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An experimental investigation of coloring matter in Canna Indica flowers

James Clinton Vogt
University of the Pacific

AN EXPERIMENTAL INVESTIGATION OF COLORING MATTER IN CANNA INDICA FLOWERS

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Department of Chemistry

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by

James Clinton Vogt

June 1961

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TABLE OF CONTENTS

INTRODUCTION

The chemist has long been attracted to the investigation of natural and artificial coloring matters for a variety of reasons, including not only color-pleasure, the industrial importance of dyestuffs and pigments, but also on account of the fact that visible color more than any other property facilitates the experimental study of organic substances whether by analysis or by synthesis. Color furnishes a standard of homogeneity or a measure of concentration, is an invaluable guide in the search for methods of separation and purification, and it at once indicates by its appearance or disappearance the occurrence of a chemical reaction.

Although the chemical nature of this coloring matter was not known by early man, the search for this knowledge was started many years ago. As long ago as 1664, Robert Boyle published "Experiments and Considerations Touching Colours," in which he examined results when extracts from flowers were treated with acids and alkalies.

In cases where plant pigments could be utilized for dyeing, the practical value became so great that as soon as a structural theory of organic chemistry had been

developed, chemical constituents of pigments were determined and the materials synthesized. This was particularly the case where production of natural substances could not keep up with the demand.

The research was carried out for the purpose of isolating and identifying the coloring matter in flowers of the canna indica (canna lily) variety. It was done by extracting the pigment from flower petals with methanol, amyl alcohol, and other solvents, and crystallizing the pigment as a salt. Physical properties such as solubility, color reactions, spectra and others were examined for this pigment. Chemical properties and degradation products were also examined in order to identify the chemical structure of the pigment.

Two important groups of pigments that account for most red, blue and yellow colors in flowers are known as anthocyanins and anthoxanthins. In general the darker pigments are due to the anthocyanin group and the lighter pigments to the anthoxanthin group. Members of both the anthocyanin and the anthoxanthin groups of pigments have a heterocyclic structure and a large number have been isolated and identified.

Red and blue water soluble pigments belong to the anthocyanin group while yellow water soluble pigments be-

long to the anthoxanthin group which contains flavone or flavonol compounds (20). The anthoxanthin pigments constitute the largest and most widely distributed group in nature, and are often associated with other pigments and tannins.

A second classification of plant pigments is the plastid group, the members of which are associated with the protoplasmic structure of the plant, and a second group which generally exists in solution in the cell sap and are called anthocyanins. The plastid pigments are not soluble in organic solvents such as ether and benzene. Included in this group are carotenes, zanthoplylls and chlorophylls. They differ considerably from the water soluble pigments. The anthocyanins belong to a larger group of glycosides. These anthocyanin pigments account for shades of blue, purple, violet, mauve, magenta, and nearly all the reds in flowers, fruits, leaves and plant stems. They are always combined with sugars, are water soluble and usually dissolved in the cell sap. They may, however, exist in either an amorphous or a crystalline state. (5)

Sugar free pigments or aglucons are called anthocyanidins. Anthocyanins were extracted early from flowers and leaves with amyl alcohol, then converted to the corresponding anthocyanidins on the addition of acids such as hydrochloric acid.

St. Jonesco (21) concluded that the presence of anthocyanins, anthocyanidins, and pseudo bases together indicates their close interrelationship and their relation to the disappearance of the red color in plants. Anthocyanidins appear characteristic of pure red organs and do not exist as colored pigments in a free condition in all colored tissue containing anthocyanin. It is further concluded that canna, among other flowers having purple-red, purple, violet or blue colorations do not contain anthocyanidins but contain an anthocyanin pigment, the coloration of which varies in each species of plant, and an intense pure yellow pigment which dissolves in amyl alcohol like the anthocyanidins but is not a pseudo base.

Robinson (10) concluded that anthocyanins and flavones can be regarded as being produced divergently from two plant substances. One is present in a limited amount and is a component of all pigments while the second is produced in an amount and variety dependent on factorial influences and interactions.

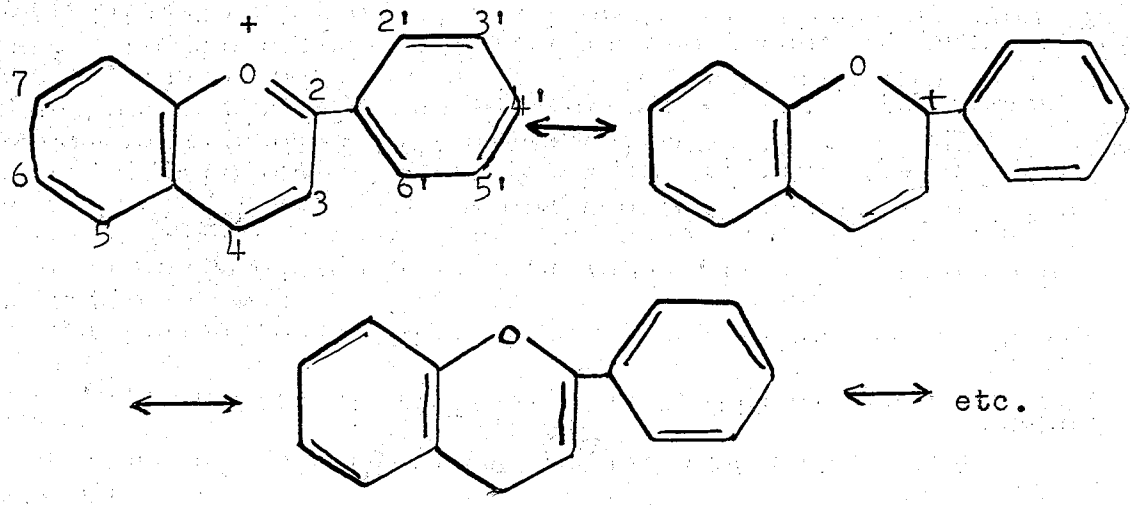
Principal factors affecting colors of anthocyanin and anthoxanthin pigments in nature are (1) nature and concentration of anthocyanin, anthoxanthin pigments and other colored substances present; (2) the state of anthocyanin and anthoxanthin pigments in solution, which is determined

by the pH of the cell sap and the presence or absence of protective colloids of the polysaccharide group; and (3) the presence or absence of other pigments such as tannins, also the effect of alkaloids and traces of iron and other metals that form complex combinations. With regard to variable colors of flower petals, Robinson (11) stated that the factor is the concentration of anthocyanin and the ratio of this to the concentration of co-pigments of the tannin and flavonol classes. Pale contains much less anthocyanin than darker colors and with higher concentration the co-pigment is unable to modify the color of all the anthocyanin. The character of the anthocyanidin resonating system is seriously affected by the presence of mineral salts and by the pH of the environment. Consequently colors of pigments of flowers sometimes vary markedly with type of soil. Thus cyanin, the pigment of red rose and blue cornflower, is pale violet in neutral solution, red in dilute acid and blue in dilute base, through which color reactions it acts as an indicator. The color of flowers, however, should not be taken as a reliable measure of the environmental pH.

Cram and Hammond (2) state that anthocyanins are one of the main classes of plant pigments. They occur in flowers and fruits as glycosides, hydrolysis of which provides colored aglycones known as anthocyanidins. Vivid blues and reds of anthocyanins are associated with the

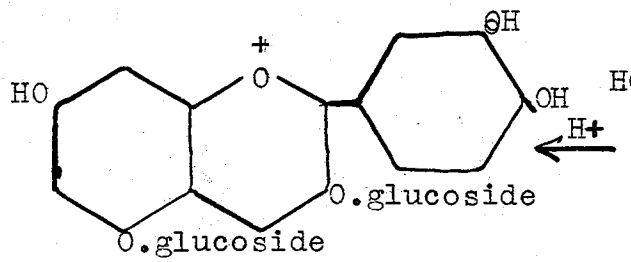
distribution of positive charge throughout an aryl-substituted chroman ring system (I).

I.

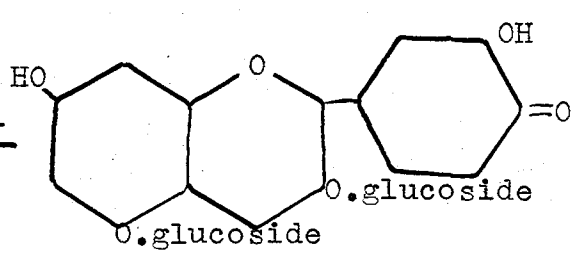


Anthocyanidins are usually isolated in the form of chloride salts, are frequently hydroxylated in some or all of the 3, 5, 7, 3', 4', 5' positions. Using this explanation, structural formulas can be shown for the red cyanin cation (II), the violet cyanin color base (III), and the blue cyanin anion (IV). Cyanins in general differ only in the number and position of hydroxyl groups and in the character of sugars to which they are attached.

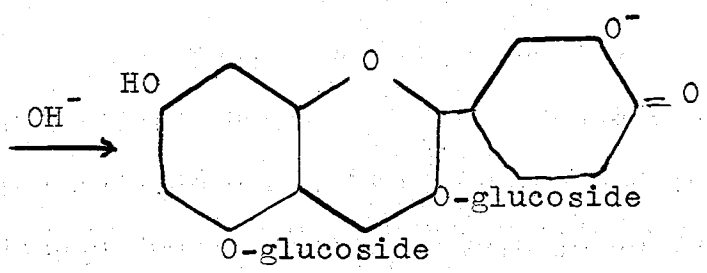
II.



III.

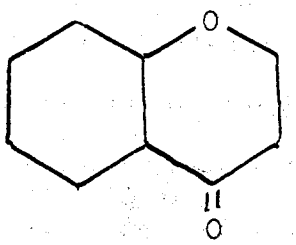


IV.

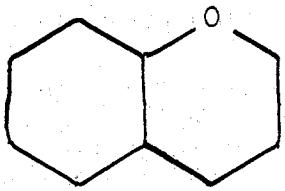


The basic unit structure of anthoxanthin is benzopyrone (V) and that of anthocyanin is benzopyrylium (VI), both of which contain the basic quadrivalant oxygen atom.

V.

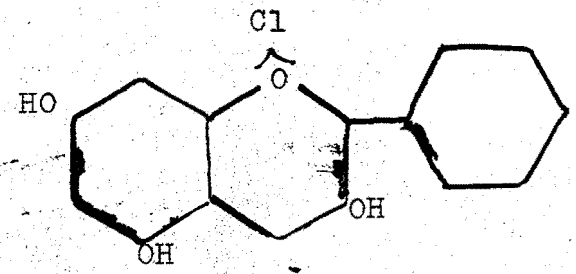


VI.



This quadrivalent oxygen atom can form additive compounds with acids producing oxonium salts. These oxonium salts of anthoxanthins are unstable, are usually precipitated by using lead acetate. Oxonium salts of anthocyanins on the other hand are very stable and frequently occur as such in the plant. Dilute acids can usually be used to isolate them from their solutions. Hydrochloric acid, for example, can be added to get readily crystallizeable compounds such as 3, 5, 7, trihydroxyflavylium chloride (VII) which is the simplest intact structural unit of all the anthocyanin salts.

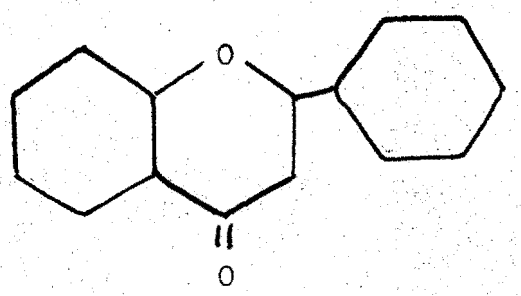
VII.



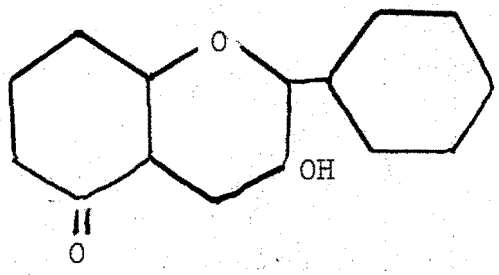
The chloride salts of anthocyanins are called anthocyanidins.

The simplest structural formula of anthoxanthins is flavone (VIII) and the basic structure of the flavonols is 3 hydroxyflavone (IX).

VIII.



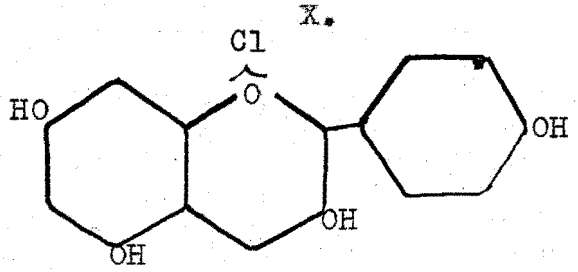
IX.



Hydroxy groups may be attached to the 3, 5, 7, 3', 4', and/or 5' positions to form the different anthoxanthins found in nature.

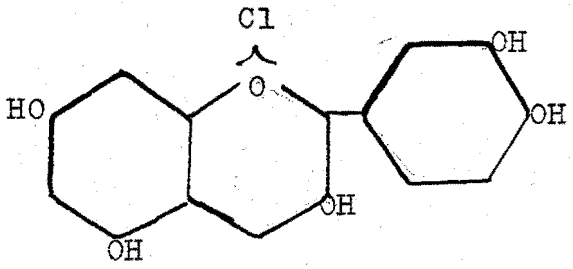
The simplest anthocyanidin found in nature is pelargonidin (X). This chloride salt of the anthocyanin from which it is derived has hydroxy groups in the 3, 5, 7, 4' positions.

X.

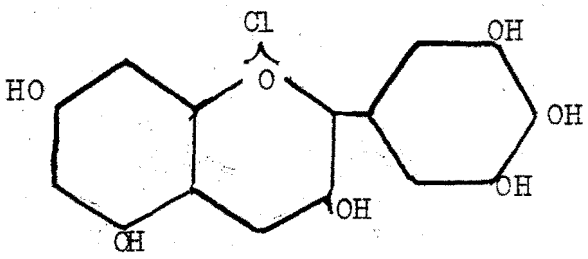


There are two other fundamental type groups; cyanidin (XI), which is 3, 5, 7, 3', 4' pentahydroxy 2 phenylbenzopyrilium chloride, and delphinidin (XII), which is 3, 5, 7, 3', 4', 5' hexahydroxy 2 phenylbenzopyrilium chloride.

XI.

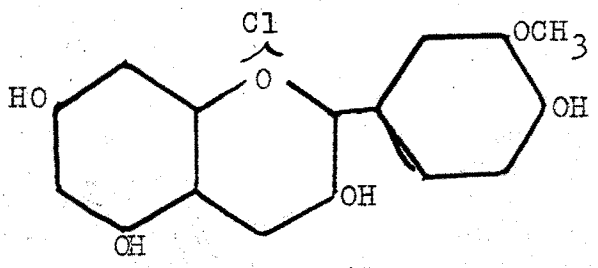


XII.

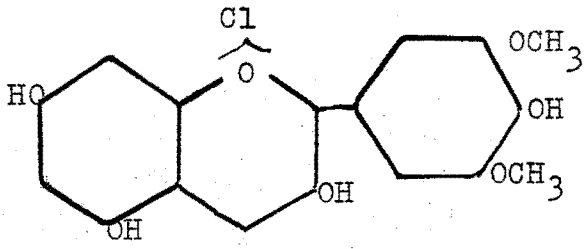


Three other fundamental type groups are ethers of cyanidin or delphinidin. These three are peonidin (XIII) which is 3, 5, 7, 4' tetrahydroxy 3' methoxy 2 phenylbenzopyrilium chloride; malvidin or syringidin (XIV) which is 3, 5, 7, 4' tetrahydroxy 3' 5' dimethoxy 2 phenylbenzopyrilium chloride; and hirsutidin (XV) which is 3, 5, 4' trihydroxy 7, 3', 5' trimethoxy 2 phenylbenzopyrilium chloride.

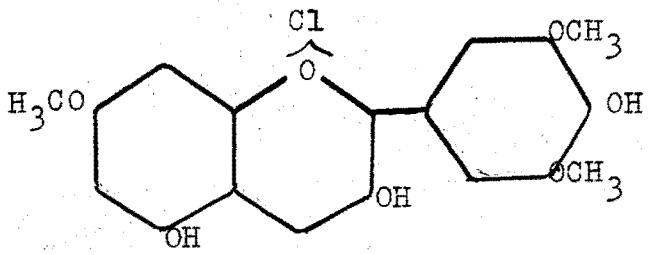
XIII.



XIV.



XV.



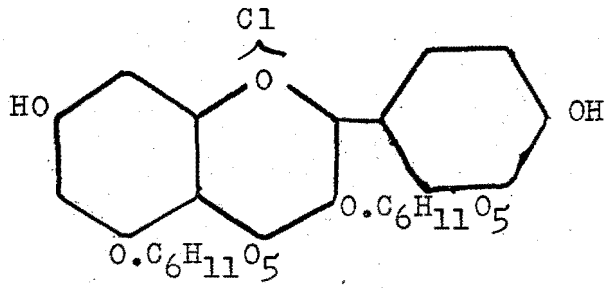
All six types have been synthesized so the structure is known.

Robinson and Robinson (17) concluded that almost the whole range of anthocyanin pigments of flowers, fruits and blossoms is derived from the first three fundamental types of anthocyanidins listed, namely, pelargonidin, cyanidin and delphinidin, by various substitutions in the hydroxy groups. They gave as exceptions the bluest anthocyanin found in the beet Celosia cristata, and Atriplex hortensis as being nitrogenous pigments. At the other end of the scale the most yellow anthocyanin noted in Papaver alpinum and the yellow Iceland Poppy is related to the flavones.

Anthocyanins are glucosides of anthocyanidins, sugars being attached to the 3 or the 3, 5 positions, the 3, 5 diglucosides being the most common. Some of the sugars are monoglucoside, monogalacticide, rhamnoside or pentoside.

All members yield, when boiled with a dilute mineral acid such as hydrochloric, an anthocyanidin and a sugar or several sugars, Gilman (5). Some of the members, for example delphinin, yield in addition to the anthocyanidin and a sugar or sugars, a third component which is invariably an organic acid. Pelargonin (XVI), the 3, 5 diglucoside of pelargonidin is typical of the 3, 5 diglucosides, the most widely distributed group of anthocyanins.

XVI.



The acid radicals can be either in ester combination with one of the hydroxyl groups of the pigment nucleus or attached to an hydroxyl of the sugar component.

Willstatter and co-workers (23, 24, 25, 26) established the main features of the chemistry of anthocyanins

which were recognized as saccharides, occasionally acylated of the anthocyanidins. They found that the anthocyanins exhibit amphoteric character, forming salts with both acids and bases. Thus the violet pigment cyanin, which can be isolated from blue cornflowers, red roses, deep red dahlias and other flowers forms a blue sodium salt and a red hydrochloride. Hydrolysis of the latter by hot aqueous hydrochloric acid into cyanidin chloride and glucose can be shown by the following equation.

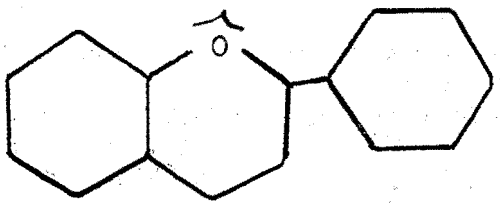
$$C_{27}H_{31}O_{16}Cl + 2H_2O = C_{15}H_{11}O_6Cl + 2C_6H_{12}O_6$$

cyanin chloride — cyanidin chloride — glucose

Other anthocyanin chlorides can be hydrolized with water to give the anthocyanidin chloride and the sugar that was attached.

The occurrence of the 2 phenylbenzopyrilium nucleus (XVII) in various anthocyanins was originally established by Willstatter (23) through an alkaline fusion of sugar free pigments. Empirical formulas of the three parent

XVII.



classes of anthocyanidins show that they differ from each other by a single oxygen atom.

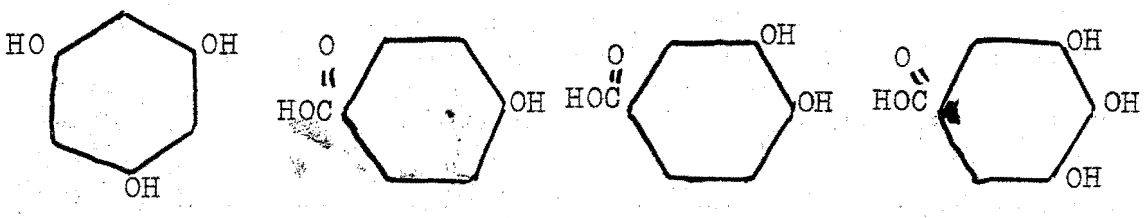
Pelargonidin chloride $C_{15}H_{11}O_5Cl$ or $C_{15}H_7OCl(OH)_4$

Cyanidin chloride $C_{15}H_{11}O_6Cl$ or $C_{15}H_6O_2Cl(OH)_5$

Delphinidin chloride $C_{15}H_{11}O_7Cl$ or $C_{15}H_5O_3Cl(OH)_6$

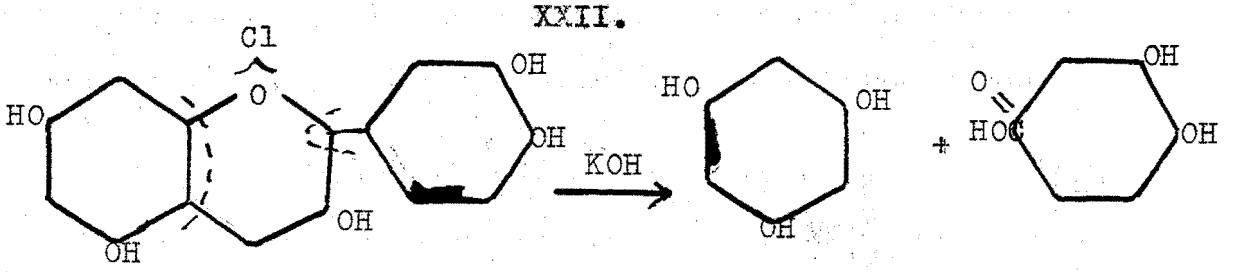
These anthocyanidins, which are free of sugars, degrade upon fusion with potassium hydroxide into two simple products, one of which is a phenol, and the other a phenolcarboxylic acid. The phenol is phloroglucinol (XVIII) in all three reactions, and the phenolcarboxylic acid is in the order named above, respectively; p-hydroxybenzoic acid, (XIX) protocatechuic acid (XX) and gallic acid (XXI).

XVIII. XIX. XX. XXI.



The following reaction (XXII) represents the degradation of cyanidin.

XXII.



Cyanidin Phloroglucinol Protocatechnic acid

Precise nature of the phenyl residue in position 2 and the points of linkage of the sugar residues have been

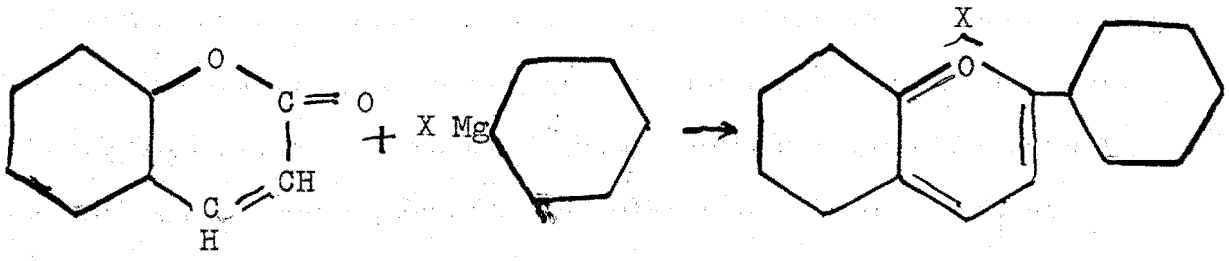
proven by Karrer (6, 7, 8) and considered very reliable. Later degradations of sugar free pigments with dilute barium or sodium hydroxide (10%) in an atmosphere of hydrogen, also degradations with hydrogen peroxide helped to establish positions of the methoxy groups of peonidin (XIII), malvidin (XIV), and hirsutidin (XV). The hydrogen peroxide used by Karrer opened the ring structure between the 2 and 3 carbon atoms without removing the sugar residues or the methoxy groups. Karrer also found the position of the sugar on the anthocyanins by methylation of the anthocyanins followed by the removal of the sugar group and the identification of the unmethylated position that the sugar had originally occupied. This latter method established the location of the sugar residue of the monoglucosides as always being in the 3 hydroxyl position of the anthocyanin nucleus. In the case of the diglucosides he found that the second sugar residue is generally, but not always, attached to the 5 position.

The procedure followed by most investigators today is the degradation of the anthocyanidin with 10% barium hydroxide in an inert atmosphere in order to prevent side oxidations.

The earliest conclusive contributions in the synthesis of anthocyanidins and the anthocyanins were made by

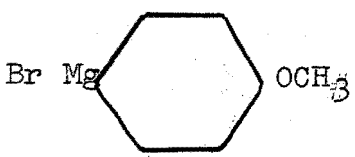
Willstatter (22, 23, 24, 25, 26) and the Robinsons (10, 12, 13, 14, 15) working independently. Willstatter, the first investigator to synthesize anthocyanidin, used Grignard reagents with 3 methoxy coumarins to produce the desired anthocyanidins. He used this method to prepare pelargonidin and cyanidin. This general method, the addition of aryl Grignard reagents to coumarins, is shown in the following equation (XXIII).

XXIII.

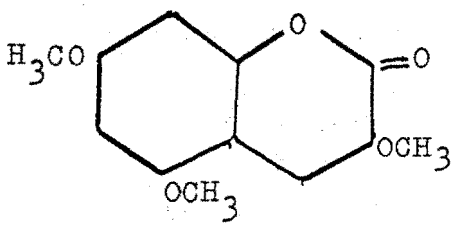


Willstatters synthesis of pelargonidin is: p-anisylmagnesium bromide (XXIV) reacted with 3, 5, 7 trimethoxycoumarin (XXV) which produces the carbinol base, pelargonidin tetramethyl ether (XXVI) which is acidified to the chloride of pelargonidin tetramethyl ether (XXVII). The ether is then heated with concentrated hydrochloric acid in a sealed tube causing the methoxy groups to hydrolize off to give pelargonidin chloride.

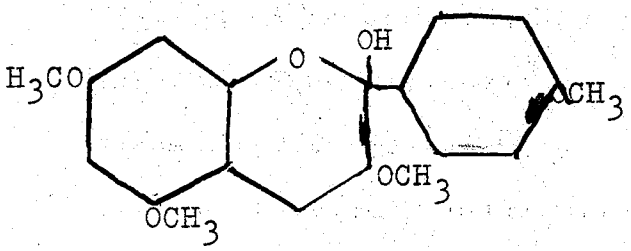
XXIV.



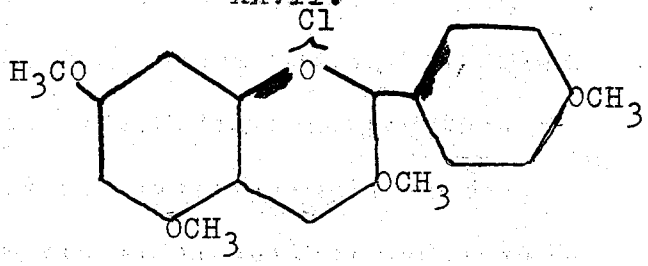
XXV.



XXVI.

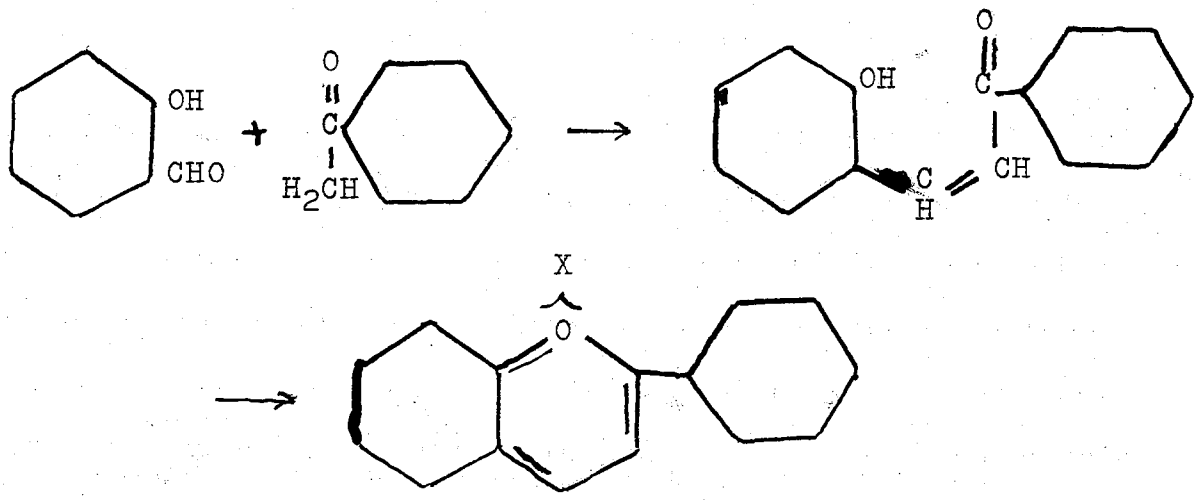


XXVII.



Robinson on the other hand employed a second general method, the condensation of o-hydroxybenzaldehydes with appropriate ketones to form 2 hydroxychalcone which is converted to the anthocyanidin with hydrochloric acid causing ring closure. This method is shown by the following equation (XXVIII).

XXVIII.



Robinson, who probably did more work on synthesis of anthocyanidins than any other investigator used this second method with success in the synthesis of all the anthocyanidin types. He even synthesized several of the natural occurring anthocyanins, a more difficult and much more

important achievement, since his method proved that anthocyanins could be made in the manner made in nature instead of being in the form of a salt. These investigations of Robinson, as well as Willstatter, have confirmed the parent types of the class, originally assigned by Willstatter, namely pelargonidin, cyanidin and delphinidin.

Other early investigations were attempted by Everest (3, 4) who reduced flavones with zinc dust and hydrochloric acid; Willstatter and Mallison (23) who reduced quercetin in methyl alcoholic hydrochloric acid to cyanidin chloride using magnesium, zinc dust or sodium amalgam as a source of hydrogen; Malkin and Nierenstein (9); Asahina and Inubuse (1) and others. None of these investigations, however, were as significant as those of Willstatter and Robinson. Some were merely qualitative, others produced exceedingly small yields, still others gave products which were difficult to isolate.

Since the members of this group are found in the sap of plant cells, they are all to some degree soluble in water, quite soluble in hydroxylic solvents and insoluble in non-hydroxylic solvents such as ether, benzene and chloroform.

Characteristic color reactions were investigated early by the Robinsons (16, 17, 18, 19). These qualita-

tive methods are considered very accurate and are useable for crude extracts since only one pigment is usually involved in the color of a plant. Some of these important properties of the anthocyanidins are summarized in the following paragraphs.

Delphinidin is bluish red in aqueous solution, pelargonidin red while the other four are violet red. In water pelargonidin, delphinidin and peonidin are readily soluble while the others are only slightly soluble. Cyanidin and delphinidin will give amyl alcohol an intense blue color on the addition of sodium acetate and ferric chloride, while the others give no reaction to this test. Fehlings solution can be reduced in the cold by cyanidin and delphinidin whereas pelargonidin must be warmed and the other three anthocyanidins must be boiled to produce the same reduction. In soda solution all types give a violet to blue color change. In aqueous solution the red color of pelargonidin fades on standing, that of delphinidin fades slowly in the cold, rapidly when heated. The other anthocyanidins must be heated for the color to fade.

Addition of 10% aqueous sodium hydroxide to a dilute solution of anthocyanidin which is then shaken with air will destroy petunidin and delphinidin at once while other

anthocyanidins are relatively stable to this test. All can be extracted to some extent by an organic mixture of anisole and ethyl isoamyl ether with 5% picric acid except delphinidin. A pink tint is imparted to a mixture of cyclohexanol and toluene by cyanidin while malvidin gives the mixture a faint blue color. Peonidin and pelargonidin are extracted in large amounts while delphinidin and petunidin are not extracted at all.

Distribution between 1% hydrochloric acid and a mixture of cyclohexanol (1 volume) and toluene (5 volumes) indicates that delphinidin and petunidin are not extracted. Malvidin imparts a faint blue color to the organic layer, cyanidin a pale rose color. Peonidin and especially pelargonidin are extracted to a considerable extent.

The Robinsons (18) concluded that certain substances called co-pigments influenced color anthocyanin and anthocyanidin solutions. These effects are detected in strongly acid solutions and the presence or absence of these substances is undoubtedly a factor to be taken into consideration. These natural occurring co-pigments include anthoxanthins (flavones and flavonol saccharides, etc.), tannins and other substances. Circumstances point to co-pigmentation of anthocyanins, for example the pelargonin salt in the flower petal. Finally probably traces of iron and other

inorganic substances may affect flower color. As an example it has been observed that when stalks of red hydrangea are immersed in very dilute aqueous ferric chloride the flowers slowly become blue. Ashes of many flowers contain 1 to 2% ferric oxide and the anthocyanin test for iron is one of the most delicate known.

As mentioned earlier these pigments are amphoteric substances which form true oxonium salts with acids. These salts are remarkably stable and have extraordinary crystallizing properties. Consequently in final stages of isolation the pigment is usually converted into its hydrochloric or picric acid salt. Acid salts of anthocyanins and anthocyanidins are usually red, metallic salts with bases blue and neutral salts purple.

Anthocyanins have an affinity for metals as noted in the canning industry by deteriorization of the tin cans and the color change of the canned fruit. This is due to the fact that metal salts of the anthocyanins are insoluble and therefore precipitated on the inside of the can.

Strong absorption powers are possessed by the anthocyanins and anthocyanidins over a wave length range of 6000 to 2000 angstrom units. The sugar free pigments and the glycosides are practically the same. Maximum absorption, the cause of color, lies in the visible spectrum with all types at approximately 5000 angstroms and all have

a band in the vicinity of 2700 angstroms.

Flowers and fruits of plants contain many different glycosidic combinations of various anthocyanins, with varying attachments of sugar molecules yielding different compounds of varying color and shades of color. The amount of anthocyanins in flowers and plants varies over a wide range. Typical extremes are cyanin, only 0.75% dry petal weight in blue corn flower and 14% in the dark green garden variety, and in red dahlias over 20.0%. Anthocyanins in addition to being found in flowers and fruits are also present in leaves of certain trees.

Isolation of the principal types usually proceeds along the following lines. Extraction is carried out with methyl or ethyl alcohol followed by hydrochloric acid which is bubbled through the extract to change the anthocyanin to its corresponding anthocyanidin. This crude chloride is then precipitated by the addition of ether, purified by redissolving in aqueous hydrochloric acid, followed by adding quantities of alcohol and ether for reprecipitating the salt. Final recrystallization can be accomplished with alcoholic hydrochloric acid or aqueous alcoholic hydrochloric acid.

Recent investigators have found that some anthocyanins can be purified through chromatographic absorption. Much work has been done in this field and many articles have been published reporting the progress made.

EXPERIMENTAL RESEARCH

Petals of Canna Indica flowers were picked from plants in the southeast corner of the College of the Pacific campus. The pure red colored petals were carefully separated for this research, and the yellow and multicolored petals discarded.

The petals were then dried in the open air for two weeks. When thoroughly dry they were mixed with dry methanol in a Warring blender. Dry hydrogen chloride gas was generated by dropping concentrated hydrochloric acid into concentrated sulfuric acid and bubbled into the methanol petal mixture until saturation. Color of the mixture was dull brownish red at the beginning of this step and a bright red color at the end indicating the formation of an anthocyanidin.

The methanol containing the anthocyanidin was then filtered out using aspirator filtration and the extracted petals were discarded as waste.

The addition of dry ether to the methanol and anthocyanidin caused the formation of a somewhat dull reddish jel like precipitate. Excess methanol was driven off by heating leaving a supersaturated solution of anthocyanidin. Evaporation was carried out under vacuum slightly over room temperature to avoid decomposition.

The supersaturated methanol solution was then cooled and the crystallized anthocyanidin separated out by filtration. The crystals were further dried in a vacuum dessicator to constant weight. Color of the crystals was reddish brown.

The crystals were tested for solubility in water and various other solvents. They were found to be somewhat soluble in water giving a light red colored solution which color faded on standing. The crystals were moderately soluble in methanol and ethanol, insoluble in ether, benzene, carbon tetrachloride and other non polar solvents. They were only slightly soluble in the "universal solvent" dimethyl formamide. In ten percent sodium hydroxide solution color changed to brownish blue indicating formation of the color base salt, the blue anion, as explained on page 6, and typical of almost all the anthocyanidins.

A second pigment extraction was carried out with amyl alcohol giving a blue green extract. The color deepened to an intense blue upon the addition of dry hydrogen chloride gas. This extract was filtered and dry ether added to the filtrate. The excess amyl alcohol was driven out by heat, the supersaturated solution cooled, filtered and the crystals dried.

The addition of sodium acetate and a trace of ferric chloride produced a color change somewhat to blue green

and on heating to a dark blue black. Cold Fehlings solution caused the color to change to black and on heating slightly to a reddish brown showing the reduction of the Fehlings solution. Sodium carbonate solution of the crystals was reddish brown in color turning to a blue wine color when warmed.

A third extraction made with a mixture of one volume of cyclohexanol and 5 volumes of toluene extracted enough pigment to color the solution a pinkish orange.

Dilute barium hydroxide was added to the solution until a dark colored precipitate settled out. A decomposition point could not be determined because of the dark color. The addition of a small amount of hydrogen chloride caused the formation of the anthocyanidin again as a precipitate, assumed to be the barium salt of canna indica anthocyanidin.

A weighed amount of dry petals was placed in a soxlet extracter and methanol used to carry out another extraction. The resulting extraction was cherry red in color, which color deepened to darker red on the addition of hydrogen chloride. Passing ether into the mixture again resulted in the formation of dull reddish colored jel like precipitate. Again the excess methanol was driven off by

heat and the supersaturated solution allowed to cool, precipitating the anthocyanidin, which was then separated by filtration. After drying these crystals to constant weight in a vacuum dessicator the mean percentage of anthocyanin in the dry petals was calculated to be 21.88 percent.

The spectrum of this canna indica anthocyanidin in ethanol was observed in a Beckman Model B spectrophotometer from 360 to 650 millimicrons.

The canna anthocyanidin was fused with potassium hydroxide, yielding as chief degradation products a phenol and a potassium salt of a phenolcarboxylic acid. Diethyl ether was added to the alkaline solution and the ether evaporated. The remaining residue melted at 218°C - 220°C, as did a mixture of the residue and phloroglucinol. Phloroglucinol, anhydrous, melts at 219°C.

The degradation products were heated with methanol and hydrogen chloride gas to give a methyl ester of the phenolcarboxylic acid. This methyl ester melted at 129°C, the melting point of methyl hydroxybenzoate which has been reported as having a melting point of 127°C - 129°C and 131°C.

These products recovered from the alkali fusion are phloroglucinol and p-hydroxybenzoic acid which would indicate that the canna anthocyanidin was composed of these units. The structure of the anthocyanidin containing these

units would be 3, 5, 7, 4' tetrahydroxy 2 phenylbenzopyrilium or pelargonidin (XXIX).

XXIX.

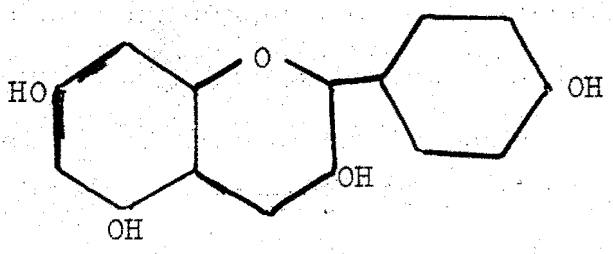
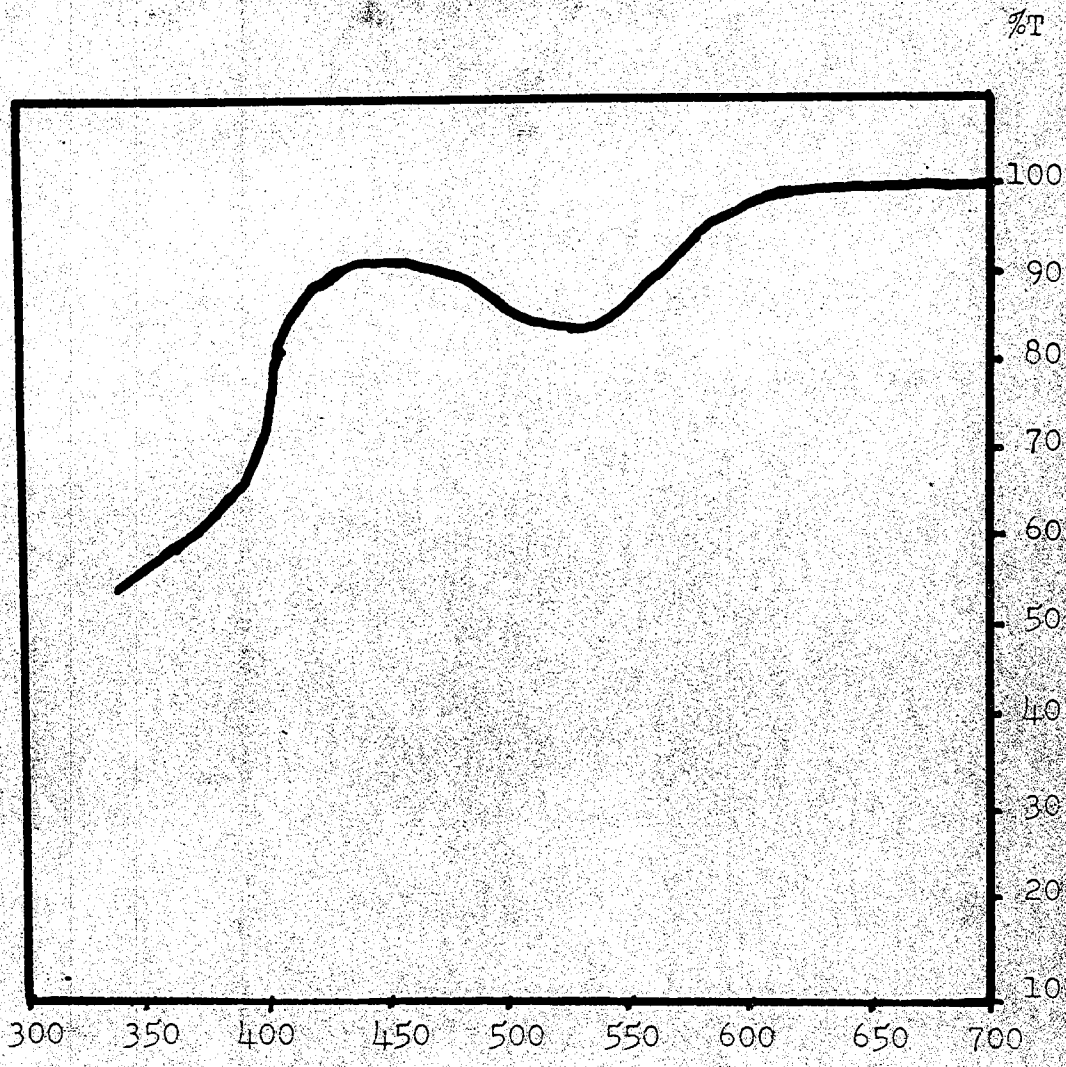


FIG. 1.

TRANSMISSION SPECTRUM OF UNKNOWN ANTHOCYANIDEN BETWEEN 360 and 650 MILLIMICRONS



SUMMARY

The pigment of Canna Indica pure red colored petals was extracted with methanol and precipitated as a chloride. Color was bright red in the acid solution indicating formation of an anthocyanidin. The anthocyanidin was crystallized out of the solution and the crystals tested for solubility. They were moderately soluble in methanol and ethanol, somewhat soluble in water, imparting a light red color to the solution, which color faded on standing. They were only slightly soluble in dimethyl formamide and insoluble in nonpolar organic solvents. The pigment colored 10% sodium hydroxide brownish blue, gave a pink color to a mixture of cyclohexanol and toluene and reduced Fehlings solution when warmed.

Degradation products of the pigment were phloroglucinol and p-hydroxybenzoic acid as proven by melting points.

A spectrum was obtained of the pigment in the range of 360 to 650 millimicrons with a Beckman spectrophotometer.

Coloring matter in the Canna Indica flowers was thus shown to be an anthocyanin since it was red in acid solution and blue in alkali.

Melting points of the degradation products show the pigment to be pelargonin.

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