THE NATURAL PLANT COLORING MATTERS.

BY JOHN H. WURDACK.

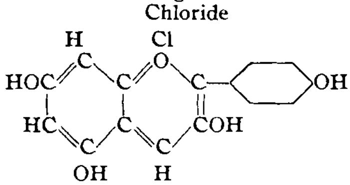
(*Continued from p. 315, April issue.*)

THE ANTHOCYANINS.

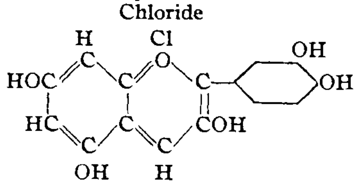
Anthocyanins are the red, violet, and blue coloring matters of flowers, many fruits, and some leaves. They are glucosidal, containing the sugars dextrose, rhamnose, or galactose, or combinations of these. They combine with acids or alkalies; indeed, the color depends on, and is an indicator of the reaction of, the cell sap: If it is acid in character we find the anthocyanin combined with a plant acid (malic, tartaric, citric, etc.) as a red or reddish pigment; if it is neutral the anthocyanin is free as a violet coloring matter; if it is alkaline the anthocyanin is found as a salt having a blue color. In some plants they are not only dissolved in the cell sap, but are also separated out as crystals or amorphous granules, separation occurring when the concentration becomes too high through loss of water during dry periods or through excessive accumulation of coloring matter.

On hydrolysis they are broken up into sugars and the corresponding coloring matters proper known as **anthocyanidins** which possess the same colors as the anthocyanins but are rarely found in plants. In some cases other hydrolytic products also appear, such as para-hydroxybenzoic acid or malonic acid. The parent anthocyanidins are **pelargonidin, cyanidin,** and **delphinidin,** whose chlorides have the following formulas:

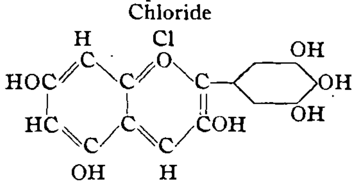
Pelargonidin Chloride



Cyanidin Chloride

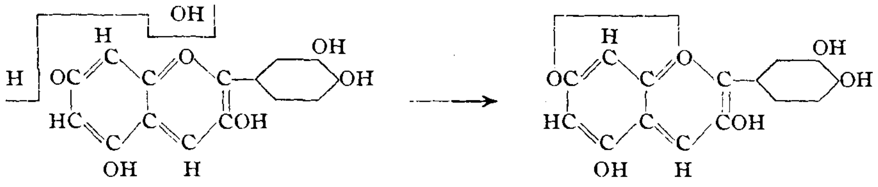


Delphinidin Chloride



In addition there are a number of homologues: **peonidin** being cyanidin monomethyl ether; **myrtillidin, petunidin,** and **ampelopsidin** being delphinidin monomethyl ethers; and **oenidin** and **malvidin** being delphinidin dimethyl ethers.

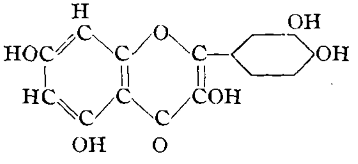
The above three formulas show the anthocyanidins united with hydrochloric acid as red colored products in which form they are most commonly obtained for examination; in the plant the red colors are instead united with organic acids, and carbohydrate groups are generally attached to the molecules, forming the glucosides. It will be observed that they are oxonium compounds containing the basic tetravalent oxygen. By replacing the chlorine with the hydroxyl group on neutralization with caustic alkalies, water simultaneously splitting off, the neutral violet color is produced as illustrated in the following hypothetical formulation:



Treatment with alkali results in the conversion of the neutral violet compounds to blue metallic salts by reaction upon the phenolic hydroxyl groups without disturbing the oxonium structure. Of course, treatment with acids results in the reverse changes, blue to violet to red. It will be noted that these color changes of the anthocyanidins (and anthocyanins, which react in the same manner) are the same as those with litmus.

The anthocyanins and the anthocyanidins in neutral or more so in alkaline solution lose their color by changing to pseudo-bases, which probably explains the loss of color in flowers; on acidulation the color returns. In the absence of excess acid the red anthocyanins (combined with acid) will also decolorize if the solution is dilute. The colorless pseudo-bases turn intensely yellow with alkali thus resembling those flavone derivatives which in neutral or acid solution are almost colorless, this fact being suggestive in growing white and yellow flowers. To illustrate the effect just described: Extract a red rose with aqueous alcohol and add caustic soda to the filtered liquid; it will turn intensely green, the green being a blend of the blue cyanin compound with the yellow produced by the action of the alkali upon the pseudo-base formed from part of the cyanin by the presence of water. If the filtered liquid is first acidified with a few drops of hydrochloric acid, there is formed the color salt only, which with the caustic soda gives a pure blue.

The anthocyanins and the anthocyanidins are closely related to the yellow colors of the flavonol group, the anthocyanins corresponding to the flavonol glucosides, and the anthocyanidins to the flavonols themselves; so far, none have been isolated corresponding to the flavones, but, in all likelihood, such will be found—the field is far from exhausted. It is possible to produce anthocyanins by reducing flavonol glucosides, and also to produce anthocyanidins in similar manner from the sugar-free flavonols; for instance, quercetin,



by treatment with magnesium and acid in the presence of mercury at 35° C. is converted to cyanidin; myricetin with magnesium powder and glacial acetic acid gives a green residue of a complex organo-magnesium derivative which with alcoholic hydrochloric acid yields delphinidin chloride.

The anthocyanins isolated by Willstätter and his co-workers from red, violet and blue flowers and fruits are as follows:

Pelargonin, callistephin, salvianin, all glucosides of pelargonidin;

Cyanin, idaein, chrysanthemin, asterin, keracyanin, prunicyanin, mecocyanin, all glucosides of cyanidin;

Peonin, a glucoside of peonidin;

Delphinin, violanin, both glucosides of delphinidin;

Oenin, a glucoside of oenidin;

Malvin, a glucoside of malvidin;

Myrtillin, althaein, both glucosides of myrtillidin;

Petunin, a glucoside of petunidin;

Ampelopsin, a glucoside of ampelopsidin.

The following tabulation of the anthocyanins and the results of their complete hydrolysis by boiling with (usually 20%) hydrochloric acid is interesting. By incomplete hydrolysis some of the diglucosides yield intermediate monoglucosides which are not mentioned here. In a few instances the information is incomplete because of the war conditions under which Willstätter and his co-workers accomplished part of the work:

Pelargonidin Group.

$C_{27}H_{31}O_{15}Cl$	$+ 2H_2O =$	$C_{15}H_{11}O_5Cl$	$+ 2C_6H_{12}O_6$	
Pelargonin Chloride		Pelargonidin Chloride	Dextrose	
$C_{21}H_{21}O_{10}Cl$	$+ H_2O =$	$C_{15}H_{11}O_5Cl$	$+ C_6H_{12}O_6$	
Callistephin Chloride		Pelargonidin Chloride	Dextrose	
???	$+ xH_2O =$	$C_{15}H_{11}O_5Cl$	$+ 2C_6H_{12}O_6$	$+ CH_2(COOH)_2$
Salvianin Chloride		Pelargonidin Chloride	Dextrose	Malonic acid in considerable amount

Cyanidin Group.

$C_{27}H_{31}O_{16}Cl$	$+ 2H_2O =$	$C_{15}H_{11}O_6Cl$	$+ 2C_6H_{12}O_6$	
Cyanin Chloride		Cyanidin Chloride	Dextrose	
$C_{21}H_{21}O_{11}Cl$	$+ H_2O =$	$C_{15}H_{11}O_6Cl$	$+ C_6H_{12}O_6$	
Idaein Chloride		Cyanidin Chloride	Galactose	
$C_{21}H_{21}O_{11}Cl$	$+ H_2O =$	$C_{15}H_{11}O_6Cl$	$+ C_6H_{12}O_6$	
Chrysanthemin Chloride		Cyanidin Chloride	Dextrose	
$C_{21}H_{21}O_{11}Cl$	$+ H_2O =$	$C_{15}H_{11}O_6Cl$	$+ C_6H_{12}O_6$	
Asterin Chloride		Cyanidin Chloride	Dextrose	
$C_{27}H_{31}O_{15}Cl$	$+ 2H_2O =$	$C_{15}H_{11}O_6Cl$	$+ C_6H_{12}O_6$	$+ C_6H_{12}O_5$
Keracyanin Chloride		Cyanidin Chloride	Dextrose	Rhamnose
$C_{27}H_{31}O_{15}Cl$	$+ 2H_2O =$	$C_{15}H_{11}O_6Cl$	$+ C_6H_{12}O_6$	$+ C_6H_{12}O_5$
Prunicyanin Chloride		Cyanidin Chloride	A hexose	Rhamnose
$C_{27}H_{31}O_{16}Cl$	$+ 2H_2O =$	$C_{15}H_{11}O_6Cl$	$+ 2C_6H_{12}O_6$	
Mecocyanin Chloride		Cyanidin Chloride	Dextrose	
$C_{28}H_{33}O_{16}Cl$	$+ 2H_2O =$	$C_{16}H_{13}O_6Cl$	$+ 2C_6H_{12}O_6$	
Peonin Chloride		Peonidin Chloride	Dextrose	

Delphinidin Group.

$C_{41}H_{39}O_{21}Cl$	$+ 4H_2O =$	$C_{15}H_{11}O_7Cl$	$+ 2C_6H_{12}O_6$	$+ 2C_6H_4OH.COOH$
Delphinin Chloride		Delphinidin Chloride	Dextrose	Para-hydroxybenzoic Acid
???	$+ 2H_2O =$	$C_{15}H_{11}O_7Cl$	$+ C_6H_{12}O_6$	$+ C_6H_{12}O_5$
Violanin Chloride		Delphinidin Chloride	Dextrose	Rhamnose
			not in molar amounts	
$C_{23}H_{25}O_{12}Cl$	$+ H_2O =$	$C_{17}H_{15}O_7Cl$	$+ C_6H_{12}O_6$	
Oenin Chloride		Oenidin** Chloride	Dextrose	
$C_{29}H_{35}O_{17}Cl$	$+ 2H_2O =$	$C_{17}H_{15}O_7Cl$	$+ 2C_6H_{12}O_6$	
Malvin Chloride		Malvidin** Chloride	Dextrose	
$C_{22}H_{23}O_{12}Cl$	$+ H_2O =$	$C_{16}H_{13}O_7Cl$	$+ C_6H_{12}O_6$	
Myrtillin Chloride		Myrtillidin* Chloride	Galactose	
$C_{22}H_{23}O_{12}Cl$	$+ H_2O =$	$C_{16}H_{13}O_7Cl$	$+ C_6H_{12}O_6$	
Althaein Chloride		Myrtillidin* Chloride	??	
$C_{28}H_{33}O_{17}Cl$	$+ 2H_2O =$	$C_{16}H_{13}O_7Cl$	$+ 2C_6H_{12}O_6$	
Petunin Chloride		Petunidin* Chloride	Dextrose	
$C_{22}H_{23}O_{12}Cl$	$+ H_2O =$	$C_{16}H_{13}O_7Cl$	$+ C_6H_{12}O_6$	
Ampelopsin Chloride		Ampelopsidin* Chloride	Dextrose	

* Monomethyl ethers
** Dimethyl ethers

Variation of color in flowers or other plant structures is due to one or several of the following causes:

1. Several different anthocyanins occurring in one plant or even one blossom, the various anthocyanins differing more or less in tint depending on the absence or presence of one or more methyl groups and upon the nature, number and method of union of the sugar molecules.

2. Difference in percentage amount, concentrated solutions differing from weaker ones not only in depth of shade but often also in tint.

3. Difference in reaction of the cell sap. In general, the anthocyanins are all blue in an alkaline cell sap, violet when neutral, and some shade of red when the reaction is acid; for instance, the blue of the cornflower is due to the potassium salt of cyanin (cyanin combined with the alkali potash), the violet of the larkspur is caused by the neutral form of delphinin, and the scarlet of the pelargonium depends on the tartrate of pelargonin (pelargonin combined with tartaric acid).

4. Admixture with yellow pigments.

The following list gives the anthocyanin content of various flowers and fruits based on dried material. A careful study of the list yields much interesting information.

Cornflower (*Centaurea cyanus*):

Blue of fields, 0.65–0.70% cyanin.
Rose-colored of fields, 3.75% pelargonin.
Dark purplish red, cultivated, 13–14% cyanin.

Rose:

White and yellow, hardly any anthocyanin.

Pink, a little cyanin.

Dark red, 2% cyanin combined with acid.

Orange Salmon-colored	, cyanin with carotin and a flavon glucoside.

Pansy:

Deep brown parts, anthocyanins with yellow pigments.

Deep yellow parts, 25% violaquercitrin and 0.6% carotin which latter is the chief source of color.

Deep bluish violet flowers, not less than 33% violanin.

Pelargonium:

Scarlet *P. zonale*, var. "Meteor," 6.6–7.1% pelargonin; up to 14.1% in old blossoms.

Violet-red var. of *P. zonale*, 2.8% of a mixture of chiefly cyanin and a little pelargonin.

Bluish rose of *P. peltatum*, 0.97% pelargonin.

Dahlia:

Deep brown-red garden dahlia (cactus dahlia), varieties: "Harold," "J. H. Jackson," "Matchless," "Night," "Othello," average not less than 19.4% cyanin.

Scarlet-red: "Rakete," 5.6% pelargonin; "Alt Heidelberg," 4% pelargonin.

Deep purple (cactus dahlia), disc florets, 23.7% cyanin; ray florets, 15.1% cyanin; some parts up to 30% cyanin.

Blue-red, contain cyanin.

Dark violet, contain pelargonin.

Orange-red Scarlet	, contain pelargonin with dahlia yellow.

Peony:

Red blossoms, 3–3.5% peonin.

Mallow:

Blue-violet blossoms of *Malva sylvestris* (wild or wood mallow), 6.4% malvin.

Hollyhock:

Blossoms of Althaea rosea, 11% althaein.

Larkspur:

Violet blossoms of *Delphinium consolida*, about 1.68% delphinin.

Salvia:

Scarlet-red flowers of *Salvia coccinea* and *S. splendens*, about 6% salvianin.

Winter Aster (Chrysanthemum):

A large number of scarlet-red, red, and dark red varieties of *Chrysanthemum indicum* contain chrysanthemin mixed in many cases with carotin and xanthophyll.

Florets of dark red "Ruby King," about 7% chrysanthemin.

Summer Aster:

Purplish red summer (China) aster (*Callistephus chinensis* Nees; syn. *Aster chinensis* L.), about 7.4% of a mixture of asterin (most) and callistephin.

Petunia:

"Karlsruher Rathaus" petunia (*P. hybrida* Hort.), about 15.4% petunin.

Poppy:

Corn poppy (*Papaver Rhoeas*) contains about 18% of a mixture of mecocyanin, which predominates, and of another anthocyanin whose anthocyanidin has not yet been obtained pure.

Scarlet gladiolus, contains a pelargonidin glucoside, probably pelargonin.

Zinnia elegans Jacq. *Gaillardia bicolor* Hook. *Helenium autumnale* L. *Gladiolus* (except scarlet) *Tulipa Gesneriana* L. *Tropaeolum majus* L. *Ribes rubrum* L. (berries)	contain cyanidin glucosides in larger or smaller amounts, together with carotin, xanthophyll and other pigments.

Grape:

Skins of fruit of *Vitis vinifera* from different sources contain oenin and some of the anthocyanidin, oenidin; some varieties contain very probably also a few units per cent. of a diglucoside.

Skins of berries of Wild Vine (*Ampelopsis quinquefolia* Michx; *Vitis hederacea* Ehrh.) contain ampelopsin.

Skins of berries of *Vitis vulpina* contain an unnamed monoglucosidic anthocyanin.

Bilberry (Whortleberry):

Skins of fruit of *Vaccinium Myrtillus,* contain myrtillin.

Cowberry (Mountain Cranberry):

Orange-colored berries of *Vaccinium Vitis-Idaea,* about 0.035% idaein combined with acid (citric, benzoic, tannic), chiefly in the skins.

Cherry:

Skins of fruit of *Prunus Avium,* contain keracyanin.

Sloe (Black Thornberry):

Skins of fruit of *Prunus spinosa,* contain prunicyanin.

Plum:

Skins of fruit of *Prunus domestica,* contain a cyanidin glucoside.

Black Wild Plum:

Contains myrtillin.

Raspberry
Mountain Ash berry } , contain a cyanidin glucoside.

The work has been progressing since this article was written two years ago. Since that time a number of other anthocyanins have been found: **Betanin** in red beets to the extent of 0.22% of beet meal, the anthocyanidin being betanidin; **raphanin** in radish peelings, the anthocyanidin being pelargonidin; **rubin,** a diglucoside of cyanidin, in a dark violet variety of radish; **rhinanthocyanin,** a product of the hydrolysis of rhinanthin, a constituent of the seeds of *Alectorolophus hirsutus.* Free anthocyanidins are reported in the flowers of *Pelargonium* and of *Papaver Rhoeas,* in the red or olive leaves of *Prunus Pissardii,* and in the fruits of *Ruscus aculeatus* and *Solanum dulcamara.*

SPRING AND AUTUMN LEAF COLORATION.

Mention has already been made how yellow leaf coloration occurs in autumn by the bleaching of chlorophyll when the vital activity of the leaf is waning. The yellow coloring agents carotin, xanthophylls and sometimes flavonols had been present throughout the entire life of the leaf but had been covered up by the more preponderant chlorophyll color, becoming visible only with the disappearance of the chlorophyll to lend beauty to the autumn scenery.

The red leaf coloration, however, does not exist preformed in the leaves, at least not as a rule. In some few instances autumn and winter reddening is due to red carotinoids, rhodoxanthin being involved in the case of many conifers, lycopin being concerned with certain conifers under tropical conditions. For the most part, however, the red color of spring and autumn leaves is due to anthocyanins.

Many people well acquainted with the beauty of autumn woods have little idea that in spring the young leaves just burst from their buds are frequently red, producing a color effect somewhat resembling that of autumn, although not so pronounced because of the dearth of leaves. This red color of unfolding leaves is usually due to anthocyanins; later some ferment changes these to sugars and the anthocyanidins which latter are then converted into colorless compounds (pseudobases?) which may become oxidized to yellow flavonols, these being hidden by chlorophyll later and in summer. In autumn a reverse change occurs, these yellow flavonols being reduced back again, resulting in anthocyanins to constitute the color in red autumn leaves.

In the production of anthocyanins light is a necessary agent; for instance, green apples will not redden if kept dark. In the case of leaves, during the waning vitality in autumn no further chlorophyll is being produced and that still present bleaches away, permitting the yellow of carotin, xanthophylls and possible flavones or flavonols to show; and if flavonols are present the light being better admitted to the leaf changes them to anthocyanins. In autumn woods the writer has frequently seen instances where an overlapping leaf left a distinct outline upon the under leaf, the shaded portion in the lower leaf being green or yellow because of more or less protection from the light, the exposed portion appearing bright red. A little reflection will enable a person to see how, depending on conditions, it is possible for leaves in autumn to show green, yellow, red, or all three colors in the same tree or even leaf.

The production of anthocyanins is also closely related to sugar content, sugars being necessary for production of glucosides. Sometimes in early autumn a tree will be found having only a few red or partially red leaves, the rest being still green. Examination of such leaves will show a break in a rib or in the petiole; the green leaves are still manufacturing sugar which is being transported to other plant parts, but in the broken leaf the path of transport was interrupted and sugar thus accumulated; and, as the chlorophyll of the broken leaf was bleached away by light, the yellow flavonols were converted in the presence of the light to the glucosidal anthocyanins. Later the same thing happens to the other leaves where sugar accumulates because the avenue of transport is interrupted by the growth of the corky layers across the bases of the leaves, which layers both cut away the leaves from the stems and also close the wounds resulting in the twig from the dropping away of the dead leaves. Any instances of premature coloration of branches or entire trees will betray the cause in some injury—a break, damage to the bark or roots, an insect or fungoid blight—causing loss of vitality and accumulation of sugar.

Another favoring agent for anthocyanin production in autumn leaves is a temperature rather low but above the freezing point, such condition abruptly checking vitality while the leaf is still full of sap. Alpine and arctic plants often have a red color because they are subjected to conditions favoring red leaf coloration; *i. e.*, low temperature which inhibits chlorophyll formation, bright light, and the production of sugar which is accumulated rather than starch because the presence of sugar in the cell sap lowers its freezing point, enabling the plant to withstand lower temperatures. Any visitor to more northern, therefore cooler, latitudes has probably observed the extreme brilliance of the reds of those autumn woods and any climber of real mountains must have observed the brightness of flower colors at high elevations where a clear cool atmosphere and bright skies prevail; for instance, the painted cup of our western states is somewhat disappointing on the plains but in the mountains its vivid, fiery crimson makes it the most conspicuous of flowers.

Perhaps merely a coincidence or possibly a necessary relationship, tannins are always associated as a component in plants with red colored leaves. We can readily recall such cases in the sumacs, red and scarlet oaks, blackberry leaves, etc.

The brown coloration of dead leaves finds an explanation in the fact that colorless water- and alcohol-soluble chromogens (probably flavones, flavonols) of leaves give golden yellow salts with alkalies which oxidize to a dark brown color and it is thought that these brown oxidation products of the yellow alkali salts of the colorless chromogens play the chief rôle in the post-mortal browning of leaves, a reaction accelerated, no doubt, by oxidizing enzymes. In some leaves the brown may blend with remaining leaf carotinoids to a golden bronze.

Our article would not be complete did it not refer to:

Phycoerythrin, the principal pigment of red seaweeds which also contain carotin and xanthophyll, and

Phycophaein, the coloring matter in dried brown seaweeds, it being a post-mortal oxidation product of colorless chromogens present in the fresh plant, carotinoids being also present.

Nor should we omit mention of the coloration of white flowers, some cultivated white leaves, etc., which, of course, is the natural cellulose color untinctured by any foreign tints. The brown color of tree bark, dead leaves and dead vegetation in general is in some cases apparently an oxidation product of flavones or flavonols as previously indicated; in others, the result of the oxidizing of tannins in cell walls when exposed to light and air.

For further information the writer would suggest reference to those books and journals of which he has made liberal use in this article; *viz.*,

Liebig's Annalen, for the original papers on chlorophyll, carotinoids and anthocyanins by Willstätter and co-workers.

Chemical Abstracts and the *British Chemical Abstracts*, for an abbreviated account in English of the above and related papers.

"Untersuchungen über Chlorophyll," by Willstätter. (Julius Springer, Berlin.)

"Carotinoids and Related Pigments," by Palmer. (Chemical Catalog Co.)

"The Natural Organic Colouring Matters," by Perkin and Everest. (Longmans, Green and Co.)

"An Introduction to the Chemistry of Plant Products," by Haas and Hill (Longmans, Green and Co.)

"A Textbook of Botany for Colleges," by Ganong. (Macmillan Co.)

A STUDY IN PHARMACY.

The article which follows is of unusual interest not only because of the value of the research, even though many of Nature's methods involved remain as unexplained mysteries, but also because it presents a study of many years ago. Liberty is taken in quoting lines, which have reference to thoughts that preceded, from one of the author's letters: "The change of starch stored in a bulb, when comes the time to change, the deposition of starch in that bulb, when comes the time for the plant's food for the coming spring to be placed therein, the same mysterious water moving upward and downward as temperature changes and produces this marvelous phenomena—staggers me when thinking the subject over."—Editor.