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DISTRIBUTION OF ALLAGOCHROME IN VASCULAR PLANTS[1]

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ABSTRACT

GARRICK, L. S., and H. M. HABERMANN. (Goucher Coll., Baltimore, Md.) Distribution of allagochrome in vascular plants. Amer. Jour. Bot. 49(10): 1078–1088. Illus. 1962.—Allagochrome is a blue-green, water-soluble pigment which can be reduced with a change in color to yellow and is autoxidizable in the presence of molecular oxygen. The development of quantitative procedures for assay has made possible an intensive study of its distribution in a wide variety of plant materials. The survey of 124 species representing 112 genera summarized in this paper indicates its presence in 55 of the species assayed. Quantitative estimates have been made of its concentrations in species showing positive tests. The widespread occurrence of allagochrome in vascular plants suggests a common function or accumulation as a metabolic product. The significance of variation in the position of absorption peaks, correlation of occurrence with known cases of cyanide-resistant respiration, and species specificity of the chemistry of allagochrome are discussed.

ALLAGOCHROME is a water-soluble pigment which was first isolated from leaves of *Helianthus annuus* L. (Habermann, 1960a,b). Its color varies not only with pH of the solution but also with its state of oxidation or reduction. In alkaline solution, the oxidized form is blue-green. It is reduced by sodium hydrosulfite with a color change to yellow and is autoxidizable. In absorption spectra of neutral and alkaline solutions of purified preparations from several species, a peak occurs at 670–675 mμ; a shoulder, at 630 mμ (see Fig. 1 for spectra of *Helianthus* allagochrome in alkaline solution). As the pH of a solution is lowered, there is a gradual loss of the characteristic absorption peak and shoulder in the red portion of the spectrum. In acid solution, allagochrome is red. The acid-base color changes, like the changes observed on oxidation or reduction, are completely reversible. Prolonged acidification, however, leads to precipitate formation.

Several kinds of evidence suggest that the allagochrome chromophore is bound to a high-molecular-weight moiety which is probably protein: (1) Estimated molecular weight of 50,000 for *Helianthus* allagochrome (determined by a diffusion technique [Northrup and Anson, 1929]) is supported by the rapid passage of the pigment through #75 grade Sephadex (the minimum molecular weight for complete exclusion is approximately 40,000). (2) Allagochrome is separated from other components of crude extracts by electrophoresis, migrating toward the positive electrode. (3) It is precipitated by many of the usual protein-precipitating agents such as alcohols, acetone, acids, ammonium sulfate, and salts of heavy metals. (4) It has a high optical density in the ultraviolet (Habermann, 1961). There is some loss of its protein-like properties on purification; e.g., following electrophoresis and passage through Sephadex, there is a drop in absorbance in the ultraviolet relative to the height of the red peak. Thus, much non-specific protein appears to be present in crude extracts.

Although the function of allagochrome in plant metabolism has not yet been unequivocally established, its involvement in respiration as a cyanide-resistant terminal oxidase (Habermann, 1961) and in photosynthesis as a catalyst of photophosphorylation (Habermann and Krall, 1961) has been suggested. A widespread distribution of the pigment would, of course, sup-

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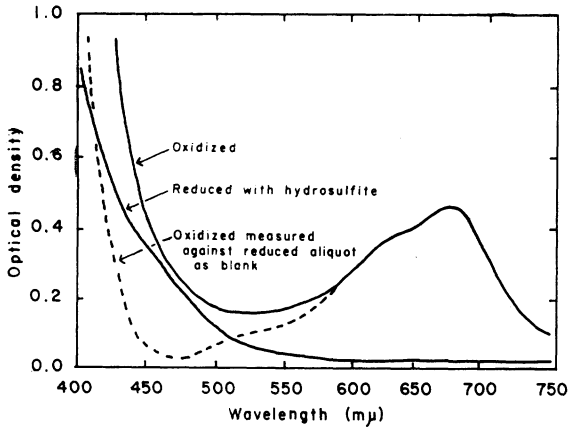


Fig. 1. Spectra of oxidized and reduced *Helianthus* allagochrome. Broken line is the spectrum of the oxidized form measured against a reduced aliquot as blank.

port the hypothesis that its involvement in respiration and photosynthesis are possible in many plant species rather than isolated phenomena. Development of quantitative procedures of assay for allagochrome has made possible a survey of allagochrome content of species representing many families of higher plants. This paper summarizes results of assays of 124 species representing 112 genera and includes determinations made with a few species of the lower plant phyla. These studies have indicated that allagochrome has a widespread occurrence among vascular plants. Where possible, correspondence of patterns of allagochrome distribution with generally accepted evolutionary trends and with known examples of cyanide-resistant respiration has been pointed out.

MATERIALS AND METHODS—*Sources of plant materials*—Most of the plants assayed were collected on the Goucher College campus or grown in the greenhouse. Field-grown materials were collected during the fall, winter, or spring, and, except for those collected in the spring, were representative of mature plants. Materials grown in the greenhouse ranged in age from young seedlings to mature plants. Some fruits and vegetables were obtained from local markets during the winter. Samples of tropical and subtropical plants were obtained from the United States Botanic Garden, Washington, D.C. Where possible, species were chosen for assay to represent a broad selection of vascular plants; samples include representatives from primitive and advanced taxa.

Assay procedure—Freshly collected, weighed specimens (usually 0.5–2.0 g) were ground with cold glycerine-NaOH buffer (pH 9.5: 0.05 M glycine, 0.02 M NaOH) in a small Waring Blendor. The homogenate was transferred to a graduate with several rinses of buffer and diluted with buffer to the desired volume (25–100 ml). Homogenates were centrifuged for 20 min at 4 C in an International HR–1 refrigerated centrifuge (10.5 cm 8-place head, 13,500 rpm, approximately 25,000×). Duplicate aliquots of the clear supernatant were transferred to matched cuvettes, and a few crystals of sodium hydrosulfite were added to one of the cuvettes to reduce the allagochrome. The absorption spectrum of each sample was measured in a Beckman DK–2 recording spectrophotometer using the reduced aliquot as a blank. The reduced form of allagochrome does not absorb in the red portion of the spectrum (see Fig. 1 for representative spectra of *Helianthus* allagochrome). Thus, the recorded red peak represents only the allagochrome present in each supernatant. Chlorophylls *a* and *b*, the main colored contaminants absorbing in the red portion of the spectrum, do not undergo color changes on addition of the reducing agent, are present in equal concentrations in the sample and reduced blank, and thus do not contribute to the recorded optical density.

Expression of results—Although purified preparations of allagochrome from several genera (*Helianthus*, *Chrysanthemum*, and *Ligustrum*) have identically shaped spectra in the red (indicating identical chromophores), there is considerable variation in weight of dried material per unit volume of solution needed for a given optical density. This suggests a species specificity in the protein/chromophore ratio in vitro. A close agreement in values of weight per ml for an optical density of 1 at the red peak for preparations from a given species with somewhat different preparative histories suggests that these specific differences in the nature of the allagochrome complex exist in vivo and do not necessarily result from complex formation during extraction.

The differences cited above make it impossible to utilize values of weight per unit volume of solution with given optical density determined for any single species for calculation of weights of allagochrome complex present in samples from other species. For this reason, the following expression (which reflects the absolute concentration of the chromophore and makes no assumptions about weights of complexing materials in vitro or in vivo) has been used as an indication of relative allagochrome concentrations:

$$\text{Allagochrome value} = \frac{\text{O.D. (at red max.)} \times \text{vol. of homog. (ml)}}{\text{weight of sample (g)}}$$

Estimation of extent of extraction—Experimental results indicated large variations with species in concentrations of the allagochrome chromophore present in supernatants from homogenates of plant materials. Such differences and later observations of differences of allagochrome content with age and position of leaves led us to question whether the initial extraction provided

TABLE 1. *Re-extraction of precipitate from centrifugation of Helianthus homogenate*

	Allagochrome value	% of total (1 + 2)
Supernatant 1	7.85	92.1
Supernatant 2	0.67	7.9
Total (1 + 2)	8.52	100.0

an adequate measure of the total extractable allagochrome. We cannot extrapolate to all species from the data obtained by extraction of allagochrome from the precipitate of the first centrifugation cited in Table 1; however, the data indicate that over 90% of the pigment is present in the first supernatant. Although a single extraction, therefore, is not strictly quantitative, it does provide a good approximation of relative allagochrome content.

RESULTS—*General distribution*—Assays for allagochrome summarized in Table 2 indicate the presence of this pigment in diverse groups of vascular plants. The pigment has not been found in ferns, but it has been detected in gymnosperms and angiosperms, both mono- and dicotyledons. The species assayed are listed in Table 2 in an order which follows phylogenetic trends with placement of the more generalized before the more specialized taxa where possible. When parts of plants other than leaves were assayed, the parts are indicated (e.g. fruit, flower, etc.). *Y* following the name of the species indicates a young plant: *D*, material collected long before processing; ? indicates that results were not fully confirmed by later assays. The notation "no measurable peak" indicates that no detectable allagochrome was present in the extracts. The absence of measurable red absorption peaks in the extracts does not necessarily imply that no allagochrome is present in a given species: it might only reflect the limitations of measuring instruments or the inadequate size of the sample extracted.

Differences in allagochrome level with age—Leaves of a few species were assayed at several stages of maturity. These determinations indicated that there are differences in allagochrome content with leaf position and maturity of the plant. Such variations have been observed in *Chrysanthemum* sp., *Helianthus annuus*, *Nicotiana rustica*, and *Lycopersicon esculentum*. *Helianthus* leaves exhibit increasing allagochrome values at successive nodes above the cotyledons.

Interspecific differences in position of red absorption peaks—Although crude extracts prepared at different times from a given species are usually consistent in the position of the red absorption peak, there are often considerable variations in peak positions for extracts of different species (often between species of the same family). In 3 species of *Labiatae*, for example, the red peaks are located at wavelengths from 10 to 30 mμ shorter than the most frequently observed peak range of 670–675 mμ (see Fig. 2 for examples of varied peak positions in crude extracts prepared from several species). Such divergent peak positions are not always maintained on purification, however. *Ligustrum* allagochrome (especially interesting because of the extreme peak location at 635 mμ in crude extracts) was purified by electrophoresis and chromatography on Sephadex. After purification, a peak position at 670 mμ was observed. In a few cases, e.g., *Plantago lanceolata* and *Lycopersicon esculentum*, crude extracts prepared at different times have shown quite different peak positions. We do not feel that such occasional inconsistencies in peak position are significant, however. Age of the plant, freshness of the sample, part of the plant, and presence of substances in the homogenates which might affect pH or the form of the chromophore could affect the position of the red absorption peak.

Relationships between occurrence and phylogeny—Table 3 shows the phylogenetic relationships of angiosperm orders postulated by Bessey (Lawrence, 1951). According to this scheme, the ancestor of each group is thought to be derived from primitive members of the preceding order. For those orders in which allagochrome has been found in at least 1 species, the range of measured allagochrome values is indicated.

Allagochrome values and cyanide-resistant respiration—Table 4 lists plants which have been reported to exhibit cyanide-resistant respiratory oxygen uptake and the allagochrome values which we have measured for these species. In all cases for which allagochrome values are available, respiratory resistance to cyanide occurs in plant materials having measurable concentrations of the pigment. No respiratory data for *Pyracantha coccinea* are available, but this plant, which has a high allagochrome content, also produces natural cyanides.

DISCUSSION—Assays of a large number of vascular plants have demonstrated the widespread occurrence of allagochrome. Whether or not allagochrome is a single pigment having the same chemical structure in all species in which it occurs is a question which can be resolved only after purification and chemical characterization of the pigment from many sources. Present spectrophotometric evidence based on the most highly purified preparations available (from *Helianthus*, *Chrysanthemum*, and *Ligustrum*) indicates that the chromophores responsible for the characteristic absorption patterns in the red portion of the spectrum are identical. Copper content (Habermann, unpublished data), as well as the proportion and nature of the high-molecular-weight moiety associated with the allagochrome chromophore, varies with the species from which the pigment is extracted.

TABLE 2. *Assays of relative allagochrome content of some vascular plants*

Order Family	Species	Wavelength of red peak (mμ)	Number of determinations	Allagochrome value O.D. × vol / wt
		Division Psilophyta		
Psilotales				
Psilotaceae	*Psilotum nudum*	670?	3	
		Division Lycophyta		
Lycopodiales				
Lycopodiaceae	*Lycopodium complanatum*	no measurable peak	1	
		Division Pterophyta		
Eufilicales				
Polypodiaceae	*Nephrolepis* sp.	no measurable peak	1	
	Cyrtomium falcatum	no measurable peak	1	
Hydropteridales				
Marsileaceae	*Marsilea* sp.	no measurable peak	1	
		Division Spermatophyta Subdivision Gymnospermae		
Coniferales				
Taxaceae	*Taxus baccata*	no measurable peak	1	
	Taxus cuspidata	no measurable peak	1	
Pinaceae	*Pinus nigra*	no measurable peak	1	
	Pinus mugo	no measurable peak	1	
	Tsuga sieboldii	670	1	20.43
	Tsuga canadensis	670–675	1	17.14
	Tsuga caroliniana	670	1	16.08
Taxodiaceae	*Cryptomeria japonica*	667–679	2	3.77
Cupressaceae	*Juniperus virginiana*	no measurable peak	1	
		Subdivision Angiospermae Class Dicotyledoneae		
Ranales				
Magnoliaceae	*Magnolia fuscata*	675	1	3.67
Piperaceae	*Peperomia* sp.	no measurable peak	1	
Berberidaceae	*Mahonia* sp.	635	1	82.13
	Berberis triacanthophora	670	1	38.15
Lauraceae	*Sassafras albidum*	no measurable peak	1	
Sarraceniales				
Sarraceniaceae	*Sarracenia purpurea*	670–675	1	0.57
Guttiferales				
Violaceae	*Viola* sp.	no measurable peak	1	
Rhoeadales				
Cruciferae	*Brassica oleracea* (D)	680	1	0.19
	Raphanus sativa (Y)	no measurable peak	1	

TABLE 2. *Continued*

Order Family	Species	Wavelength of red peak (mμ)	Number of determinations	Allagochrome value O.D. × vol / wt
Malvales				
Malvaceae	*Hibiscus* sp.	680	1	1.19
Urticales				
Moraceae	*Ficus elastica*	675?	1	
Geraniales				
Geraniaceae	*Pelargonium zonale*	no measurable peak	1	
Rutaceae	*Triphasia* sp.	670–675	1	
Simaroubaceae	*Ailanthus altissima*	674–676	2	8.79
Euphorbiaceae	*Ricinus communis*	no measurable peak?	5	
	Poinsettia sp.	no measurable peak?	1	
	Croton sp.	no measurable peak?	1	
Balsaminaceae	*Impatiens* sp.	no measurable peak	1	
Centrospermae				
Phytolaccaceae	*Phytolacca americana*	665–678	12	1.55
Chenopodiaceae	*Beta vulgaris* (garden beet) (D)	no measurable peak	1	
	Beta vulgaris (chard)	670's		
	Spinacia oleracea (Y)	678?	1	
Polygonaceae	*Rumex* sp.	680?	3	
Nyctaginaceae	*Bougainvillea spectabilis*	no measurable peak	1	
Caryophyllaceae	*Dianthus barbatus*	no measurable peak	1	
Ericales				
Ericaceae	*Rhododendron catawbiense*	no measurable peak	1	
	Rhododendron microphytum	no measurable peak	1	
	Kalmia latifolia	no measurable peak	1	
	Pieris japonica	no measurable peak	1	
Ebenales				
Sapotaceae	*Achras sapota*	no measurable peak	1	
Primulales				
Plantaginaceae	*Plantago lanceolata*	635	2	4.61
	Plantago lanceolata	678–680	2	6.87
Plumbaginaceae	*Plumbago* sp.	no measurable peak	1	
	Statice sinuata	no measurable peak	1	
Myrsinaceae	*Ardisa* sp.	no measurable peak	1	

TABLE 2. *Continued*

Order Family	Species	Wavelength of red peak (mμ)	Number of determinations	Allagochrome value O.D. × vol / wt
Gentianales				
Oleaceae	*Ligustrum japonicum*	635	3	54.66
	Forsythia sp. (flowers)	630–640	1	1.03
Apocynaceae	*Carissa bispinosa*	666–670	1	7.87
Polemoniales				
Boraginaceae	*Myosotis* sp.	650	1	2.09
Solanaceae	*Lycopersicon esculentum*			
	65 days old	no measurable peak	1	
	137 days old	650	1	4.13
	147 days old	660	1	4.66
	Nicotiana rustica			
	80 days old	670	1	0.12
	133 days old	670	1	11.99
	6 months old (leaves)	664–673	1	20.74
	6 months old (flowers)	665–675	1	2.79
	Capsicum frutescens (D) (fruit)	673	2	
	Francisca sp.	no measurable peak	1	
Scrophulariales				
Scrophulariaceae	*Antirrhinum majus*	no measurable peak	1	
Lamiales				
Labiatae	*Glechoma hederacea*	640–642	1	16.38
	Prunella vulgaris	638–642	1	44.79
	Leonurus cardiaca	660–665	2	13.30
Rosales				
Leguminosae	*Mimosa pudica* (Y)	no measurable peak	1	
	Trifolium pratense	no measurable peak	1	
	Lathyrus sp.	no measurable peak	1	
	Vicia villosa	no measurable peak	1	
	Glycine max	no measurable peak	1	
	Phaseolus limensis (D) (pcd)	no measurable peak	1	
	Calliandra sp.	no measurable peak	1	
Rosaceae	*Agrimonia parviflora*	no measurable peak	1	
	Rubus allegheniensis	no measurable peak	1	
	Duchesnea indica	no measurable peak	1	
	Prunus laurocerasus	no measurable peak	1	
	Pyracantha coccinea	673	1	43.99

TABLE 2. *Continued*

Order Family	Species	Wavelength of red peak (mμ)	Number of determinations	Allagochrome value O.D. × vol / wt
Saxifragaceae	*Saxifraga* sp.	no measurable peak	2	
Crassulaceae	*Aeonium cuneatum*	670–676	1	53.37
Loasales				
Cucurbitaceae	*Cucumis sativa* (D) (Y) (fruit) (leaves)	no measurable peak	2	
Cactales				
Cactaceae	*Opuntia* sp.	no measurable peak	2	
Celastrales				
Vitaceae	*Parthenocissus quinquefolia*	no measurable peak	1	
Celastraceae	*Schaefferia* sp.	667–675	1	12.75
Buxaceae	*Buxus microphylla*	no measurable peak	1	
Aquifoliaceae	*Ilex aquifolium*	664–666	1	54.24
	Ilex opaca	665	1	19.87
	Ilex canariensis	668–670	1	77.56
	Ilex crenata	670	1	24.82
Umbellales				
Araliaceae	*Aralia* sp.	665–670	1	27.17
	Hedera helix	665	1	28.84
Umbelliferae	*Daucus carota*	670	1	6.54
	Apium graveolens (D)	no measurable peak	1	
	Petroselinum crispum (D)	673–675	2	
Rubiales				
Rubiaceae	*Coffea arabica*	670–673	1	23.12
	Ixora sp.	no measurable peak	1	
Caprifoliaceae	*Lonicera japonica*	660–667	3	14.27
	Diervilla corvaensis	668–672	1	17.49
Valerianaceae	*Valeriana officinalis*	632–638	1	6.74
Asterales				
Compositae	*Helianthus annuus*			
	young leaves (1st 3 nodes above cotyledons)	673	8	5.78
	older leaves (1st 3 nodes above cotyledons)	673	6	13.31
	Aster simplex	673	2	27.59
	Aster novae-angliae	673	3	54.55

TABLE 2. *Continued*

Order Family	Species	Wavelength of red peak (mμ)	Number of determinations	Allagochrome value O.D. × vol / wt
	Chrysanthemum sp.	674–683	4	44.28
	Chrysanthemum sp. (Y)	673	1	2.62
	Chrysanthemum maximum	663–673	1	1.47
	Eupatorium altissimum	675–678	2	61.52
	Solidago altissima	672–674	2	59.68
	Solidago juncea	672–674	1	37.94
	Rudbeckia hirta	668–675	2	8.99
	Taraxacum officinale	670–674	4	7.67
	Taraxacum officinale (flowers)	666–683	1	5.92
	Helichrysum sp. (Y)	670–680	1	0.35
	Class Monocotyledonae			
Alimatales (Pandanales)				
Typhaceae	*Typha* sp.	no measurable peak	1	
Arales				
Araceae	*Symplocarpus foetidus*	665–670	1	
	Spathiphylum sp.	670?	1	
	Philodendron sp.	674?	5	
	Zantedeschia ethiopica	no measurable peak	1	
Lemnaceae	*Lemna minor*	no measurable peak	2	
Liliales				
Liliaceae	*Allium cepa* (D)	no measurable peak	1	
	Cordyline sp.	no measurable peak	1	
	Asparagus officinalis	no measurable peak	1	
Commelinaceae	*Tradescantia* sp.	no measurable peak	1	
Amaryllidaceae	*Narcissus* sp.	no measurable peak	1	
Iridaceae	*Iris* sp.	no measurable peak	1	
	Gladiolus sp.	no measurable peak	1	
Bromeliaceae	*Ananas comosus* (D)	no measurable peak	2	
Palmales				
Palmaceae	*Rhapis* sp.	no measurable peak	1	
Graminales				
Cyperaceae	*Cyperus* sp.	no measurable peak	1	
Gramineae	*Zea mays* (Y)	no measurable peak	1	
	Avena sativa (Y)	no measurable peak	1	
Orchidales				
Orchidaceae	*Orchis* sp.	635–640	1	2.27

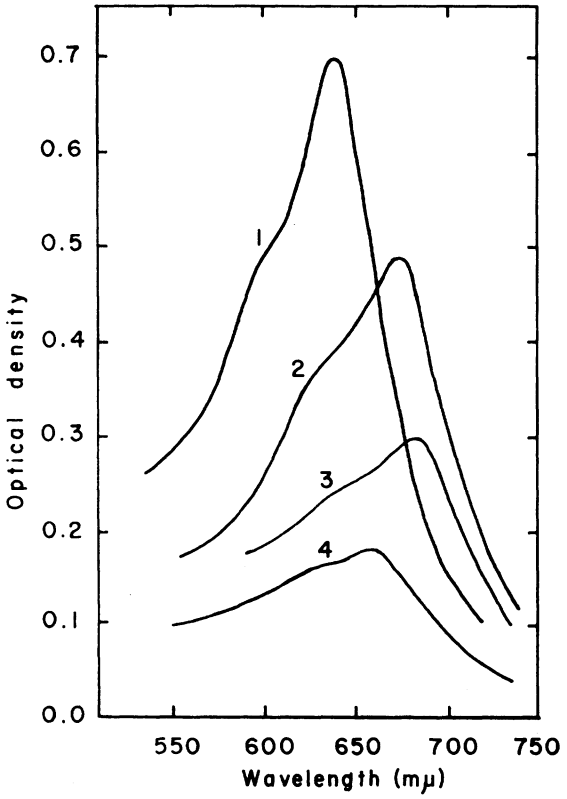


Fig. 2. Representative spectra illustrating varied peak positions of crude allagochrome extracts (all spectra measured against reduced aliquots). Species and peak positions: 1—*Prunella vulgaris*, 640 mμ; 2—*Helianthus annus*, 673 mμ; 3—*Plantago lanceolata*, 680 mμ; 4—*Lycopersicon esculentum*, 660 mμ.

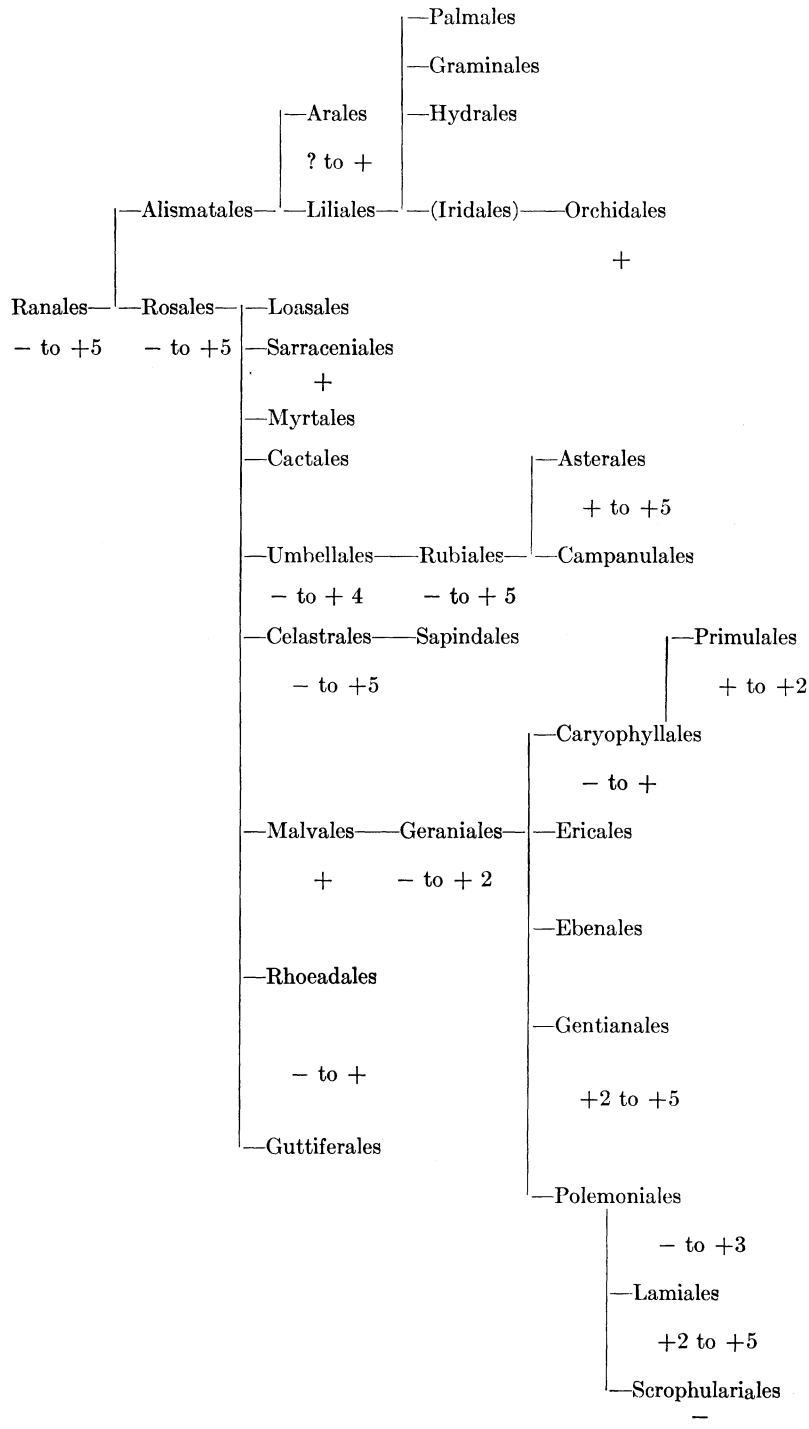
The significance of the variation in peak positions of crude extracts (which often appears to be species specific) is not yet known. Acidification causes a color change from blue-green to red and thus shifts the absorption peak to shorter wavelengths (patterns of spectral shifts with pH will be reported in detail elsewhere [Habermann, unpublished data]). The presence of high concentrations of plant acids might in part explain the variation in peak position but does not explain why the peak shift occurs *without* a concomitant change from the usual peak and shoulder pattern to a single broad peak: Such changes are observed with purified solutions in buffers of increasingly acid pH. The shift in peak position on purification of allagochrome prepared from leaves of *Ligustrum japonicum* (from 635 to 670 mμ) suggests that aspects of the chemical environment of the homogenate other than pH and state of oxidation or reduction can affect the position of the allagochrome absorption peak.

The form in which allagochrome exists in vivo undoubtedly depends on the level of oxidation or reduction which is extant wherever the pigment is located in the aqueous phase of the cell contents. The pigment is extremely soluble in water and for this reason we have not yet been able to determine its intracellular location. Normally, any oxidized allagochrome would be completely masked (or indistinguishable from) the chlorophylls. The cotyledons of dark-grown seedlings of white mutant sunflowers, however, are frequently a striking bluish-green, the color of the oxidized form of allagochrome. Although measurable amounts of protochlorophyll are

TABLE 3. *Plants with some respiratory resistance to cyanide*

Family	Species and reference where information on cyanide resistance was found	Presence and range of allagochrome values (OD × vol × wt^{-1})
Compositae	*Helianthus annuus* (Habermann, 1961)	present; 5.78–13.31
Umbelliferae	*Daucus carota* (Beevers, 1961; Marsh and Goddard, 1939)	present; 6.54
Araceae	*Symplocarpus foetidus* (Beevers, 1961; Hackett, 1957)	present
	Philodendron sp. (flowers) (Beevers, 1961; Yocum and Hackett, 1957)	flowers not assayed; leaves indefinite
	Arum sp. (spadix) (Beevers, 1961; Hackett and Simon, 1954)	not assayed but pigment is present in some Araceae
	Sauratum sp. (Beevers, 1961)	
	Peltandra sp. (Beevers, 1961; Yocum and Hackett, 1957)	
Solanaceae	*Solanum tuberosum* (Beevers, 1961; Thimann, Yocum and Hackett, 1954)	not assayed but pigment is present in some Solanaceae; range: 0.12–11.99

TABLE 4. *Summary of suggested angiosperm phylogeny (after Bessey, 1915, according to Lawrence, 1951) with ranges of allagochrome values[a] found in each order*



[a] Relative allagochrome values of positive assays:

O.D. × vol × wt^{-1}:		
	0 to 5	= +
	5 to 10	= +2
	10 to 20	= +2
	20 to 40	= +4
	40 or greater	= +5

(—) indicates that some species of the order which were assayed tested negatively.

also present, the pigment responsible for this coloration is quite possibly allagochrome, which was first observed in extracts from this source (Habermann, 1960a,b). During extraction, color due to the oxidized form of the pigment seems to depend on the supply of oxygen.

More thorough analysis of the changing patterns of distribution of allagochrome with age of plant materials may eventually provide additional information concerning its function. It has thus far been found in seeds, seedling cotyledons, leaves, flowers, and fruits (a distribution which is certainly compatible with function as a terminal oxidase). Further data are needed to evaluate the correlation between presence of allagochrome and cyanide-resistant respiration indicated in Table 3. Beevers (1961), Bendall and Hill (1956), and Thimann, Yocum, and Hackett (1954) have suggested an electron pathway alternative to the cyanide-sensitive normal cytochrome system. The discovery of a cyanide-insensitive oxidation of cytochrome b_7 in the aroid spadix (Bendall and Hill, 1956) does not preclude the possibility of other cyanide-insensitive pathways.

Further studies of allagochrome distribution and its species specificity in composition may provide an additional tool for establishing taxonomic affinities, especially in the higher plant groups. This pigment appears to meet the requirements for usefulness in establishing phylogenetic pathways: it is variable at least in the ratio of chromophore to protein in preparations from different sources, and it is not universal in its distribution.

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