[CONTRIBUTION FROM THE BOTANICAL AND THE CHEMICAL INSTITUTES OF THE IMPERIAL UNIVERSITY OF TOKYO.]

STUDIES ON ANTHOCYANINS: COLOR VARIATION IN ANTHOCYANINS.

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Historical.

The term "anthocyanin" has been used since A. Marquart[1] to represent the plant pigments responsible for the various colors found in flowers, fruits and autumn foliage. Such pigments behave like indicators toward acid and alkali. In the plant they are mostly dissolved in cell-fluids, but are sometimes present as crystals according to H. Molisch,[2] who identified them in the latter state under the microscope. Furthermore, that they are chemical individuals was determined by R. Willstätter and A. E. Everest,[3] studying the blue pigment of corn-flower (*Centaurea cyanus*), on which fruitless researches had already been made by Moro,[4] and by Frémy and Cloez.[5] V. Grafe[6] reported that there were a few pigments which occurred as glucosides. But that all anthocyanins are glucosides was proved by R. Willstätter and his collaborator. According to the latter authors cyanin was, for example, hydrolyzed into one molecule of cyanidin and two of glucose.

On the other hand, the relationship between the anthocyanins and the flvaone and flavonol series, yellow pigments of the plants, was studied by many investigators, among whom W. Stein,[7] and H. Hlasiwetz and L. Pfaundler[8] reduced some flavonol derivatives (morin, luteolin, quercetin and quercitrin) and obtained anthocyanin-like red solutions. R. Comb[9] reduced a yellow pigment in the leaves of *Ampelopsis hederacea* with sodium amalgam, and sought to show that the product was identical with the anthocyanin in the red autumn leaves of the same plant. Nevertheless, some authors[10] seemed to be of the opinion that the anthocyanins were *oxidation* products of the yellow pigments, because the former often occur associated with such oxidizing enzymes as oxidase or peroxidase. Reason-

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[1] Farben der Blüten (1835), (F. Czapek, *Biochem. der Pflanzen*, **1**, 586).

[2] *Bot. Ztg.*, **1905**, p. 159, and for the further literature, see L. Buscallioni and G. Pollacci, "Le antocianine e il loro significato biologico," **1902**.

[3] *Ann.*, **401**, 189 (1913).

[4] *Ann. Sci. Nat.*, **3**, 160 (1849).

[5] *J. prakt. Chem.*, **62**, 269 (1854).

[6] *Sitzb. Akad. Wiss., Wien*, **115**, 975 (1906); **118**, 1033 (1909); **120**, 765 (1911).

[7] *J. prakt. Chem.*, **88**, 351 (1862); **89**, 280, 491 (1863); (morin, quercetin and quercitrin).

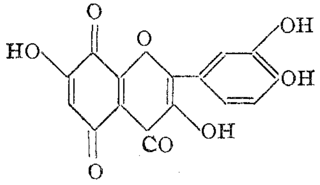
[8] *Sitzb. Akad. Wiss., Wien*, **50**, 6 (1864).

[9] *Compt. rend.*, **157**, 1002 (1913); *Ber. botan. Ges.*, **31**, 570 (1914).

[10] W. N. Jones, F. Keeble, E. P. Armstrong, M. Wheldale, *Proc. Roy. Soc.*, **1911–1914**; *J. Genetics*, **1911–1915**.

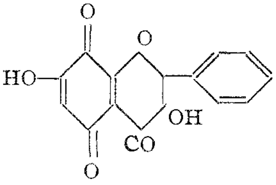
ing from this point of view M. Nierenstein[1] obtained red querceton [I] and chryson [II] by oxidizing quercetin and chrysin, respectively, with chromic acid,

[I]



Querceton.

and [II]

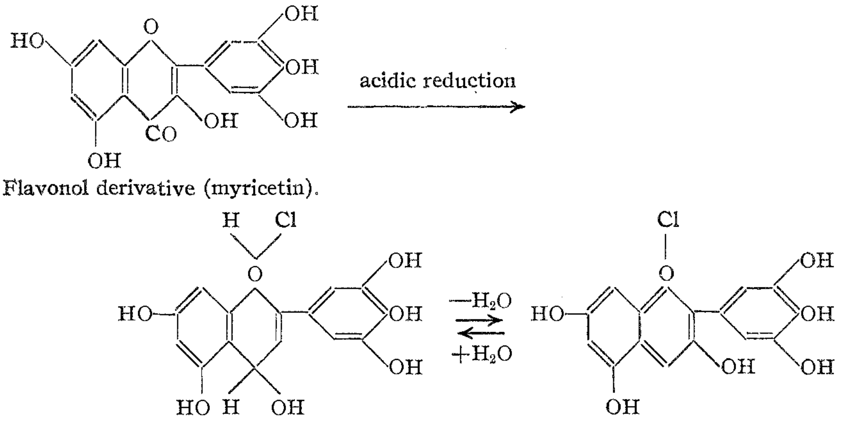


Chryson.

These substances are quite different from the anthocyanins.

The anthocyanin molecules, according to R. Willstätter and A. E. Everest[2] contain phenopyrylium ring and the oxygen in the ring combines with acids forming oxonium salts. In his recent paper A. E. Everest[3] states that red pigments having the properties of anthocyanidin can readily be obtained by reducing the flavone and flavonol series, and that quite similarly glucosides of the series yield red glucoside pigments of the anthocyanins (quercetin (Kahlbaum), and flavonols extracted from yellow and white flowers of *Narcissus*, *Tulipa*, *Primula*, *Chieranthus*, etc.).

On this basis he put forward the scheme



Flavonol derivative (myricetin).

Intermediate product.

Anthocyanidin (delphinidin chloride).

to represent the change mentioned, which R. Willstätter[4] approved.

Going a step further, we inquired how and why various colors, such as red, blue, violet, etc., can be produced from one and the same pigment. To this R. Willstätter[5] replied stating that the violet antho-

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[1] *Ber.*, **44**, 3487 (1911); **45**, 499 (1912).

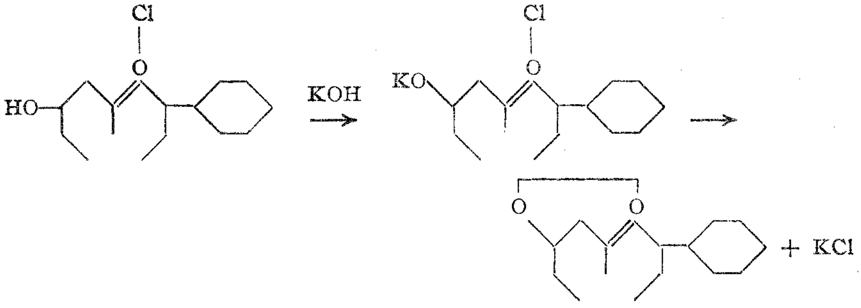
[2] *Loc. cit.*

[3] *Proc. Roy. Soc.*, **87**, 444 (1914); **88**, 326 (1915).

[4] R. Willstätter and H. Mallison, *Sitzb. kgl. preuss. Akad.*, **1914**, p. 769.

[5] *Ann.*, **401**, 189 (1913), *Sitzb. kgl. preuss. Akad.*, **1914**, p. 403.

cyanin would be an inner salt formed by neutralizing a red oxonium salt. The reaction



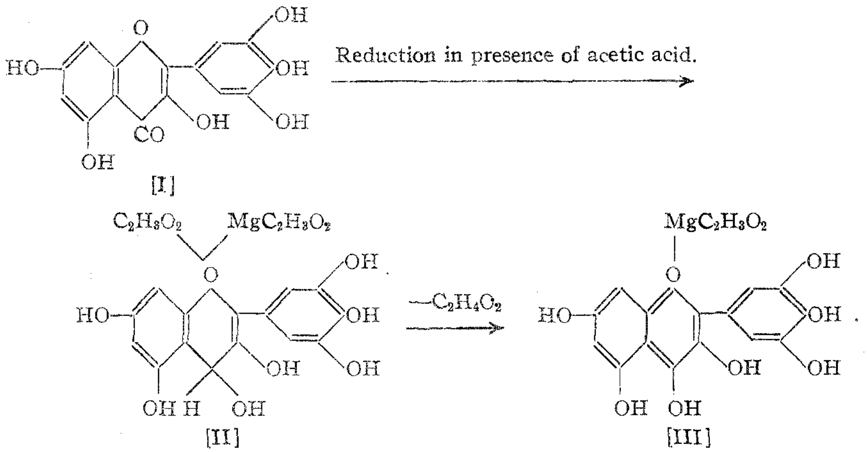
would represent this change, and the blue modification would be a phenolate of the inner salt formed with excess of alkali. The author himself added that the constitution of the red oxonium salts alone had been proved experimentally, but the assumption of inner salts and of phenolates was extremely hypothetical and lacked experimental support. The alkali-phenolates could not possibly be formed in cells, the cell-fluid being apparently acidic. The green or bluish green color produced by adding alkali to the red anthocyanin solutions is very unstable and changes into yellow or brown; the inner salt formed by neutralizing carefully with calcium carbonate has a weak reddish violet color, lacks that beautiful tint often seen in nature, and even the color soon fades away. All these observations are not at all explained by R. Willstätter's hypothesis, and, indeed, argue against it.

Recently we have been engaged in some experiments with certain flavonol derivatives, and have observed some new phenomena, with which we can, we believe, explain better the variation of the colors due to the anthocyanins.

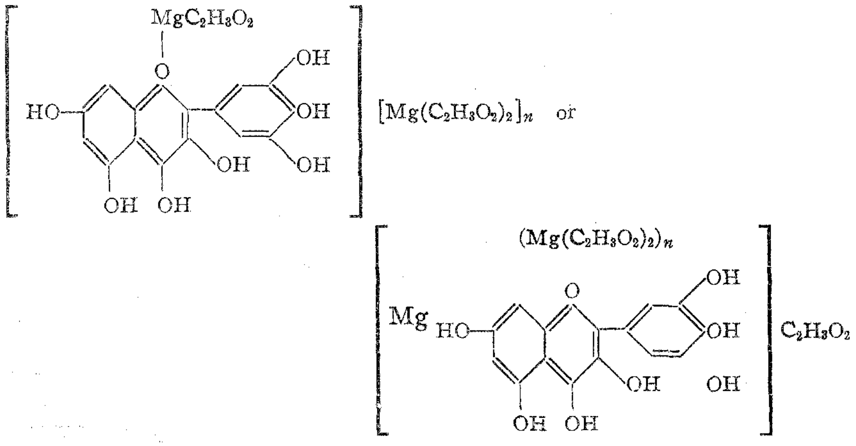
Theoretical.

All investigators, without exception, have invariably used metallic zinc or magnesium and excess of inorganic acid for the reduction of the flavone and the flavonol series, obtaining the red anthocyanin pigments, the oxonium salts of R. Willstätter. Quercetin gives, for example, allocyanidin at 0° and cyanidin at a higher temperature. We were, however, of opinion that organic acids might be used in place of inorganic acids for the reduction; and, in fact, such acids were found to be good reagents for the purpose. With monobasic acids for the most part we obtained pigments colored from deep green to bluish green, the tints of the colors being a little different from one another according to the reagents employed, though we had expected some red pigments. Moreover, we were able to isolate some of the pigments and to examine their properties.

On reduction with metallic magnesium and glacial acetic acid, myricetin gives green compounds of the composition $C_{15}H_{11}O_8MgC_2H_3O_2[Mg(C_2H_3O_2)_2]_n$, *n* being 2 or 4. The course of the reaction should be as follows: first, the reduction of myricetin [I] takes place with the formation of a phenopyrylium ring [II], whereupon the metal organic compound [III] results by eliminating acetic acid from the ring.



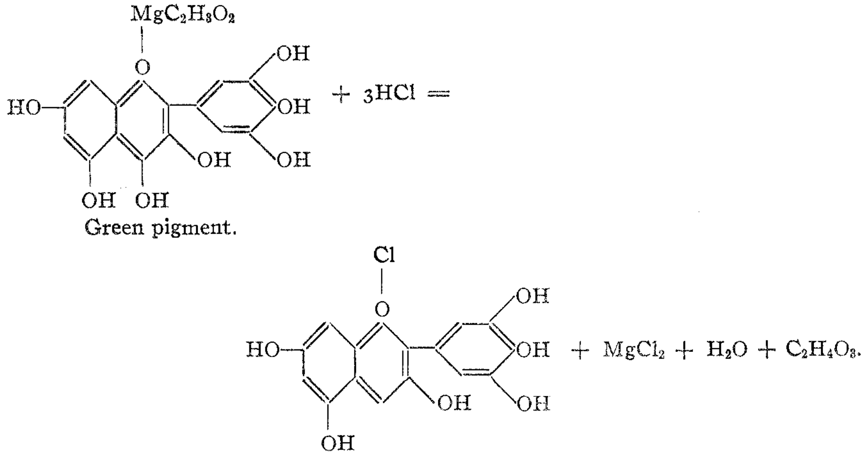
As the acetates of the bivalent metals often tend to form complex compounds, it was to be expected that addition of magnesium acetate would take place, giving



according to A. Werner's coördination theory.

Quite similarly myricitrin, a rhamnoside of myricetin, gives with the same reagents a deep blue product coördinated with 4 molecules of magnesium acetate.

The above green or blue pigments are soluble in water as well as in alcohol with the same colors, and give neutral solutions. But the addition of dil. acid to the solutions changes the colors to red. The reaction should proceed as follows:

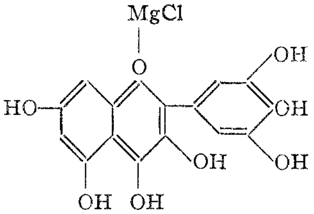


Green pigment.

Red oxonium salt.

From this it is easy to understand why Willstätter and the others have always obtained red pigments by the reduction of the yellow pigments.

Even with inorganic acids green or blue pigments are formed under certain conditions. We obtained from myricetin treated with alcoholic hydrochloric acid a deep green pigment having the formula

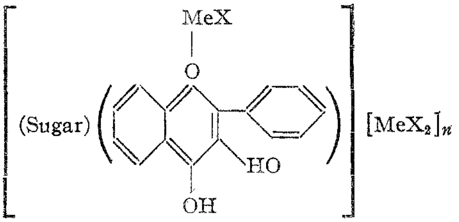


Here the addition of magnesium chloride did not, as we had expected, take place, probably because of its lesser tendency to form complex salts. The compound dissolves in water and alcohol, maintaining its original color, and the reaction is quite neutral.

That all the above compounds have deep colors, or, in other words, that their absorption bands are displaced far toward the red end of the spectrum, is attributed on one hand to the fact that the phenopyrylium ring of the green or blue pigments has one more hydroxyl group than that of the oxonium salts, and on the other, to the fact that magnesium forms the complexes with its auxiliary valence which together play the role of bathochromism. In the case of the reduced glucoside flavonol, one of the hydroxyl groups is substituted by a sugar molecule, which shifts the absorption band hypsochromatically, *i. e.*, toward the violet end of the spectrum.

On the basis of what we have stated, and of the experiments carried out with the pigments of many flowers we can put forward a new explanation on the various flower colors, as follows:

1 The metal organic or complex compounds of reduced flavonol glucosides



are the most important factor in the production of flower colors.

2. The "blue" anthocyanins are the complex compounds of reduced flavonol glucosides, which possess several hydroxyl groups belonging to the flavonol nucleus[1] besides those of sugar molecules, and the metal with which they are coördinated is probably calcium or magnesium, for salts of these metals are always present in the plant cells.

3. The "violet," "violescent red" or "red" pigments are either the analogous metallic complex compounds of flavonol glucosides, which contain fewer of the auxochrome hydroxyl groups,[2] or are a mixture of the blue pigments and their decomposition products by excess of acids, *i. e.*, the red oxonium salts of R. Willstätter.

Our conception of the color variation of the anthocyanins is, we believe, free from the objections raised in the preceding pages, and, moreover, the blue pigments are accessible, while the phenolates and inner salts are wanting an experimental support.

The deposition of anthocyanins in the cell-fluid in the colloidal particles which H. Molisch[3] often observed, and the sparing solubility of many blue or bluish violet anthocyanins in alcohol, are quite consistent with the magnitude of the complex molecules.

According to A. Guilliermond's[4] microscopic study the anthocyanin pigments seem to be formed in the cell plasm. It is highly probable that the complex compounds may be formed in this neutral colloidal substratum.

The behavior of the natural anthocyanin solutions toward the salts of alkaline earths and heavy metals has confirmed the theory we proposed. We studied the color changes of the alcoholic extracts of various flowers, when the salts mentioned were added to their solutions. In any

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[1] Such as myricetin and quercetin.

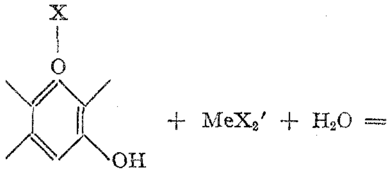
[2] Kempferol gives a beautiful carmin-red product by reduction with magnesium and glacial acetic acid. See Table III.

[3] *Loc. cit.*

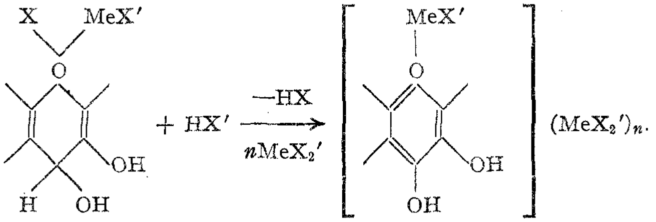
[4] *Rev. gen. botan.*, **25**, 295 (1914).

case, the colors always shifted in the sense of deepening colors, *i. e.*, bathochromatically; say from red or violescent red to beautiful blue or violet, and sometimes the colors were intensified. It was quite remarkable what gay and fresh colors were produced from the colorless solutions. Moreover, we were able to show that this color reaction depends upon the constitution of the anthocyanins, especially upon the number and the position of the hydroxyl groups.

This agrees completely with what we have stated in the foregoing pages on the color production of the anthocyanins; and the changes are to be represented with the following formulas:

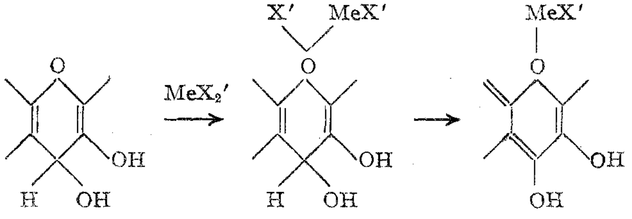


Nucleus of the anthocyanin
as an oxonium salt.



Blue, violet or red complex compound.

With the colorless modification of the pigment, which is a pseudobase,[1] the change must proceed as follows:



Colorless pseudobase. Colored complex compound.

The reversible color change of the extracts of some petals caused by temperature effect[2] is interpreted as the formation and decomposition of the complex compounds. We also observed the same color change with the solutions of the pigments synthesized by us.

The color change of hydrangea and other flowers caused by iron salts

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[1] R. Willstätter and A. E. Everest, *Loc. cit.*

[2] H. Molisch, *Botan Ztg.*, 1889, p. 17; H. Fitting, *Z. Botanik.*, 4, 105 (1912).

and alum, studied by Lachemeyer and his pupils[1] and afterwards by H. Molisch[2] is nothing but the complex formation, as we see with the extracts containing anthocyanins.

Experimental.

Myricetin and Myricitrin.—The yellow pigments employed throughout these experiments were extracted from the bark of *Myrica rubra*[3] cultivated in Wakayama Prefecture.[4] The bark was pulverized, and warmed for a few hours with 80% alcohol (5 liters of alcohol to a kilogram of the bark). When the alcohol became deep orange, heating was stopped. On cooling, the alcohol was decanted and then the bark was pressed to squeeze out any absorbed alcohol. The extract was distilled to a small volume, and raw myricitrin crystallized out on cooling. Upon recrystallization twice from hot water, pure myricitrin was obtained. To obtain myricetin, myricitrin was boiled with one or two per cent. hydrochloric acid for a few hours, when a yellow substance separated out. Filtered and recrystallized from alcohol, pure myricetin was obtained in nearly theoretical yield. We also tried the method adopted by A. G. Perkin[5] for the preparation of myricetin from the *Myrica rubra* extract, and found it very troublesome, because a muddy substance was always associated with myricetin, which was very hard to separate, especially with poor samples.[6]

1. Reduction of Flavonols with Magnesium and Some Organic Acids.

Myricitrin gave an intense blue coloration when dissolved in alcohol and reduced with magnesium and glacial acetic acid or its higher homologs, but myricetin gave a green coloration with the same treatment. The results obtained with myricetin are given in Table I.

From the table it is easy to see that monobasic organic acids give the reduction products from green to bluish green with the exception of highly dissociated acids. With di- and tri-basic acids red oxonium salts are formed, which is easily explained by their slight tendency to form magnesium compounds with the oxygen in the pyrylium ring. And it is also clear that the solvents have very little effect on the color reaction.

The addition of water or dil. acid to the above reduction product changes the colors mostly to red.

In Table II are given the results obtained by adding a drop of water or dil. hydrochloric acid to myricetin reduced in acetone.

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[1] *J. prakt. Chem.*, 1, 46 (1834).

[2] *Loc. cit.*; M. Miyoshi, *Botan. Centr.*, 83, 345 (1900).

[3] In chemical literature it is called incorrectly *Myrica nagi*.

[4] A southern littoral prefecture in Japan lying between 135–136° E., 33.5–34.5 N.

[5] *J. Chem. Soc.*, 99, 1721 (1911).

[6] Rutin and quercetin were prepared from the commercial extracts of the buds of *Sophora japonica*. We greatly appreciate the kindness of Dr. Y. Hirose who sent us some other samples.

TABLE I.

Reducing agents: Magnesium and each of the following acids:	Color: Myricetin dissolved in Abs. alcohol.	Acetone.	Ether.
1. Formic	red	red	weak yellow
2. Acetic	deep green	brownish green	blue
3. Propionic	deep green	brownish green	......
4. Butyric	green	brownish green	......
5. Valeric	green	brownish green	green
6. Lactic	yellow	yellowish green	yellowish green
7. Oxalic	pink	......	......
8. Citric	light red	......	......
9. Benzoic	green	green	......
10. *m*-Nitrobenzoic	yellow	yellow	......
11. Cinnamic	yellowish brown	......	......
12. Monochloroacetic	violescent red	violet	light green
13. Trichloroacetic	brown	brownish red	violet
14. Camphoric	green	green	brownish green

TABLE II.

Acid used for the reduction.	Color after the addition of Water.	Dil. hydrochloric acid.
1. Formic	yellow	red
2. Acetic	red	red
3. Propionic	red	red
4. Butyric	weak pink	pink
5. Valeric	red	red
6. Lactic	unchanged	orange
7. Monochloroacetic	orange	red
8. Trichloroacetic	brown	red
9. Benzoic	unchanged	brownish red
10. *m*-Nitrobenzoic	unchanged	deep yellow

This change of the color must be due to increasing hydrogen concentration caused by its introduction or the dilution, which is rendered more probable by the fact that no change was observed in case of adding water to the reduction products of myricetin with magnesium and acetic acid, if the free acid was eliminated before addition. Moreover, the complex formation took place, and a blue or green color was regained, when a piece of say calcium chloride, magnesium acetate, manganese chloride, copper chloride, etc., was introduced to the reddened pigments. But salts of monovalent metals did not cause this change, for these salts do not form complexes.

Quercetin and its glucoside, quercitrin, behaved just the same as myricetin and myricitrin with the same treatment, while kempferol gave only a red pigment. Some of the results[1] obtained with other members of the flavone and the flavonol series are given in Table III.

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[1] Obtained by Dr. K. Kimotsuki.

TABLE III.

Reduced with magnesium and acetic acid in abs. alcohol.	Resulting color.
Quercetin	Green
Quercitrin	Purple
Rutin	Purple
Kempferol	Red
Chrysin	Purple
Toringin	Pink

II. Other Metals as Reducing Agents.

The reduction of myricetin was carried out with some other metals than magnesium. The results are given in the following tables:

TABLE IV.

	Color.		
	Zinc in		Aluminium with Hg in alcohol.
Acid.	Alcohol.	Ether.	
1. Acetic............	green	unchanged	unchanged
2. Monochloroacetic..	bright green	unchanged	green
3. Trichloroacetic....	greenish yellow	orange	green

TABLE V.

	Color.		
Acid (dil.).	Mg.	Fe.	Zn.
1. HCl..............	red	orange	red
2. HBr..............	pink	yellow	nearly colorless
3. HNO_3............	brownish red	brown	nearly colorless
4. H_2SO_4............	orange	yellow	nearly colorless
5. H_3PO_4............	red	....	......
6. H_3PO_3............	red	....	......

III. Isolation of Green and Blue Pigments.

Two g. of myricetin was dissolved in 200 cc. of absolute alcohol, and fine magnesium powder added. Glacial acetic acid was added to it drop by drop, whereupon the reduction took place with the evolution of heat, which was moderated by cooling in cold water. After 20 cc. of the acid had been added, the reduction product was kept standing for a while, and filtered to remove the metal. The green pigment was precipitated by adding ether to the filtrate. The precipitate was filtered quickly, removed into a beaker, washed well with ether, and filtered again. The washing was repeated 5 or 6 times to remove any trace of the myricetin. There resulted a hygroscopic green, solid substance, soluble in water and alcohol, giving green neutral solutions. This substance was dried and kept *in vacuo*.

Calc. for $C_{15}H_{11}O_8MgC_2H_3O_2[Mg(C_2H_3O_2)_2]_4$: Mg, 12.51%; CH_3COOH, 55.6%. Found: Mg, 12.55%; CH_3COOH, 58.9%.

From myricitrin, a glucoside of myricetin, a greenish blue glucoside pigment was obtained with the same treatment as given above. It is soluble in water and less so in alcohol, giving neutral solutions.

Calc. for $C_{21}H_{21}O_{12}MgC_2H_3O_2[Mg(C_2H_3O_2)_2]_4$: Mg, 10.73%; CH_3COOH, 47.6%. Found: Mg, 10.64%; CH_3COOH, 53.3%.

Again from myricetin a light green substance was obtained with the same treatment, using more carefully dehydrated reagents.

Calc. for $C_{15}H_{11}O_8MgC_2H_3O_2[Mg(C_2H_3O_2)_2]_2$: Mg, 10.6%; CH_3COOH, 43.7%. Found: 10.2%; CH_3COOH, 46.2%.

Both of the green substances obtained from myricetin on being dissolved in alcoholic hydrochloric acid gave carmine-red solutions, from which brownish red crystalline powder (delphinidin chloride) separated on careful evaporation.

To estimate acetic acid, the substance was decomposed with sulfuric acid, submitted to steam distillation and the distillate then titrated. As regards the high values obtained, they must be due to a further decomposition, producing volatile acidic or phenolic substances. To ascertain if this were the case we carried a few experiments and followed the course of distillation. As the distillation proceeded, the acidic distillate increased to a maximum point, and then decreased constantly. When, however, nearly half of the acid had been distilled, suddenly the acidic distillate increased to a certain point, and then decreased gradually to the end.

In the above reduction where alcoholic hydrochloric acid was employed in place of acetic acid, there was obtained from myricetin a deep green compound which showed crystalline prisms under the microscope and was no longer hygroscopic. It was soluble in water and alcohol, giving neutral green solutions.

Calc. for $C_{15}H_{11}O_8MgCl$: Mg, 6.42%; Cl, 9.36%. Found: Mg, 6.72%; Cl, 9.40%.

IV. Experiments with Flowers.

In the preceding pages we have stated that the pigments obtained by reducing flavonols are complex or metal-organic compounds. In the following we will give a few experimental results concerning the complex formation of the natural anthocyanidins with metallic salts. For this purpose we prepared alcoholic (80%) extracts of petals, pericarps, leaves, etc. (5 : 1), and each salt was added to each extract little by little till the characteristic color had fully developed. Comparing the kind and intensity of the colors produced with those (No. 1–720) in "*Code des Couleurs*" (C. C.) by P. Klincksieck and Th. Valette, we made a precise record, from which a few examples will be given in the following table. For the sake of brevity, the following abbreviations are used instead of giving the statements according to the C. C.: R = red; RO = reddish orange; O = orange; OY = yellowish orange; Y = yellow; YG = yellowish green; G = green; GB = greenish blue; B = blue; BV = bluish violet; V = violet; VR = violescent red. An apostrophe represents dark colors, such as RO′ = chestnut brown; BV′ = indigo, etc., while a small letter represents lighter colors, such as r = pink; v = lilac, etc.

TABLE VI.

	Plant(flower). Salt.	$CaCl_2$.	$SrCl_2$.	$BaCl_2$.	$MgCl_2$.	$ZnCl_2$.	$Al_2(SO_4)_3$.	$SnCl_2$.	$Pb(C_2H_3O_2)_2$.	Cr_2Cl_6..	$(NH_4)_2MoO_4$.	$MnCl_2$.	Fe_2Cl_6.	$NiCl_2$.	$CoCl_2$.	$Cu(C_2H_3O_2)_2$.	$HgCl_2$.	CH_3COOH.	HCl.	NaOH.	K_2CO_3.
1	*Delphinium Ornatum*..............	BV	V	YG	GB'	G	YG	GB	YG	G'	Y	B	YG'	G	GB	YG	YG	VR	VR	Y	YG
2	*Platycodon grandiflorum*............	BV	V	V	BV	GB	BV	GB	G	BV'	BV	BV	OY'	B	V	G	oy	VR	VR	YG	G
3	*Campanula punctata*...............	BV	VR	r	V	G	vr	OY	YG	VR'	yg	V	OY'	B	V	Y'	oy	VR	VR	Y	YG
4	*Monochoria Korsakowii*.............	BV	BV	BV	BV	GB	VR	B	G	V'	GB	BV	G'	GB'	BV	G	YG	VR	VR	YG	G
5	*Primula japonica*..................	BV	V	ro	VR	GB	o	V	YG	v	o	V	RO'	BV	V	RO'	VR'	R	R	Y	YG
6	*Amsonia ellipitica*.................	B	BV	vr	B	GB	YG	BV	G	VR'	YG'	B	Yg'	V	VR	G	y	R	R	G	G
7	*Shortia soldanelloides*..............	V	V	vr	V	B	VR	V'	G	VR	OY'	VR	V'	VR	VR	RO	y	R	R	OY	OY'
8	*Hosta coèrulea*.....................	V	VR	VR	V	B	VR	VR	G	VR	O'	V	VR'	VR	VR	VR	VR'	VR	VR	G	G
9	*Galtonia candicans*.................	V	V	V	V	B	v	BV	G	V	GB	V	B'	V	V	G	g	VR	R	G	G
10	*Rhododendron lidifolium* v...........	VR	VR	vr	VR	VR	VR	V	oy	VR	G'	VR	R'	VR	VR	R'	vr	VR	R	YG	Y'
11	*Lycoris radiata*....................	VR	VR	VR	VR	V	VR	V	GB	VR	BV	VR	V	VR	VR	VR	ro	R	R	OY'	Y'
12	*Paeonia Albiflora*..................	VR	VR	r	VR	R	vr	VR	g	R	RO	RO	V'	R'	VR'	R	VR'	R	R	G	G
13	*Dendrobium nobile*.................	VR	VR	vr	R	O'	vr	RO	OY	vR	vr	vR	O'	R	R	RO	vR	R	R	O'	YG'
14	*Rosa gallica*.......................	VR	VR	vr	VR	vr	VR'	V	G	VR'	O'	VR	V	VR'	VR'	Ro'	ro'	R	R	O'	YG'
15	*Papaver Rhoeas*....................	VR	VR	VR	VR	BV	VR	V	VR	VR	V	VR	V	VR	VR	RO	vr	R	R	B'	VR'
16	*Hipeastrum reginae*................	R	R	RO'	Rv	OY	VR	VR'	G	R	VR'	VR	VR'	R	R	RO	VR	R	R	VR'	VR'
17	*Rhododendronindicum* v *marcranthum*	R	R	r	VR	BV	VR	V	G	VR'	V	R	YG	VR	R	O'	v	R	R	YG	YG
18	*Vaccinium Vitis-idaea* (fruit)........	R	R	R	R	VR	...	VR	G'	R	RO'	R	R'	R	VR	R'	ro	R	R	YG'	YG'
19	*Pelargonium zonale*................	R	R	R	R	VR	VR	Ro	OY	R'	RO	R	VR'	R	R	RO	VR	Ro	Ro	G	G
20	*Hemerocallis flava*.................	RO	RO	O	RO	GB'	ro'	VR	G	VR'	YG	R	BV'	R	R	...	OY	Ro	R	YG'	oY'
21	*Punica granatum*..................	rO	O	O	rO	rO	O	RO	oy	RO	O	RO	V'	RO	RO	RO	O	O	RO	G	G
22	*Phytolacca acinosa* (fruit)...........	RO	VR	VR	VR	RO	g	VR	RO	VR'	...	vR	O	O	R	yG	VR	vR	Vr	OY	YG
23	*Portulaca grandiflora*...............	Ro	R	R	VR	RO	g	VR'	O	VR'	...	Ro	OY	OY	RO	Yg	ro	vR	Vr	RO'	RO'
24	*Celosia cristata*....................	Ro	Ro	...	R	O	...	VR	O	RO'	...	OR	OY	OY	RO	OY	Ro	R	VR	OY	OY

From Table VI the anthocyanin solutions[1] mostly colorless, acquired with calcium chloride a deep blue or bluish violet (1–6), violet (7–9), violescent red (10–15), red (16–19) or orange-red (20–24)[2] color. The chlorides of strontium, barium, magnesium and manganese reacted nearly the same as that of calcium. The actions of nickel and cobalt salts resembled one another in most cases. It was quite remarkable that copper acetate colored the extracts orange-red and chromium chloride, violescent red. Zinc, tin, and lead salts gave characteristic colored precipitates.

Hence it is highly possible that the great variety of colors of the anthocyanins are due to the metal organic complex compounds, and accordingly it is very hard to comprehend the theory of R. Willstätter's "alkaliphenolates and neutral inner salts."

The colors of the complex compounds depend on the constitution of the anthocyanin molecules, especially on the number and position of the hydroxyl groups and their substituents (such as methoxyl and sugar groups), as we see in the above table from the behaviors of the pigments of *Delphinium*, *Paeonia*, *Rosa*, *Vaccinium*, and *Pelargonium* toward the salts, the constitutions of which have already been determined by R. Willstätter. Among chemically unknown anthocyanins those in *Papaver*, *Punica* and also those grouped by L. Weigert as "Rübenrot" gave a remarkable color reactions toward the salts.

In contrast with the alkali phenolates, the complex compounds are very stable toward water and also in weakly acidic solutions.

We observed a reversible color change when we heated the complex compounds. As the temperature rose, green color changed into blue, blue into violet and violet into red, and *vice versa*. Certain salts of barium, chromium, mercury, etc., gave characteristic colors to the anthocyanin extracts when they were heated.

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[1] The original pigment of the anthocyanins in the cell-fluid (in the form of complex compounds) often remained untouched on being extracted, although the hydrogen-ion concentration was greatly diminished; or even when they were extracted under any other conditions. The solutions were sometimes faintly colored and tended soon to fade. The pigments of blue flowers (*Centaurea*, *Delphinium*, *Gentiana*, etc.) were dissolved in 80% alcohol only by a part with a light violet color. In these cases the pigments are of high molecular weight.

[2] The salts of other monobasic acids may be used in place of the chloride, the resulting colors being slightly different according to the anions.