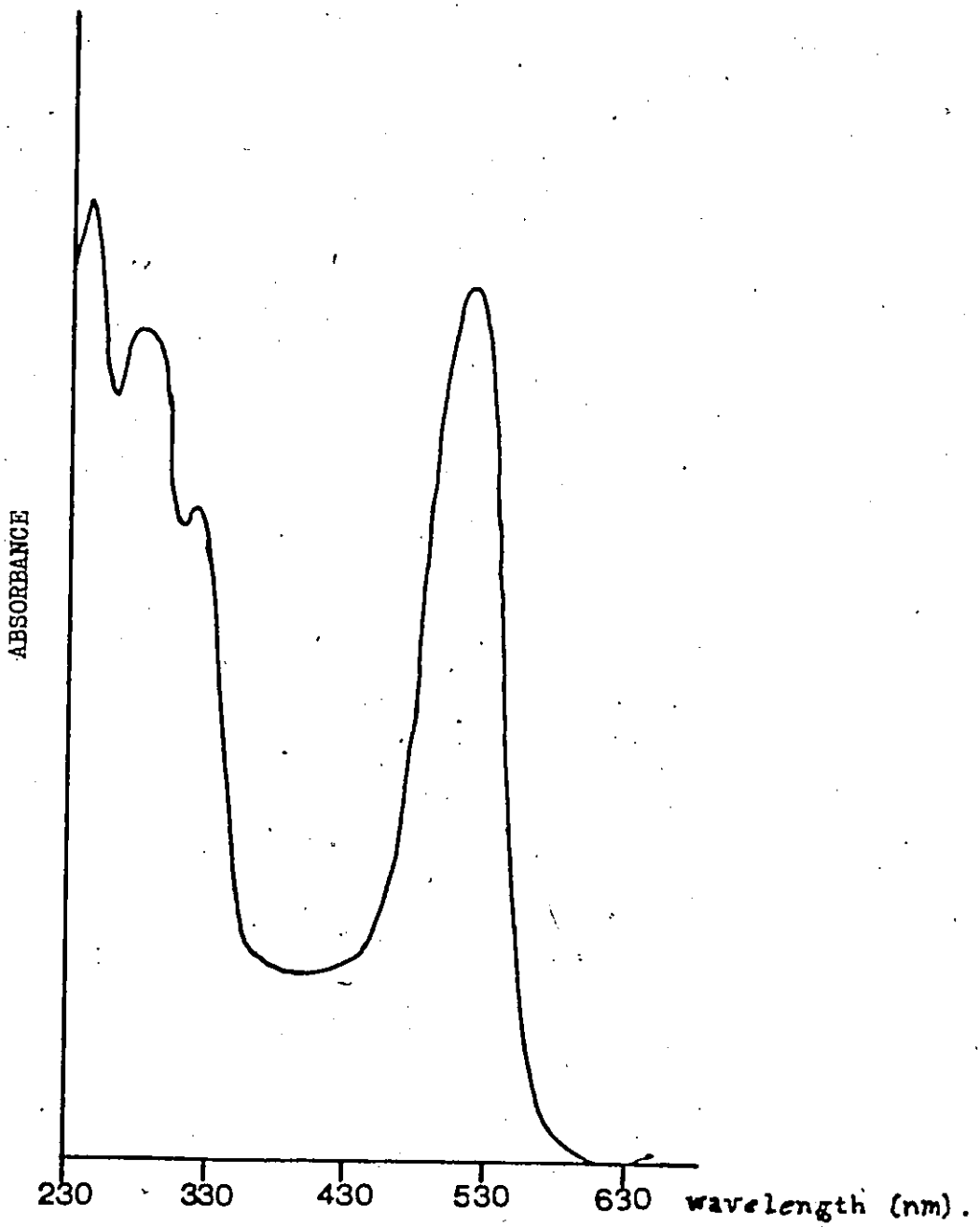


Fig 5 Characteristic spectrum of Pg-glycoside acylated isolated from I. *herzogii* flower extract.

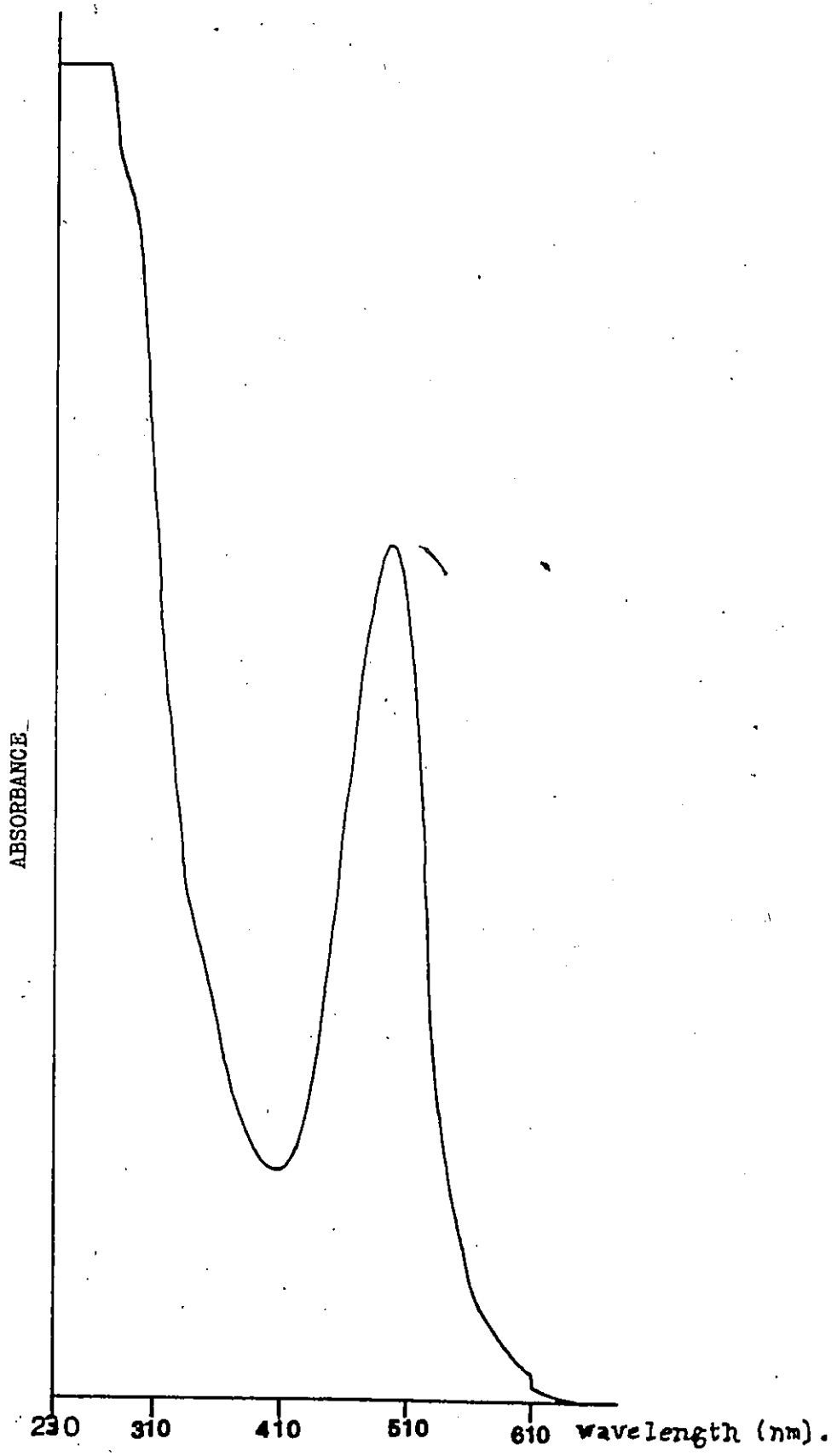


Fig 6 Characteristic spectrum of Au-glycoside isolated from hybrid flower extract.

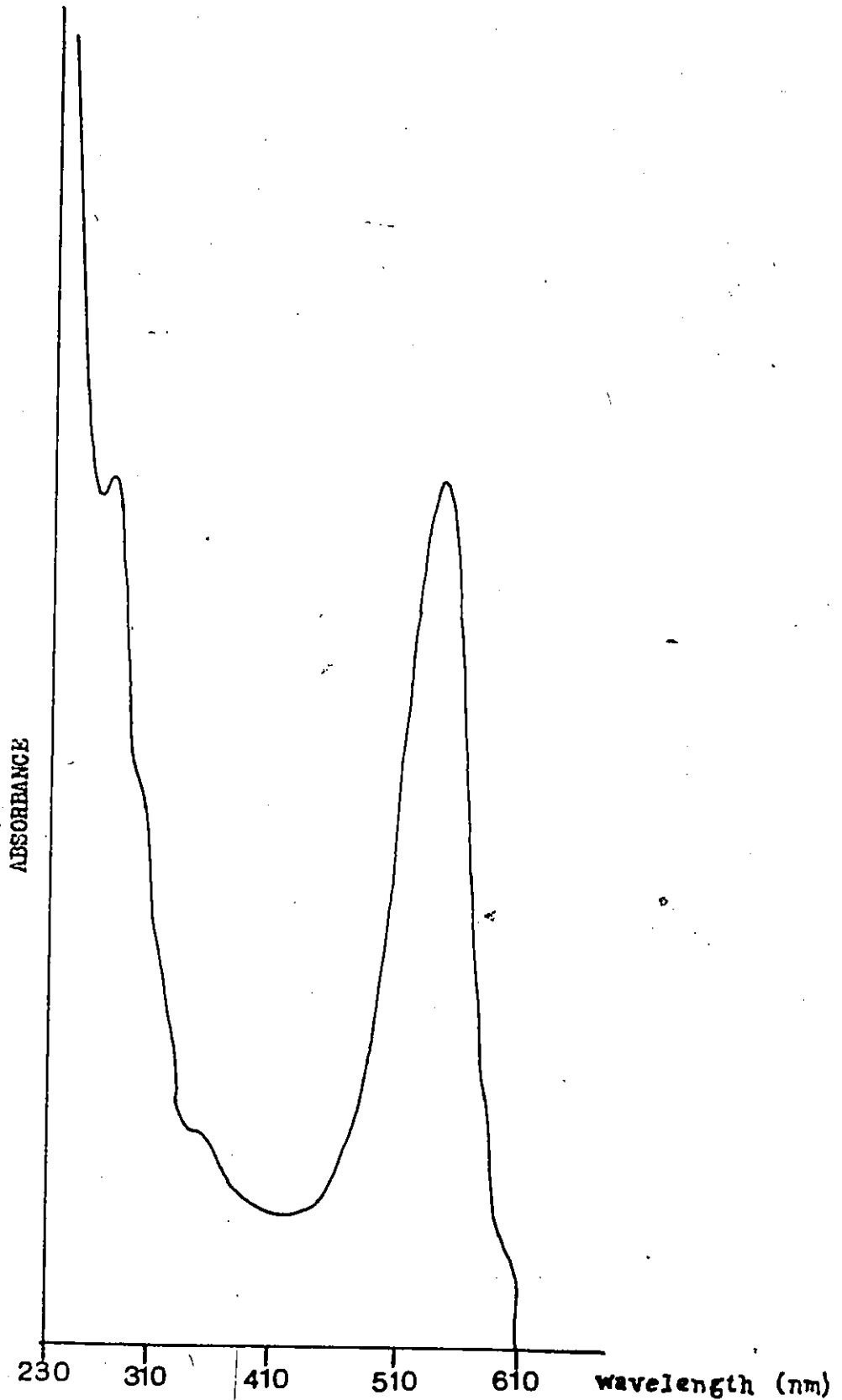


Fig 7 Characteristic spectrum of Mv-3,5 diglycoside isolated from I. platypetala flower extract.

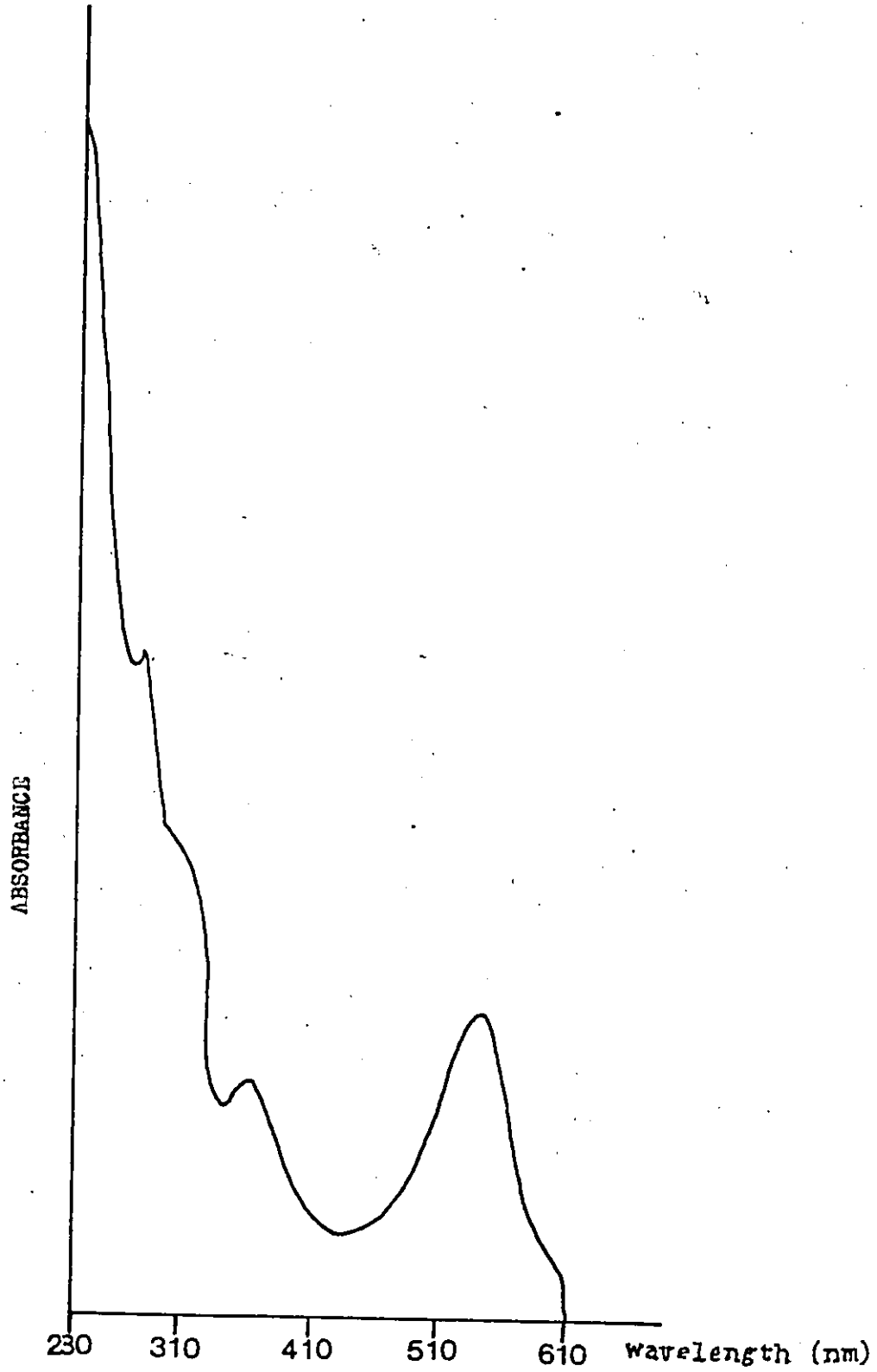


Fig 8 Characteristic spectrum of Mv-3-glycoside acylated isolated from I. platypetala flower extract.

Table 3. Chromatographic and spectral properties of pigments from vegetative tissues identified and used as standards

Pigment Bands*	Rf Values X 100 in			Color in±		Spectral Characters		Aglycone	Sugar	Tentative Identification@
	BAW	1% HCl	HOAC-HCl	vis	UV	MeOH-Cl λmax(nm)	Shift from EtOH-Alcl$_3$ Δλ(nm)			
From I. platypetala stem and leaves										
I	33	06	16	Ppk	Pabs.	535,285	21	Dp#	glucose rhamnose	Dp-3 bioside
II	46	06	25	Ppk	or. r. abs.	532,410, 285	18	Cy	glucose	Cy-3, mono glucoside
III	28	11	28	Ppk	or. r. abs.	532,285	18	Cy	glucose rhamnose	Cy-3 bioside
IV	21	22	53	Ppk	or. r. abs.	532,285	18	Cy	glucose xylose arabinose	Cy-3, trioside
from I. sultani I leaves										
I	17	05	24	P	or. r. abs.	540,280	0	Mv	glucose arabinose	Mv-3 bioside

* Pigment bands were those from chromatogram developed in HOAC-HCl solvent descendingly.

± Color abbreviations: P = purple, pk = pink, or. r. = orange-red abs. = absorbing.

Dp= delphinidin, Cy= cyanidin, Mv= malvidin.

@ Because the solvent system, BuHCl (Harborne, 1967), was not routinely used in these studies, the possibility that some acylated pigments were also present cannot be excluded.

Table 4. Chromatographic and spectral properties of pigments from floral tissues

Pigment Bands*	Rf Values X100 in				Color in		Spectral characters		Tentative Identification
	BAW	Bu:Hcl	1% Hcl	HoAc-Hcl	vis.	UV	MeOH-Hcl λ_{max} (nm)	Shift with EtOH-Alcl$_3$ $\Delta\lambda$ (nm)	
From I. herzogii									
I	14	-	23	27	or.y.	y.flt.	510,270	0	Pg-glycoside 'F'
II	16	-	25	48	or.y.	y.flt.	508,365	0	Pg-glycoside 'H'
III	32	-	27	49	or.	y.abs.	508,410	0	Pg-glycoside 'I'
IV	35	-	26	54	or.y.	Bt.y.flt.	510,310, 280	0	Pg-3,5 diglycoside acylated 'D'
V	54	-	12	53	or.	y.flt.	510,435, 270	0	Pg-3, monoglycoside 'C'
VI	58	-	21	65	or.	Bt.y.	510,316, 276	0	Pg-3,5 diglycoside acylated 'E'
From I. schlechterii									
1. (no. 5)	32	15	53	70	or.	or.abs.	522,430, 270	0	Pg-3, monoglycoside 'B'
2. (no. 8,12,23,&34)	06	-	41	55	or.y.	y.abs.	515,270	0	Pg-glycoside 'K'
3. (no. 8,12,23,&34)	10	-	18	48	or.y.	y.abs.	512,270	0	Pg-glycoside 'L'
4. (no. 8,10,11,12,23&34)	13	-	26	50	or.y.	y.flt.	510,270	0	Pg-glycoside 'M'
From I. sultani									
1. (I & III)	22	05	26	50	or.y.	y.flt.	522,270	0	Pg-glycoside 'A'
2.	34	-	25	48	or.pk.	y.abs.	510,270	0	Pg-glycoside 'J'

4. (no. 8,10,11,12,23&34)	13	-	26	50	or.y.	y.flt.	510,270	0	Pg-glycoside 'H'
From I. sultani									
1. (I & III)	22	05	26	50	or.y.	y.flt.	522,270	0	Pg-glycoside 'A'
2.	34	-	25	48	or.pk.	y.abs.	510,270	0	Pg-glycoside 'J'
From I. sultani									
(III & IV)	21	-	21	53	or.pk.	or.y.flt.	516,280	0	Pg-glycoside 'G'
From Hybrid									
(Tangerine x 366029)	-	05	42	64	or.	abs.	491,350	0	Au-glycoside
From I. capensis	08	-	13	29	p.pk.	or.r.abs.	530,280	18	Cy-glycoside
From I. holstii									
I	21	12	15	52	Ma	or.y.flt.	526,275	0	Pn-3,5 diglycoside
II	42	51	13	55	Ma	Bt.or.y. flt.	526,310, 270	0	Pn-3,5 diglycoside acylated
From I. platypetala									
(no.21-B)									
I	40	04	20	34	Ma	or.flt.	540,310	0	Mv-3,5 diglycoside acylated
II	61	02	25	45	Ma	or.r.	540,280	0	Mv-3,5 diglycoside
III	65	08	17	38	P	Bt.or.r.	540,310, 275	0	Mv-3, glycoside acylated
From I. platypetala									
(no. 21-A and 21-D)	36	-	02	09	Ppk.	or.abs.	546,280	0	Mv-glycoside

* Pigment bands listed are from chromatograms developed in BAW solvent, descendingly.

Color abbreviations: or.y.= orange-yellow, y. = yellow, Ma = magenta, Ppk = purple-pink, or.r. = orange red, P = purple abs. = absorbing, Bt. = bright, flt. = fluorescent.

groups present (Fig. 9). An increase in hydroxylation decreases Rf, whereas an increase in methoxylation increases Rf in an aqueous solvent such as Forestal. Anthocyanidins with free O-dihydroxylic groups in the B-ring show bathochromic shifts of 18-35 nm in visible maxima in the presence of aluminum chloride at pH 2 to 4. The six aglycones Pg, Au, Cy, Pn, Dp and Mv, thus identified are listed in Table 5. Most were separated through use of Forestal, BAW and formic acid solvents but it was necessary to use tert-butanol-2N-HCl-HOAC-H_2O (6:1:1:2 v/v) solvent (Clevenger, 1971) to differentiate peonidin from cyanidin.

(b) Sugars

With reagent AHP, sugars give characteristic color reactions in visible light: hexoses (glucose, galactose and a methyl-pentose, rhamnose) a brown color and pentoses (xylose and arabinose) a pink color.

Five major recurring phenolic compounds identified only by color in UV light (yellowish green and purple blue) and approximate Rf values in two solvents (Table 6) were observed on chromatograms.

B. Pigments in vegetative tissues

(i) Anthocyanins

The distribution of various glycosides in stems and leaves is presented in Table 7.

Cyanidin, 3-bioside was the most common pigment, being present in approximately 80% of species and varieties examined. Cyanidin 3-mono and 3-triosides were also present in about 35% of taxa. All three glycosides of cyanidin occurred together in stem and leaves of I. linearifolia & I. platypetala and in leaves of I.

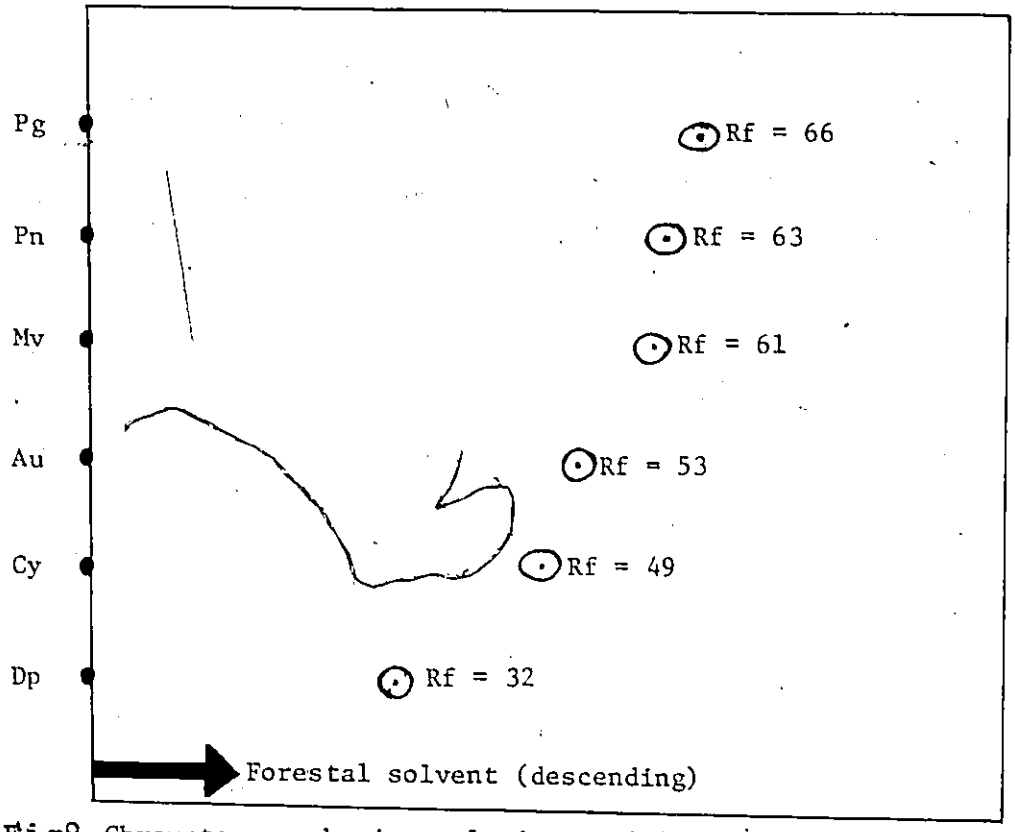


Fig9 Chromatogram showing relative positions of different anthocyanidins

Pg= pelargonidin
Pn= peonidin
Mv= malvidin
Au= aurantinidin
Cy= cyanidin
Dp= delphinidin

Table 5. Chromatographic and spectral properties of anthocyanidins obtained from hydrolysis of purified anthocyanins and of acyanic tissues of Impatiens species.

Pigments	Rf values (X100) in Forestal	Formic acid	BAW	Color in ± Vis light	UV	MeOH-HCl λmax. (nm)	Shift with EtOH-AlCl$_3$ Δλ (nm)
Unknowns							
1)	69	30	78	or.r.	y.	520,270	0
2)	52	23	58	or.r.	abs.	499,285	0
3)	49	21	52	Ma.	or.	536,275	18
4) •	63	28	71	Ma.	or.y.	532,275	0
5)	33	12	31	P.	or.r.	546,275	21
6)	59	26	49	P.	or.r.	542,280	0
Standards							
Pelargonidin	68*	32	79	or.r.	y.	520,270	0
	68*	33	80	r.		520,270	0
Aurantinidin	standard not available						
	53**	24	52	or.r.		499,286	0
Cyanidin	49*	21	51	Ma.	or.	536,275	18
	49**	22	68	Ma.		535,277	18
Peonidin	standard not available						
	63**	30	71	Ma.		532,277	0
Delphinidin	32*	13	31	P	or.r.	546,275	21
	32**	13	42	P		546,277	23
Malvidin	58*	27	52	P	or.r.	542,280	0
	60**	27	58	P		542,275	0

* Rf values are those of standards run along with unknowns simultaneously.

** Rf values of standards as in the literature (Harborne, 1967).

• The Rf value of peonidin was 54 in tert butanol-2N Hcl-HOAc-H_2O (6:1:1:2, v/v/v/v) solvent system (Clevenger, 1971)

± color abbreviations: abs. = absorbing, Ma = Magenta, or = orange, P = purple, r = red, y = yellow

Table 6. Chromatographic properties of other flavonoids

Pigment	Rf values X 100 in		Color in±	Designation
	BAW	HOAc-HCl	UV	
1.	(60-70)	(10-15)	y. gr.	Other flavonoid I
2.	(30-35)	(20-25)	y. gr.	Other flavonoid II
3.	(35-40)	(40-50)	Pu	Other flavonoid III
4.	(5-10)	(60-65)	y. gr.	Other flavonoid IV
5.	(80-90)	(75-80)	PuBl. flt.	Other flavonoid V

± color abbreviations: y. gr. = yellow green
Pu = Purple
PuBl. = Purple-Blue
flt. = fluorescent

TABLE 7. PIGMENTS OBSERVED IN VEGETATIVE ORGANS

STANDARD PIGMENT	STEM														LEAVES										ROOTS	
PLANT SPECIES	Cy-3 monoglycoside	Cy-3 bioside	Cy-3 trioside	Dp-3-bioside	Mv-3-bioside	Leucopelargonidin	Leucocyanidin	Leucodelphinidin	Leucomalvidin	other flavonoid I	other flavonoid II	other flavonoid III	other flavonoid IV	other flavonoid V	Cy-3 monoglycoside	Cy-3 bioside	Cy-3 trioside	Dp-3 bioside	Mv-3 bioside	Leucopelargonidin	Leucocyanidin	Leucodelphinidin	Leucomalvidin	other flavonoid I	other flavonoid II	other flavonoid III	other flavonoid IV	other flavonoid V	Anthocyanins	Leucoanthocyanidin
1. I. balfourii Hook F.																													
1	2	3	0	0	0	0	2	0	0	2	1	1	2	1	0	0	0	0	0	0	2	0	0	0	1	1	2	1	1	3
2	2	3	0	0	0	0	2	0	0	2	1	1	2	1	0	0	0	0	0	0	2	0	0	0	1	1	2	1	1	3
3	2	3	0	0	0	0	2	0	0	2	1	1	2	1	0	0	0	0	0	0	2	0	0	0	1	1	2	1	1	3
2. I. balsamina L.*																													
1 1 H H p^F p^F var.	2	0	0	1	0	1	3	0	0	2	0	2	1	2	0	0	0	0	0	1	2	1	0	2	0	3	1	2	2	2
white flowered var.	0	0	0	0	0	0	2	0	0	0	2	2	1	2	0	0	0	0	0	1	2	1	0	0	2	2	2	3	0	2
3. I. capensis Meerb.																													
Local	0	2	0	0	0	0	2	0	0	1	0	2	3	0	0	1	0	0	0	0	2	1	0	2	1	2	1	1	1	3
N.Y.	1	2	0	0	0	0	2	0	0	2	0	2	3	1	0	1	0	0	0	0	3	1	0	2	1	3	2	3	-1	-1
Frankfort	1	2	0	0	0	0	2	0	0	2	0	3	3	2	0	1	0	0	0	0	3	1	0	2	1	3	2	3	-1	-1
4. I. linearifolia Warb.																													
P.I. 354263 (#18)	2	3	1	2	0	0	2	1	0	1	1	2	3	2	2	3	1	2	0	0	2	1	0	1	1	2	3	2	1	2
P.I. 354266 (#19)	2	3	1	2	0	0	2	1	0	1	1	2	3	2	2	3	1	2	0	0	2	1	0	1	1	2	3	2	1	2
P.I. 354267 (#20)	2	3	1	2	0	0	2	1	0	1	1	2	3	2	2	3	1	2	0	0	2	1	0	1	1	2	3	2	1	2
5. I. platypetala Lindl.																													
P.I. 349629 A (#21-A)	2	3	1	1	0	0	2	1	0	1	1	2	2	2	2	3	1	1	0	0	2	1	0	3	2	1	2	3	1	2
P.I. 349629 B (#21-B)	2	3	1	1	0	0	3	1	0	1	1	2	2	2	2	3	1	1	0	0	2	2	0	3	2	1	2	3	2	2
P.I. 349629 D (#21-D)	2	3	1	1	0	0	2	1	0	1	1	2	2	2	2	3	1	1	0	0	2	1	0	3	2	1	2	3	1	2
6. I. Schlechterii Warb.																													
P.I. 354254 (#5)	0	1	1	0	0	0	2	0	0	0	2	0	2	1	0	0	0	0	0	0	2	0	0	1	2	1	2	3	2	1
P.I. 354257 (#8)	0	3	1	1	0	0	3	1	0	2	0	0	2	2	0	3	0	0	0	1	3	0	0	2	0	2	1	0	1	2
P.I. 354261 (#10)	0	3	0	1	0	0	3	1	0	0	2	2	1	1	0	3	0	0	0	0	2	0	0	2	0	3	2	0	1	3
P.I. 354264 (#11)	0	3	0	1	0	0	3	1	0	2	1	2	2	2	0	3	0	0	0	0	2	0	0	2	0	3	2	0	1	2
P.I. 354265 (#12)	0	3	0	1	0	0	3	1	0	2	1	2	2	2	0	3	0	0	0	1	2	0	0	2	0	3	2	0	1	2
P.I. 354259 (#23)	1	3	0	0	0	1	3	0	0	1	0	2	2	1	1	3	0	0	0	1	3	0	0	2	0	1	2	0	1	1
P.I. 367705 (#34)	2	3	0	1	0	0	2	0	0	2	0	2	3	2	2	3	0	0	0	0	2	0	0	2	0	1	3	0	1	3
7. I. Bultani Hook F.																													
I Scarlet flowered var.	0	2	0	0	2	0	2	1	0	0	0	1	2	2	1	3	1	0	1	0	2	2	0	2	0	2	1	2	1	2
II Magenta flowered var.	0	2	0	0	1	0	2	1	0	1	1	1	2	0	0	1	0	0	1	0	2	1	0	2	1	2	1	0	1	2
III Red & White flowered var.	1	1	0	0	2	0	2	1	0	2	1	0	1	1	0	1	0	0	1	0	2	1	0	2	1	1	2	0	1	2
IV Pink flowered var.	0	2	0	0	1	0	2	1	0	1	1	0	2	1	0	1	0	0	1	0	2	1	0	3	1	3	1	0	1	1
V White flowered var.	0	0	0	0	0	0	2	1	0	1	2	0	1	2	0	0	0	0	0	0	2	2	0	2	0	2	1	0	0	3
8. I. herzogii K. Schum	0	2	0	1	0	-1	-1	-1	-1	1	0	2	1	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
9. I. holstii Hook F.																													
1	1	0	0	0	3	0	2	1	0	0	2	1	1	3	1	2	0	0	0	0	2	2	0	3	1	2	2	3	1	2
2	1	0	0	0	3	0	2	1	0	0	2	1	1	3	1	2	0	0	0	0	2	2	0	3	1	2	2	3	1	2

P.I. 354261 (#10)	0	3	0	1	0	0	3	1	0	0	2	2	1	1	0	3	0	0	0	0	2	0	0	2	0	3	2	0	1	3
P.I. 354264 (#11)	0	3	0	1	0	0	3	1	0	2	1	2	2	2	0	3	0	0	0	0	2	0	0	2	0	3	2	0	1	2
P.I. 354265 (#12)	0	3	0	1	0	0	3	1	0	2	1	2	2	2	0	3	0	0	0	1	2	0	0	2	0	3	2	0	1	2
P.I. 354259 (#23)	1	3	0	0	0	1	3	0	0	1	0	2	2	1	1	3	0	0	0	1	2	0	0	2	0	1	2	0	1	1
P.I. 367705 (#34)	2	3	0	1	0	0	2	0	0	2	0	2	3	2	2	3	0	0	0	0	2	0	0	2	0	1	3	0	1	3
7. I. Sultani Hook F.																														
I Scarlet flowered var.	0	2	0	0	2	0	2	1	0	0	0	1	2	2	1	1	1	0	2	0	2	2	0	2	0	2	1	2	1	2
II Magenta flowered var.	0	2	0	0	1	0	2	1	0	1	1	1	2	0	0	1	0	0	1	0	2	1	0	2	1	2	1	0	1	2
III Red & White flowered var.	1	1	0	0	2	0	2	1	0	2	1	0	1	1	0	1	0	0	1	0	2	1	0	2	1	1	2	0	1	2
IV Pink flowered var.	0	2	0	0	1	0	2	1	0	1	1	0	2	1	0	1	0	0	1	0	2	1	0	3	1	3	1	0	1	1
V White flowered var.	0	0	0	0	0	0	2	1	0	1	2	0	1	2	0	0	0	0	0	0	2	2	0	2	0	2	1	0	0	3
8. I. herzogii K. Schum	0	2	0	1	0	-1	-1	-1	-1	1	0	2	1	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
9. I. holstii Hook F.																														
1	1	0	0	0	3	0	2	1	0	0	2	1	1	3	1	2	0	0	0	0	2	2	0	3	1	2	2	3	1	2
2	1	0	0	0	3	0	2	1	0	0	2	1	1	3	1	2	0	0	0	0	2	2	0	3	1	2	2	3	1	2
3	1	0	0	0	3	0	2	1	0	0	2	1	1	3	1	2	0	0	0	0	2	2	0	3	1	2	2	3	1	2
4	1	0	0	0	3	0	2	1	0	0	2	1	1	3	1	2	0	0	0	0	2	2	0	3	1	2	2	3	1	2
10. I. pallida Hutt** CULTIVARS	0	2	0	0	0	0	2	0	0	2	0	2	3	3	0	1	0	0	0	0	2	1	0	2	1	3	2	3	-1	-1
11. 'A' flame	1	0	2	0	0	0	2	1	0	2	0	1	0	2	0	1	0	0	0	0	2	1	0	0	1	0	2	3	1	1
12. Aloha	0	2	0	0	0	0	2	0	0	2	0	2	1	0	0	0	0	0	0	0	2	0	0	2	0	1	2	3	-1	-1
13. Arabesque	2	3	0	1	0	0	2	0	0	2	0	1	2	0	2	2	0	1	3	0	2	1	0	2	0	0	2	2	1	3
14. Cheers	0	0	0	0	0	0	2	1	0	1	0	2	1	0	0	0	3	1	0	0	2	1	0	2	0	0	1	0	1	4
15. Hybrid (Tangerine X P.I. 366029)	0	0	2	0	0	0	2	0	0	2	0	1	1	0	0	0	0	0	0	0	2	0	0	2	1	1	0	3	1	5
16. Sweetsue	0	1	3	2	0	0	2	0	0	2	1	0	1	0	0	1	3	1	0	0	2	2	0	2	1	1	0	2	1	2
Unidentified Species																														
17. P.I. 349582 (#1)	0	1	0	1	0	0	2	1	0	0	1	1	0	2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
18. P.I. 349582-A (#1-A)	0	1	0	1	0	0	2	0	0	0	1	1	0	2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	2
19. P.I. 349583 (#2)	0	2	0	0	0	0	2	0	0	0	0	0	1	2	-1	-1	-1	-1	-1	0	0	0	-1	-1	-1	-1	-1	-1	-1	-1
20. P.I. 349586 (#3)	0	1	0	1	0	0	2	0	0	1	0	1	2	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
21. P.I. 349585 (#6)	0	3	2	2	0	0	2	0	0	1	0	1	2	0	0	2	2	0	2	0	2	2	0	2	0	1	2	2	-1	-1
22. P.I. 349584 (#10)	0	1	0	1	0	0	2	0	0	1	0	1	0	2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

* only I. holstii had Fg-3 glycoside in stem.

Numbers indicate approximate quantity, 0 = absent or not detectable, 1 = in trace amount.

2 = in poor amount, 3 = in fair amount, 4 = in good amount, 5 = in very good amount, -1 = not analyzed (see Table 1)

** I. pallida Hutt was tentatively identified on the basis of flower color and angle and length of spur.

** See footnote to Table 3 re acylated pigments

sultani, whereas only cyanidin-trioside was present in leaves of Hybrid.

Of other aglycones, delphinidin and malvidin were found as biosides only in about 40% and 15% of plants, respectively. Delphinidin and malvidin always occurred with cyanidin but all three aglycones were present only in leaves of the cultivar Arabesque. Pelargonidin-glycoside was observed only in I. balsamina stem tissues.

(ii) Leucoanthocyanidins

Among aglycones obtained from the hydrolysis of residual tissues, cyanidin was found in stem and leaves of all the species and varieties analysed. Delphinidin was present in about 50% of the taxa. Pelargonidin was detected only in I. balsamina and I. schlechterii. no malvidin was detected.

(iii) Other flavonoids

All five compounds were present, compounds III, IV, V in a high percentage (approx. 80%) of plants, compound II at approx. 55% frequency. All five were present altogether only in three species, I. capensis, I. linearifolia and I. platypetala.

Pigments of roots were examined only in I. balsamina, I. holstii, I. linearifolia (no. 18) and I. platypetala and were found to contain a glycoside of cyanidin as the major pigment (Thakur and Nozzolillo, 1978).

C. Pigments in floral tissues

(i) Anthocyanins

The results of flower pigment analyses are presented in

Table 8, and show that the glycosides of pelargonidin were of numerous variety, 13 glycosides of which two were acylated. Glycosides 'A' 'B' 'C' and 'D' were present in about 50% of taxa and F,G,H,I,J,K,L and M in 20% of taxa. Malvidin glycosides accompanied by acylated derivatives were identified only in four species, I. balfourii, I. platypetala, I. sultani II and P.I. no. 349583 (no. 2). The 3,5 diglycoside of peonidin and its acylated derivative was detected in I. holstii and I. sultani II flower petals, whereas a glycoside of cyanidin was present only in I. capensis as a major pigment.

The glycosides of aurantinidin, a rare pigment were found in only two cultivars, Hybrid and Sweetsue, both known to be derived from Tangerine (Arisumi, pers. commun.), which is the commercial name for a horticultural variety of I. aurantiaca (Clevenger pers. commun.).

(ii) Leucoanthocyanidins

Aglycones obtained by acid hydrolysis of acyanic flower tissues were principally cyanidin, present in all of the taxa, and pelargonidin, in about 45% of the taxa examined. Delphinidin was detected only in 3 taxa, I. balfourii, I. platypetala and P.I. 349583 (no. 2) and malvidin in only two plants, I. balfourii and I. platypetala (no. 21-B).

(iii) Other flavonoids

All five compounds were present, compounds I, II, III and IV in a high percentage (approx. 80%), compound V in approx. 40% of taxa examined. Only I. capensis had all five compounds invariably present.

TABLE 8. PIGMENTS OBSERVED IN FLOWERS*

PLANT SPECIES	Pg. 3,5 diglycoside 'A'	Pg. 3 monoglycoside 'B'	Pg. 3 monoglycoside 'C'	Pg-3,5 diglycoside acylated "D"	Pg. 3,5 Diglycoside acylated "E"	Pg-glycoside"F"	Pg-glycoside "G"	Pg-glycoside "H"	Pg-glycoside "I"	Pg-glycoside "J"	Pg-glycoside "K"	Pg-glycoside "L"	Pg-glycoside "M"	Au-glycoside	Cy-glycoside	Pn-3,5 diglycoside	Pn-3,5 diglycoside acylated	Mv-3,5 diglycoside acylated	Mv-3,5 diglycoside	Mv-3, glycoside acylated	Mv-glycoside	Leucopelargonidin	Leucocyanidin	Leucodelphinidin	Leucomalvidin	other flavonoid I	other flavonoid II	other flavonoid III	other flavonoid IV	other flavonoid V
1. I. balfourii Hook F.																													
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	1	2	2	0	1	1
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	1	2	2	0	1	1
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	1	2	2	0	1	1
2. I. balcamina L.																													
1 1 H H pF pF var.	4	3	4	2	1	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	2	1
white flowered var.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	1	2	1
3. I. capensis Meerb																													
local	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	2	2	3	2
N.Y.	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Frankfort	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	1	3	2	1
4. I. linearifolia Warb.																													
P.I. 354263 (#18)	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	1	0
P.I. 354266 (#19)	2	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	2	0	1	0
P.I. 354267 (#20)	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
5. I. platypetala Lindl.																													
P.I. 349629 A (#21-A)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	3	1	0	2	1	0	2	1	1	1
P.I. 349629 B (#21-B)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	3	0	0	2	1	1	1	2	1	3
P.I. 349629 D (#21-D)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	3	1	0	2	1	0	2	1	1	4
6. I. schlechterii Warb.																													
P.I. 354254 (#5)	4	2	2	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	2
P.I. 354257 (#8)	4	2	1	1	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	2	2	0	0	1	2	2	1
P.I. 354261 (#10)	3	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2	0	0	1	3	1	2
P.I. 354264 (#11)	3	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2	0	0	1	2	0	2
P.I. 354265 (#12)	4	2	1	1	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	2	2	0	0	1	0	2	2
P.I. 354259 (#23)	3	2	1	2	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	2	2	0	0	2	0	1	0
P.I. 367705 (#34)	2	4	0	2	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	2	2	0	0	1	2	1	2
7. I. Sultani Hook F.																													
I Scarlet flowered var.	3	2	2	2	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0	1
II Magenta flowered var.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	2	0	0	0	0	2	0	0	1	1	2	0
III Red & White flowered var.	1	0	2	2	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1	0	2
IV Pink flowered var.	2	2	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	3	1	2
V white flowered var.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0	1	2	0	2
8. I. herzogii K. schum	3	2	4	3	2	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	2	1	2	2
9. I. holstii Hook F.																													
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	0	0	0	0	0	2	0	0	2	1	2	2
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	0	0	0	0	0	2	0	0	2	1	2	2

local	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	2	2	3	2	1
N.Y.	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Frankfort	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	1	3	2	1	1
4. I. linearifolia Warb.																														
P.I. 354263 (#18)	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	1	0	0
P.I. 354266 (#19)	2	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	2	0	1	0	0
P.I. 354267 (#20)	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
5. I. platypetala Lindl.																														
P.I. 349629 A (#21-A)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	3	1	0	2	1	0	2	1	1	1	3
P.I. 349629 B (#21-B)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	3	0	0	2	1	1	1	2	1	3	0
P.I. 349629 D (#21-D)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	3	1	0	2	1	0	2	1	1	4	0
6. I. schlechterii Warb.																														
P.I. 354254 (#5)	4	2	2	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	2	0
P.I. 354257 (#8)	4	2	1	1	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	2	2	0	0	1	2	2	1	2
P.I. 354261 (#10)	3	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2	0	0	1	3	1	2	0
P.I. 354264 (#11)	3	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2	0	0	1	2	0	2	0
P.I. 354265 (#12)	4	2	1	1	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	2	2	0	0	1	0	2	2	3
P.I. 354259 (#23)	3	2	1	2	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	2	2	0	0	2	0	1	0	2
P.I. 367705 (#34)	2	4	0	2	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	2	2	0	0	1	2	2	2	2
7. I. Sultani Hook F.																														
I Scarlet flowered var.	3	2	2	2	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0	1	2
II Magenta flowered var.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	2	0	0	0	0	2	0	0	1	1	2	0	2
III Red & White flowered var.	1	0	2	2	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1	0	2	2
IV Pink flowered var.	2	2	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	2	2	2
V White flowered var.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	3	1	1	1
8. I. herzogii K. schum	3	2	4	3	2	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0	1	2	0	2	2
9. I. holstii Hook F.																														
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	0	0	0	0	0	2	0	0	2	1	2	2	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	0	0	0	0	0	2	0	0	2	1	2	2	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	0	0	0	0	0	2	0	0	2	1	2	2	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	5	0	0	0	0	0	2	0	0	2	1	2	2	0
18. Aloha	3	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	2	0	1	0
13. Arabesque	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	3	2	0	3	3
14. Cheers	1	2	1	1	0	2	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	3	1	0
15. Hybrid (tangerine P.I. 36629)	2	0	0	0	0	2	0	2	0	0	0	0	0	3	0	0	0	0	0	0	0	2	1	0	0	1	0	1	0	0
16. Sweetsue	3	2	1	4	1	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	2	1	0	0	1	2	0	1	0
19. P.I. 349583 (#2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	0	0	0	2	0	1	0	2	1	0	2	2

Numbers indicate approximate quantity: 0 = absent or non-detectable, 1 = in trace amount,
2 = in poor amount, 3 = in fair amount, 4 = in good amount, 5 = in very good amount. (See Table)
-1 = not analysed

2 of 2

* Except for the pigments listed in Table 4, many of the determinations in this table are tentative and based largely comparative identification from chromatograms.

IV. Analysis of Pigment Data

A. Construction of the key

(i) based on vegetative characters

The means and variance of characters summarized in Tables 7 and 8 for seven known species, I. balfourii, I. balsamina, I. capensis, I. linearifolia, I. platypetala, I. schlechterii and I. sultani, were computed. Characters showing invariance (Table 9), were used as the basis for construction of the following key:

(ii) based on vegetative characters for seven known species

1. Cyanidin monoglycoside detectable in stem ----------2
1. Cyanidin-monoglycoside undetectable in stem ----------6
2. Cyanidin-bioside present in stem ----------3
2. Cyanidin-bioside undetectable in stem ----------5
3. Cyanidin-trioside detectable in stem ----------4
3. Cyanidin-trioside undetectable in stem ----------I. balfourii
4. Delphinidin-bioside in poor amount in stem ----------I. linearifolia
4. Delphinidin-bioside in trace amount in stem -------I. platypetala
5. Leucodelphinidin present in leaves ----------I. balsamina
5. Leucodelphinidin undetectable in leaves ----------I. schlechterii (# 34 only)
6. Inseparable group of three species, I. capensis, I. schlechterii (exoept # 34), I. sultani.

(iii) based on floral characters for seven known species

In addition to key construction with vegetative characters another key based on pigments of flowers was also made, the key is as follows:

TABLE 9 .

MEANS OF CHARACTERS SHOWING INVARIANCES (BLANKS ARE MISSING CASES)

	STEM														LEAVES												ROOTS	
	C_y-3, monoglyconide	C_y-3, bioside	C_y-3, trioside	D_p-3, bioside	M_v-3, bioside	Leucocyanidin	Leucodelphinidin	Leucopelargonidin	Leucomalvidin	other flavonoid I	other flavonoid II	other flavonoid III	other flavonoid IV	other flavonoid V	C_y-3, monoglyconide	C_y-3, bioside	C_y-3, trioside	D_p-3, bioside	M_v-3, bioside	Leucocyanidin	Leucodelphinidin	Leucopelargonidin	Leucomalvidin	other flavonoid I	other flavonoid II	other flavonoid III	other flavonoid IV	other flavonoid V	Anthocyanins	Leucoanthocyanins
I. balfourii Hook F.	2	3	0	0	0	2	0	0	0	2	1	1	2	3	0	0	0	0	0	2	0	0	0	0	1	1	2	1	1	3
I. balsamina L.	2	0	0	1	0	3	0	1	0	2	0	2	1	2	0	0	0	0	0	2	1	1	0	2	0	3	1	2	2	2
I. capensis Meerb		2	0	0	0	2	0	0	0					0	0	1	0	0	0		1	0	0	2	1					
I. linearifolia Warb.	2	3	1	2	0	2	1	0	0	1	1	2	3	2	2	3	1	2	0	2	1	0	0	1	1	2	3	2		
I. platypetala Lindl.	2	3	1	1	0		1	0	0	1	1	2	2	2	2	3	1	1	0	2	0	0	0	3	2	1	2	3		
I. Schlechterii Warb.								0									0	0	0		0		0							
I. sultani Hook F.			0	0		2	1	0	0									0												

FLOWER

	P_g-3,5, diglycoside 'A'	P_g-3, monoglycoside 'B'	P_g-3, monoglycoside 'C'	P_g-3,5, diglycoside acylated 'D'	P_g-3,5, diglycoside acylated 'E'	P_g-glycoside 'F'	P_g-glycoside 'G'	P_g-glycoside 'H'	P_g-glycoside 'I'	P_g-glycoside 'J'	P_g-glycoside 'K'	P_g-glycoside 'L'	P_g-glycoside 'M'	A_u-glycoside	C_y-glycoside	P_n-3,5, diglycoside	P_n-3,5, diglycoside acylated	M_v-3,5, diglycoside acylated	M_v-3,5, diglycoside	M_v-3, glycoside acylated	M_v-glycoside	Leucocyanidin	Leucodelphinidin	Leucopelargonidin	Leucomalvidin	other flavonoid I	other flavonoid II	other flavonoid III	other flavonoid IV	other flavonoid V
I. balfourii Hook F.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	1	0	2	2	0	1	2	0
I. balsamina L.	4	3	4	2	1	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	2	1	0
I. capensis Meerb																														
I. linearifolia Warb.																														
I. platypetala Lindl.						0	0	0	0	0	0	0	0	0	0		0					2	1	0				1		
I. Schlechterii Warb.						0	0	0		0				0	0	0	0	0	0	0	0			2	0	0				
I. sultani Hook F.								0	0		0	0	0	0	0				0	0	0				0					

Numbers indicate approximate quantity: 0 = absent or not detectable
1 = in trace amount
2 = in poor amount
3 = in fair amount
4 = in good amount

A. Partial key for seven known species with floral characters

1. Mv-3,5 diglycoside detectable in flowers ----------I. platypetala
1. Mv-3,5 diglycoside undetectable in flowers ---------2.
2. Mv-3 glycoside acylated detectable in flowers ------I. balfourii
2. Mv-3 glycoside acylated undetectable in flowers --------3
3. Pg-glycoside 'A' detectable in flowers ----------4
3. Pg-glycoside 'A' undetectable in flowers ----------5
4. Pg-glycoside 'E' detectable in flowers ----------I. balsamina
4. Pg-glycoside 'E' undetectable in flowers ----------7
5. Pg-glycoside 'B' detectable in flowers ----------I. linearifolia
5. Pg-glycoside 'B' undetectable in flowers ----------6
6. Cy-glycoside detectable in flowers ----------I. capensis
6. Cy-glycoside undetectable in flowers ----------I. sultani II
7. Inseparable group of I. schlechterii and I. sultani (I, III & IV).

B. Discriminant analysis of vegetative pigment data

Since three-species, I. capensis, I. schlechterii and I. sultani were in an inseparable group, discriminant analysis was done. Discriminant scores were obtained as shown in the following text table and plot:

Species	Highest group*	Discriminant Scores	
I. capensis		Func. 1	Func. 2
1.	I. capensis	0.670	0.416
2	I. capensis	1.144	0.044
3	I. capensis	2.449	-0.020

* i.e., the most probable identification as determined by the analysis (see plot on p. 48a).

Text table cont'd

I. schlechterii			
1	*I. sultani*	-0.822	-0.026
2	I. schlechterii	-0.623	-0.009
3	I. schlechterii	-0.798	-1.002
4	I. schlechterii	0.380	-0.967
5	I. schlechterii	0.380	-0.967
6	*I. capensis	0.555	0.026
7	I. schlechterii	-0.925	-0.904
I. sultani			
1	I. sultani	-0.886	2.937
2	I. sultani	-0.046	0.497
3	*I. schlechterii	-1.514	-0.921
4	I. sultani	-0.046	0.497
5	I. sultani	0.081	0.399
I. herzogii			
	I. capensis	0.705	0.615
I. pallida			
	I. capensis	2.274	-1.013
A 'flame	I. schlechterii	-2.230	-0.840
Aloha	I. schechterii	-0.209	-0.985
Arabesque	I. schlechterii	-0.925	-0.904
Hybrid	I. capensis	0.670	0.416
Sweetsue	I. schlechterii	-1.514	-0,921
Cheers	I. schlechterri	-2.819	-0.858
P.I. no. 349582	I. sultani	-1.615	2.125
P.I. no. 349582-A	I. sultani	-1.615	2.125

*** indicates the variety or introduction which fell into a different species than what was expected.**

Text table cont'd

P.I. no. 349583	I. sultani	-1.061	1.944
P.I. no. 349586	I. sultani	-1.615	2.125
P.I. no. 354255	I. sultani	-1.061	1.944
P.I. no. 366029	I. sultani	-1.316	0.759

Classification function coefficients were calculated and applied to test known species as in the following text table:

Constant	Other flavonoid I in stem	other flavonoid III in stem	other flavonoid IV in stem	Leucocyanidin in Stem	Species
1) -13.99956	-3.79117	6.36996	4.02771	3.77119	I. capensis
2) - 5.82645	0.27049	2.22584	2.11711	2.3350	I. schlechterii
3) - 5.34780	-1.54494	1.58506	1.89961	3.80725	I. sultani

C. Testing of keys

(i) with known species

For identification of known species with these classification function coefficients, the value of characters used for discriminant analysis was multiplied with its respective coefficient and the constant was added. The maximum value coinciding with the species was identified as that species. For example in the case of I. holstii, the value for characters: 1) other flavonoid I in the stem = 0

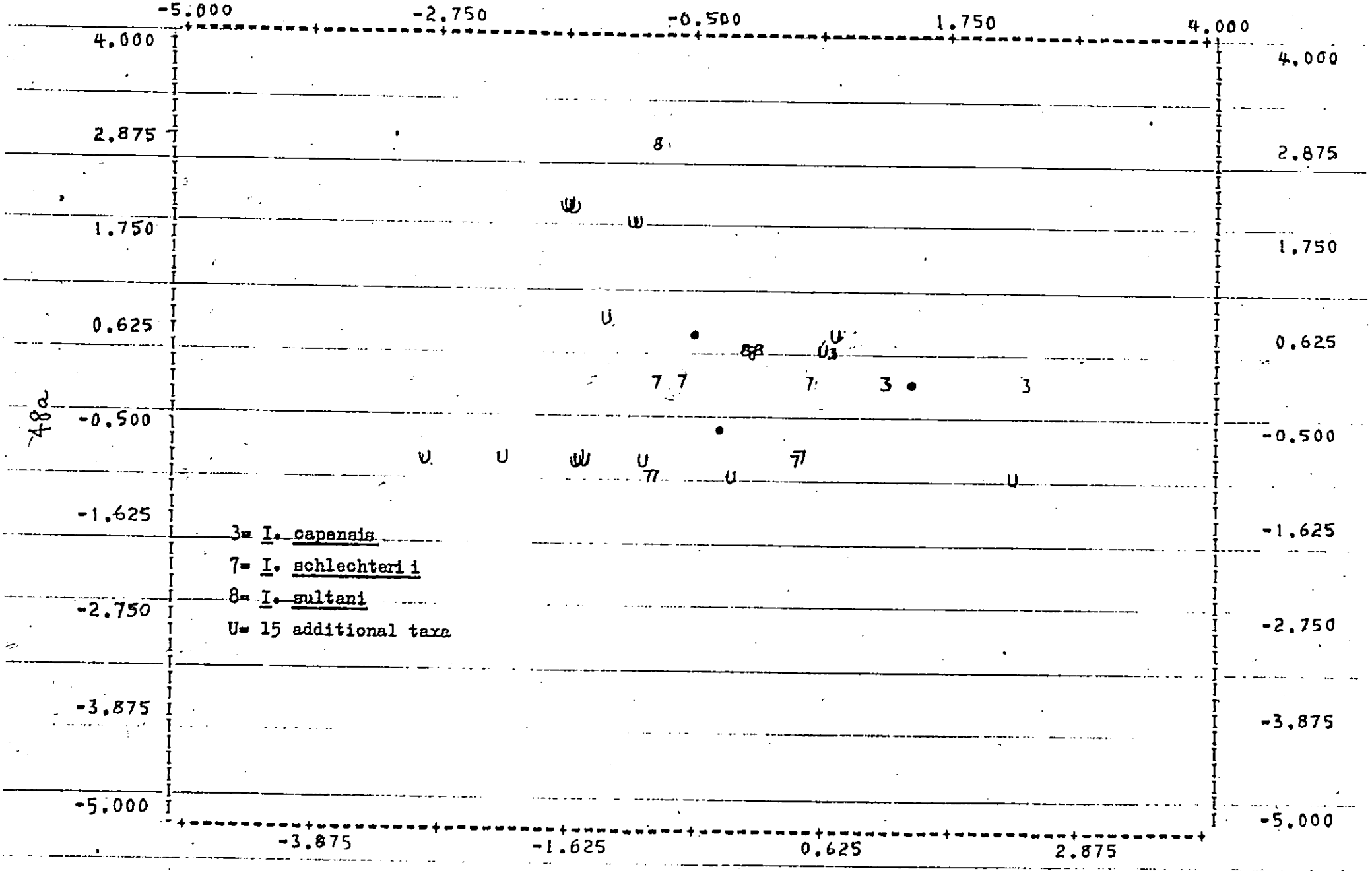
2) other flavonoid III in the stem = 1

3) other flavonoid IV in the stem = 1

4) leucocyanidin in the stem = 2

and when these values were used with the classification function coefficients for each of I. capensis 1, I. schlechterii 2, and I. sultani 3, the following calculations resulted:

OT OF DISCRIMINANT SCORE 1 (HORIZONTAL) VS. DISCRIMINANT SCORE 2 (VERTICAL). • INDICATES A GROUP CENTROID.



1) (-13.99956) (0) (6.36996) (4.02771) (3.77119X2) = 3.94049

2) (-5.82645) (0) (2.22584) (2.11711) (2.3350X2) = 3.18650

3) (-5.34780) (0) (1.58506) (1.89961) (3.80725X2) = 5.75137.

As can be seen the maximum value was obtained in 3) and the species under test can thus be identified as I. sultani.

(ii) with unknown species

Once these seven known species were classified all "unknowns" (I. holstii, I. herzogii (with no repeats), the cultivars, A'flame, Aloha, Arabesque, cheers, hybrid (Tangerine X P.I. no. 366029), Sweetsue and the unidentified species with P.I. no. 349582, 349582-A, 349583, 354255 and 366029 were tested with the key. All unknowns but the cultivar Arabesque fell into the inseparable group of I. capensis, I. schlechterii and I. sultani. The cultivar Arabesque came out with I. balfourii but could be separated from it by the presence of malvidin bioside in leaves. The remaining "unknowns" were then subjected to discriminant analysis as demonstrated above with the following results:

Taxa closest to I. capensis: I. pallida and cheers;

Taxa closest to I. schlechterii: A'flame, Aloha, Arabesque, Hybrid and Sweetsue;

Taxa closest to I. sultani: I. holstii, and P.I. nos. 349582, 349582-A, 349583, 349586, 354255 and 366029.

Testing of each individual species (known and unknown) was similarly carried out to see whether it fell in the group to which it actually belonged and the results are shown in the following

text table (see also the text table on p. 46).

Actual group	No. of cases	Predicted Group Membership		
		Group 1	Group 2	Group 3
1. I. capensis	3	100.0%	0.0%	0.0%
2. I. schlechterii	7	14.3%	71.4%	14.3%
3. I. sultani	5	0.0%	20.0%	80.0%
Ungrouped case	14	21.4%	35.7%	42.9%

Percent of "Grouped" cases correctly classified: 80.0%

D. Construction of key based on simplified analyses

The computer key described above is useful only if the anthocyanin pigments are already known. For a plant for which such data is not available, it is still possible to get an identification by using information relatively easy to obtain: These are: presence and site of pigments in organs and tissues, and nature of aglycones.

A simplified key based on vegetative characters only

1. Pigments of only cyanidin type in stem ----------2
1. Pigments of cyanidin and other types as well in stem ------4
2. Anthocyanins absent in leaves ----------I. balfourii
2. Anthocyanins present in leaves ----------3
3. Only cyanidin in leaves ----------I. capensis
3. Cyanidin, delphinidin and pelargonidin in leaves ---I. schlechterii (only PI no. 354259)
4. Cyanidin and delphinidin in stem ----------5
4. Cyanidin, delphinidin and/or other pigments in stem -------7
5. Pigments localised in epidermis, upper cortex and near vascular

bundles in stem cross sections at 5th internode ----------6

5. Pigments localised in epidermis, all across cortex and pith in stem cross sections at 5th internode ----------I. platypetala

6. Pigment localised in epidermis and mainly upper cortex of petioles ----------I. linearifolia

6. Pigment localised in epidermis, cortex and near vascular bundles of petioles ----------I. schlechterii
(all other six varieties)

7. Cyanidin, delphinidin and malvidin in stem -----I. sultani

7. Cyanidin, delphinidin and pelargonidin in stem -I. balsamina.

N.B.- The usefulness of this key is of course restricted to the above seven species most commonly encountered in horticulture.

Discussion and Conclusions

In the present study a total of ten species and twelve cultivars of Impatiens have been examined for their anthocyanins, anthocyanidins, leucoanthocyanidins and a limited number of other flavonoids. Simpler glycosides of pigments were found in vegetative tissues, cyanidin 3-glycosides being the most common, Delphinidin 3-bioside second in abundance, and malvidin, 3-bioside and pelargonidin glycosides rather rare. In floral tissues, glycosides and acylated glycosides of pelargonidin were most common, followed by those of malvidin and peonidin. Glycosides of cyanidin were detected in flowers of only one species (I. capensis) and those of delphinidin not at all. Aurantinidin glycoside, a rare pigment (Clevenger, 1964; Jurd and Harborne, 1968) was detected in flowers of two cultivars, Hybrid and Sweetsue, both derived from I. aurantiaca.

Hydroxylated anthocyanidins such as pelargonidin, cyanidin and delphinidin are considered as primitive pigment characters whereas methoxylated anthocyanidins such as peonidin and malvidin are considered as advanced characters (Harborne, 1967). It was observed in this study that malvidin glycosides were very rare in the stem tissues but present in flower petals, thus supporting the previous observations (Clevenger, 1971) that stem pigments are more primitive structurally than those of flower pigments.

Among anthocyanidins obtained by acid hydrolysis of acyanic tissues, cyanidin was the most common in stem, leaves and flowers. Delphinidin was present in stem and leaves of 50% of taxa but rarely in flowers. Pelargonidin was obtained in flower petals of 45% of

taxa but in stems and leaves of only one species. Malvidin was detected in flowers of only two taxa and not at all in stems and leaves.

The genus Impatiens is characterized by the presence of both anthocyanins and leucoanthocyanidins (Bate Smith and Lerner, 1954; Clevenger, 1971), although primarily an herbaceous taxon. It was observed in this study that when both anthocyanins and leucoanthocyanidins were present in the same tissues of a plant, there was no correlation between the type of aglycone derived from anthocyanidin and from leucoanthocyanidin. For example, delphinidin was detected only in leuco-form in flower petals of I. balfourii, I. herzogii, I. platypetala and P.I. no. 349583.

As reported by Nozzolillo (1972) for seedlings of several dicotyledonous plants, the site of pigmentation in various tissues is species specific. Similar observations were made with Impatiens, but since the pattern of pigmentation changes with the stage of development within a species, only sections of comparable age were examined. For example, among Impatiens species, I. platypetala could be separated from I. linearifolia and I. schlechteri on the basis of the localisation of the pigmented cells in stem tissues. I. linearifolia and I. schlechterii could be separated from each other on the basis of pigment localisation in petiole cross sections.

The kinds of pigments present in tissues are characteristic of a species, but several species may share the same pigmentation. In the present study for example, similar glycosides of pelargonidin were found in flower petals of both I. linearifolia and I. schlechterii

and in stems and leaves of I. linearifolia and I. platypetala, similar glycosides of cyanidin and delphinidin were found.

Few chemotaxonomic studies of the genus Impatiens using the flavonoid pigments have been done so far. In chemotaxonomic studies it is very important to identify the species accurately. This is extremely difficult in Impatiens, because of the confused and controversial state of its nomenclature (Clevenger, 1971). For example, the plants of I. capensis and I. pallida are vegetatively similar and often grow together (Wood, 1975). However, they differ in flower colors and shape and size of spur. Fernald (1950) regarded I. capensis and I. pallida as two species and described differences in the morphological characteristics of their flowers. Bohm and Towers (1962), Hegnauer (1964) and Russel and Woodland (1979) studied the phenolic compounds such as napthoquinones, coumarins and phenolic acids of both species and were able to differentiate them on the basis of the presence of vanillic acid only in I. pallida. The plants identified as I. pallida in the present study were distinguished morphologically from I. capensis by the yellow color of the petals and the right-angle of the spur but were found to contain anthocyanidins and leucoanthocyanidins in vegetative tissues similar to those of I. capensis. Hence, it still remains an open question as to whether the plants identified as I. pallida in this study were indeed a separate species from I. capensis.

A second example is found in the two species, I. holstii and I. sultani which Bailey (1949) separated on the basis of morphological differences in their leaves and flowers. Wood (1975) on the

other hand mentioned both I. sultani and I. holstii as synonyms of I. walleriana and Elias (1967) also described I. sultani as a synonym of I. walleriana. In the present study plants identified as I. sultani and as I. holstii were analysed for their pigment characters and both types were found to contain malvidin glycosides in stem tissues, a result which lends support to Wood's decision that the two are one species.

Of the 7 species investigated most intensively in the present study, two were I. capensis and I. sultani as already discussed. The remaining 5 were I. balfourii, I. balsamina, I. linearifolia, I. platypetala and I. schlechterii In the following text, chemotaxonomic studies done on each species to date are summarized.

1) I. balfourii has been examined previously only for phenolic acids by Bohm and Towers (1962) and Hegnauer (1964). In the present study vegetative and floral tissues of this species were analysed for anthocyanins, anthocyanidins and leucoanthocyanidins for the first time. No anthocyanins are present in the leaves but leucocyanidin was detected there. In stems both leucocyanidin and a cyanidin-3-bioside and 3-monoside were found. Flowers, on the other hand, contain only an acylated malvidin-3-glycoside along with leucocyanidin, leucodelphinidin and leucomalvidin.

2) The flavonoid pigments of I. balsamina have been studied in detail (Beale, 1942; Clevenger, 1958; Bohm & Towers, 1962; Hegnauer, 1964; Hagen, 1966 and Strack & Mansell, 1975). Complex mixtures of pelargonidin 3-, 3,5-glycosides and their acylated derivatives have been identified in flower petals of I. balsamina, genotype llHHprpr by Hagen (1966) and Strack and Mansell (1975) whereas simpler pigments were present in stem

tissues. Similar results were obtained with this genetic line in the present study.

3) Clevenger (1958 and 1971) studied anthocyanidins and leucoanthocyanidins in I. capensis and I. pallida, and both species were found to contain pigments of cyanidin type in stems and flowers. In the present study, the anthocyanins of stem and leaf (petiole) tissues have been identified as a cyanidin-3-bioside together with smaller amounts of a cyanidin-3-monoside. A cyanidin glycoside was also detected in the petals. Only leucocyanidin was detected in stem and flowers, but traces of leucodelphinidin were also present in the leaves.

4) Anthocyanins, lecuoanthocyanidins and other flavonoids have never previously been examined in vegetative and floral tissues of I. linearifolia. They were found in this study to comprise a cyanidin -3-trioside, bioside and monoside, a delphinidin bioside and both leucocyanidin and leucodelphinidin in stem and leaves. Flower tissues (petals) on the other hand contained only pelargonidin 3- and 3,5-glycosides and leucocyanidin and leucopelargonidin.

5) Clevenger (1971) examined hydrolysed tissue extracts of I. platypetala for anthocyanidins and found cyanidin, delphinidin, and malvidin. In the present study, the anthocyanins of stem and leaf were found to be similar to those of I. linearifolia. Floral pigments on the other hand consisted of malvidin 3 and 3,5-glycosides and malvidin 3 and 3,5-acylated glycosides, together with leucocyanidin, leucodelphinidin, and in one selection, a trace of leucomalvidin.

6) Clevenger (1971) also examined some varieties of I. schlechteri and found cyanidin and delphinidin in vegetative tissues, but pelar-

gonidin in floral tissues. In the present study, stem tissues were found to contain cyanidin and delphinidin biosides, some cyanidin monoside and leucocyanidin, leucodelphinidin and leucopelargonidin. Leaf tissues contained only a cyanidin monoside and bioside and leucocyanidin and leucopelargonidin. Flower tissues on the other hand contained several species of pelargonidin 3- and 3,5-glycosides and a 3,5-acylated glycoside and only leucocyanidin.

7) Robinson and Robinson (1931) identified a pelargonidin complex 3,5-dimonoside, closely resembling monardaein in the orange-scarlet flowers of I. holstii and a peonidin complex 3,5-dimonoside in a deep bluish-rose flowered variety. Klozova and Rokosova (1961) found malvidin glycosides in stems and those of peonidin in petals of red-violet flowers and of peonidin and pelargonidin in petals of a scarlet flowered variety of I. holstii. The magenta flowered variety analysed in this study also contained malvidin glycosides in the stem and peonidin glycosides in flower petals. Other varieties analysed for anthocyanins in the present study also contained glycosides of cyanidin and malvidin in stems and leaves. Orange, pink and red flowered varieties contained only pelargonidin glycosides in the petals.

Technical problems with the studies of flavonoids, especially anthocyanin pigments are numerous. It is hard to get synthetic standards. If the glycosides of one aglycone are numerous within a plant, it is hard to identify each glycoside due to instability of pigments and difficulties of getting sufficient amounts of each one. Quantitative estimation of individual anthocyanin among several species is also difficult, because the total amount of pigments present in tissues of various plants differ. Moreover environmental factors

also affect the pigments quantitatively, although not qualitatively (Alston and Turner, 1963). For these reasons most of the studies done to date are based on aglycone identification in this genus (Clevenger, 1971 and Hegnauer, 1964). Clevenger (1971) analysed nineteen species or varieties for the presence of anthocyanidins and leucoanthocyanidins and Hegnauer (1964) examined phenolic acids and leucoanthocyanidins in seventeen species of Impatiens.

This present study, and others, of flavonoid pigments in only a small part of the genus Impatiens indicates that an analysis of these pigments in vegetative and floral tissues does aid significantly in solving the problems of species and cultivar identification. Asen (1977) for example, distinguished two New Guinea white Impatiens sister seedling cultivars D-191-1 and D-191-7 on the basis of quantitative differences in flavonols (Kaemferol-glycosides) as determined by high pressure liquid chromatography.

If vegetative chemical characters can be used for classification purposes, they will be preferred. It is often difficult to get flowers. Once flowers are available, species can often be differentiated by their morphological characteristics only. Thus chemical characters in vegetative tissues seem to be more promising from the practical point of view because stems and leaves are more readily available.

When an attempt was made to relate chromosome numbers of species with the complexity of pigments, it was observed that the chromosome number (n) is 7 in both I. balsamina and I. capensis, but the pigments are of complex glycosides of pelargonidin type in I. balsamina flower petals and of primitive cyanidin glycosidic type

in I. capensis flower petals. Thus there is no direct relation of ploidy with complexity of pigment characters.

In conclusion I would like to put here Larsen's statement (as quoted in Asen, 1977): "All inherent morphological manifestations of varietal differences must ultimately have a biochemical difference but not all biochemical differences are necessarily reflected morphologically. Thus, biochemical differences should be more numerous than morphological differences."

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Appendix 1

Source of Authentic Samples and Chemicals:

Standard anthocyanins, cyanin and malvin, and anthocyanidins, cyanidin, pelargonidin, and delphinidin were purchased from Fluka AG, Buchs SG, Switzerland.

Standard sugars, D (+) glucose, D (+) galactose, D (+) rhamnose, D (+) xylose and L (+) arabinose were purchased from Sigma Chemical Co., St. Louis, Missouri, USA, and Fisher Scientific Co., Fair Lawn, New Jersey and Ottawa Br.

Phthalic acid was obtained from Eastman Organic Chemicals, Rochester, N.Y. Butanol was purchased from Fisher Scientific Co., Ottawa, Ontario. Iso-amyl alcohol and propanol from the same company.

Ethyl acetate, petroleum ether and acetic acid were purchased from Mallinckrodt Chemical Works Ltd., Montreal and Toronto Branches.

Hydrochloric acid was purchased from J. T. Baker Chemical Co., Phillipsburg, New Jersey. 08865.

MN Polyamide D-6 powder was obtained from Macherey Nagel & Co., W. Germany.

Aniline and pyridine were purchased from Fisher Scientific Co., Ottawa Br.

Formic acid was purchased from B.D.H. Co., England.

Appendix 2

Sources and Chromosome numbers of *Impatiens* species (see Table in Thakur & Nozzolillo, 1978)

Plant Species	Sources*		Chromosome numbers	References
A. 1. *I. balfourii* Hook F.	1	S	14 (2n)	Khoshoo (1957, 1955).
			7 (n)	Chinnappa & Gill (1974).
2. *I. balsamina* L. (llHHprpr)	2	S	7 (n) 14 (2n)	Chinnappa & Gill (1974). Rao (1972).
3. *I. balsamina* L. (white)	3	S	14 (2n)	Rao (1972).
4. *I. capensis* Meerb.	4	C	7 (n)	Khoshoo (1957, 1955).
5. *I. linearifolia* (P.I. 354266) (no. 19)	5	C	32 (2n)	Arisumi (1973).
6. *I. linearifolia* (P.I. 354267) (no. 20)	5	C	32 (2n)	Arisumi (Pers. Commun.).
7. *I. platypetala* (P.I. 349629) (no. 21).	5	C	16 (2n)	Arisumi (Pers. Commun.).
8. *I. schlechterii* Warb.	5	C	32 (2n)	Arisumi (Pers. Commun.).
9. *I. sultani* Hook F.	6	C	16 (2n)	Khoshoo (1957).
10. *I. herzogii* K. schum.	5	C	polyploid	Arisumi (Pers. Commun.).
11. *I. holstii* Hook F.	7	S	16 (2n)	Khoshoo (1957).
12. *I. pallida* Nutt	4	C	10 (n)	Chinnappa & Gill (1974).

(Appendix 2, cont'd)

B. Cultivars (Scientific names not given so far)				
13. Aflame	5	C	32 (2n)	Arisumi (Pers. Commun.).
14. Aloha	5	C	32 (2n)	Arisumi (Pers. Commun.).
15. Arabesque	5	C	32 (2n)	Arisumi (Pers. Commun.).
16. Cheers	5	C	32 (2n)	Arisumi (Pers. Commun.).
17. Hybrid	5	C	20 (2n)	Arisumi (Pers. Commun.).
18. Sweetsue	5	C	40(amphiploid)	Arisumi (Pers. Commun.).
C. Unidentified species				
19. P.I. 349582 (no. 1)	5	C	32 (2n)	Arisumi (Pers. Commun.).
20. P.I. 349582A (no. 1A)	5	C	32 (2n)	Arisumi (Pers. Commun.).
21. P.I. 349583 (no. 2)	5	C	32 (2n)	Arisumi (Pers. Commun.).
22. P.I. 349586 (no. 3)	5	C	32 (2n)	Arisumi (Pers. Commun.).
23. P.I. 354255 (no. 6)	5	C	32 (2n)	Arisumi (Pers. Commun.).
24. P.I. 366029 (no. 26)	5	C	8 (2n)	Arisumi (Pers. Commun.).

*Sources:

1. Conservatoire et Jardin Botanique, Geneva, Switzerland
2. R. L. Mansell, Department of Biology, University of South Florida, Tampa, Florida, USA. Genotype (llHHprpr).
3. Stokes Seeds, St. Catherine, Ontario.
4. Local collection in the Ottawa area in the wild (seedlings and mature plants).

5. T. Arisumi, U.S.D.A., B.A.R.C., Plant Genetics and Germplasm Institute, Beltsville, Maryland, USA 20705.

6. Local plantings.

7. Hortus Botanicus, Coimbra, Portugal.

S = Seeds C = Cuttings

* These numbers indicate accession numbers assigned by Arisumi.