PIGMENTS OF THE MENDELIAN COLOR TYPES IN MAIZE: ISOQUERCITRIN FROM BROWN-HUSKED MAIZE.*

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INTRODUCTION.

At Cornell University, Emerson has been the leader in an important series of genetical investigations of maize, in which the Mendelian factorial composition of many color varieties has been established. In a recent memoir1 he has dealt especially with what he terms plant colors; i.e., colors due to pigments (other than those of the chloroplasts) which are commonly seen in the husks, the staminate inflorescence, the foliage generally, and the stem.

Of such plant colors he has established the genetical relations of six main types as follows: (1) purple, (2) sun red, (3) dilute purple, (4) dilute sun red, (5) brown, (6) green. In a former paper in this Journal2 we have pointed out the importance of chemical studies of such color series as this in which the genetical factorial analysis has been made. Only by the chemical investigation of genetically known material may we hope to come to any satisfactory understanding of the meaning of the Mendelian analysis, and of the actual operation of the factors which in such an analysis are known as symbols.

The plant color series in maize immediately suggests a parallelism with similarly related varieties of other plants in which flower color (due to cell sap pigments of the flavonol and anthocyanin groups) varies from white to various shades of yellow, red, and purple. Such a parallelism has already been confirmed3 by the isolation of a quercetin glucoside from

* Published by permission of the Secretary of Agriculture.
1 Emerson, R. A., Cornell Univ. Agric. Exp. Station, Memoir XXXIX, 1921, 158.
brown-husked maize (Type 5) and by the occurrence of a corresponding anthocyanin (not yet isolated) in purple (Type 1).

In order that the geneticist may perceive the feasibility of a chemical attack on the problem of interpreting genetical factors, it does not seem out of place to state briefly the simple chemical relationship of the cell sap pigments to one another. Without some chemical knowledge of the pigments, the geneticist obviously cannot advance beyond the symbolic expression of his results.

The yellow sap pigments derived from flavone may be subdivided into two groups—flavone and flavonol. The flavone pigments are derivatives of the mother substance flavone (I), or phenyl-2-phenopyrone-4,4 while the flavonol pigments are derivatives of flavonol (II), which is characterized by the fact that the hydrogen in the pyrone nucleus is replaced by hydroxyl. The anthocyanidin pigments are derivatives of phenyl-2-phenopyrylium (III).4

![Chemical structures]

Compounds containing this latter nucleus are characterized by the fact that they form difficultly soluble, well crystalline oxonium salts, consequently the natural anthocyanidins are described as oxonium chlorides, regardless of the fact that the coloring matters probably exist in the plant as combinations with organic acids.

In the plant, hydroxy and methoxy derivatives of the three classes of pigments exist as glucosides. The anthocyanidin glucosides are the naturally occurring anthocyanins. The anthocyanidins in the form of their oxonium salts may be looked upon as formed from the corresponding flavonols by three steps: (a) reduction (by the addition of 2 atoms of hydrogen). (b) combination with an acid, in the plant cell probably an organic acid, but in the laboratory generally hydrochloric acid, and (c) by the elimination of a molecule of water. The three steps representing the transition from flavonol to anthocyanidin may be illustrated by the formation of cyanidin chloride from quercetin. Quercetin is not only a typical flavonol and one which is found widely distributed in nature, but it is also the particular one which is concerned in the pigmentation of maize.

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4 The numbering of the rings is that indicated by Decker and von Fellenberg (Decker, H., and von Fellenberg, T., Ann. Chem., 1907, ccclix, 296).
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Quercetin (yellow).

Pseudobase (colorless).

Cyanidin chloride (red).

(For the sake of simplicity the above reaction shows the transformation from flavonol to anthocyanidin, both of which are aglycones or non-sugar products of the splitting of the glucoside molecule. The reactions in the plant are doubtless concerned with the glucosides, since the flavonol glucosides which occur in the plant are water-soluble and more easily reduced than the flavonols themselves.) The delicate equilibrium that must be maintained in a plant cell in order that one or another cell sap pigment may be present is indicated by the ease with which reversal of this last step takes place. Merely by treatment with hot water, pelargonidin chloride (the anthocyanin of the scarlet geranium is a glucoside of pelargonidin) passes into the colorless pseudobase, by the addition of 1 molecule of water and loss of hydrochloric acid. Alcoholic extracts of anthocyanins, although deeply colored at first, frequently become rapidly decolorized by this reaction, which may possibly be found to afford an explanation of the variation of some flowers from white to red,° which is known to depend, in *Primula sinensis* var. *rubra*, upon the temperature at which the flowers develop, the flowers being white at 30–35°C, and red at 15–20°C.

It is obvious that the chemical relationships of these plant pigments are such that relatively slight changes in the condition of the cell might lead to large visible effects.

In both flavonols and anthocyanidins there is a variable number of hydroxyl groups which are capable of being condensed with sugars. Compounds with glucose, rhamnose, and galactose are known. Others will doubtless be found. Depending upon the position of the substituted hydroxyls of any one flavonol or anthocyanidin, and also upon the particular carbohydrate which condenses with it to form the glucoside, there is an opportunity for the existence of a large number of isomeric pigments of somewhat different properties. With one, two, or more hydroxyls

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substituted, there is also an opportunity for several series of isomers, although only a monoglucoside series and a diglucoside series are known. Finally, variation in the position of the hydroxyls among the several mother substances provides possibilities for still more stereoisomers. Reference to the work of Perkin and Everest shows that many flavones and flavonols have already been isolated. Willstätter and his students have made great advances in the chemistry of the anthocyanins.

The early interest was centered in the yellow plant pigments because of their value as dyes. Consequently the glucosides, of great importance from the genetical standpoint, have been less studied than the flavones and flavonols. In the recent work on the anthocyanins the glucosides have received relatively more attention, but the geneticist finds little in the past work on flavonol glucosides and anthocyanins that enables him to argue that any particular yellow pigment is the mother substance of a particular purple. It is merely inferred that the most easy transition, involving the least readjustment of the molecule, is the one that takes place in the plant. Thus, if the yellow color of a certain plant were known to be due to a glucoside of quercetin, and if a genetically related purple type of the same species existed, one might logically expect the purple color to be due to a homologous glucoside of cyanidin. Or, if both yellow and purple pigments existed in the same variety, under circumstances that made it appear likely that one pigment was the mother substance of the other, there would be ground for expecting an equally simple relationship. What little direct evidence there is that supports this view has been found by Everest, who has shown the coexistence in purple-black pansies of the closely related pair myricetin (C_{15}H_{10}O_{8}) and delphinidin (C_{16}H_{10}O_{7}), the former yielding the latter by reduction. To render the proof more satisfactory one would wish to show not only the close correspondence between the flavonol and the anthocyanidin, but also a correspondence in the sugar and the position of its attachment. It is obvious that a large field is here thrown open for investigation.

No compounds are more widespread in plants than the yellow and purple pigments under consideration. The characters de-

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pending upon their presence, absence, or distribution have often been the subject of genetical research, for the reason that color characteristics are frequently due to allelomorphic factors. If not allelomorphic, the color factors are interacting and the Mendelian ratios are interpreted from this standpoint. Thus, it is frequently the case that a simple monohybrid ratio is obtained in crosses between yellow and purple varieties. The cross between purple and brown-husked maize affords a case in point. The first hybrid generation is purple. The second shows segregation of purple and brown in a 3:1 ratio.

Color characters may be mutually exclusive, not coexisting in the same plant or tissue, or non-exclusive, as in the case of flowers in which both yellow and purple pigment coexist. Distribution factors may, in the latter case, cause a definite color pattern, as in variegated purple and yellow pansies, or the pigments may be mixed. An example of the intimate association of yellow and purple (or blue) is possibly indicated by the green flowers of certain hybrids between purple and yellow alfalfa, in which the resultant color is green. (It is not certain, however, that plastid pigments are not here involved.)

It goes without saying that it would be a great gain to both the physiologist and the geneticist if the place of these pigments in metabolism were known, and if it were possible to trace the train of reactions by which the same mother substance gives rise to different pigments in organisms of different genetical constitution. Hypothetical chemical explanations of the allelomorphism of color characters have been proposed, but all require complete or partial verification. For example, it has been suggested by Wheldale that, in the simplest case, there would be at least two factors responsible for anthocyanin formation in flowers, one, designated C, representing chromogen, and another, designated R, representing an enzyme which is supposed to act upon C with the production of color. It is generally believed that in some cases a flavonol glucoside may itself act as a chromogen for the formation of an anthocyanin, as in the case of the purple-black pansy already mentioned.

The available evidence would seem to indicate that cases of extreme simplicity are not often to be expected. For example, a flavonol from blue flowered *Delphinium consolida* L. has been isolated and examined by Perkin and Wilkinson\(^\text{11}\) and found to be kaempferol. Willstätter and Mieg\(^\text{12}\) prepared an anthocyanidin from a purple variety of the same species, and named it delphinidin. The flavonol corresponding to delphinidin is not kaempferol, but myricetin. Here we have a lack of the correspondence such as Everest found in his pansy. It must be emphasized, however, that in the case of *Delphinium* the two investigators used different varieties, and that the genetical relations are totally unknown, and probably complex, for there is evidence from another species of *Delphinium* of the complexity of pigmentation in this genus, showing that even a yellow species without known blue or purple forms may be a genetically unresolved mixture of different yellow types. That Perkin and Pilgrim\(^\text{13}\) found three flavonols in the yellow flowers of *Delphinium consolida*, chosen at random, is therefore not surprising.

Thus far no series of color types of known genetical constitution has been thoroughly examined chemically, although an excellent beginning in this work was made by Wheldale and Bassett.\(^\text{14}\) Their work concerned *Antirrhinum*, from which they were successful in isolating flavones (not flavonols) from two yellow varieties. The deep yellow variety, with the Mendelian constitution YYii contained both luteolin and apigenin. A dominant factor, I, transformed the deep yellow into ivory, or very light yellow, the homozygous ivory type having the formula YYII, and containing only the pale colored apigenin. Other factors brought about the formation of two different anthocyanins, which were unfortunately not obtained in satisfactory condition for a determination of constitution or identity. Since Wheldale and Bassett did their work before the publication of Willstätter's brilliant researches, it is hoped that the *Antirrhinum* material may still be worked out satisfactorily. However, it must be noted that no


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natural anthocyanins corresponding to the flavones are yet known, and the anthocyanins of Wheldale and Bassett may therefore have been very different in their properties from the anthocyanins prepared by Willstätter.

Geneticists who are working with cotton and have pure lines of color types at their disposal might turn to good account Perkin’s investigations of the flavonol glucosides of several types of *Gossypium*. Additional data have been contributed by Viehoever, Chernoff, and Johns. In cotton there is a series of Mendelian color types of which the basic work on the flavonols has been done, leaving only the anthocyanins to be worked out from the beginning.

We have already pointed out in another paper the value of Emerson’s maize material for this problem. Although probably no better than cotton, the maize is superior to *Antirrhinum* in containing flavonol rather than flavone glucosides, with the obvious advantage that the anthocyanins will probably prove to have been worked out, or at least to be very similar to those described by Willstätter.

The maize series affords green types of different factorial composition which give, on crossing, a brown type. The brown type, obtained in the homozygous condition by inbreeding (self-pollination), will give simple Mendelian ratios when crossed with purple, the purple character being dominant in the first hybrid generation, and segregation in a 3:1 ratio taking place in the second generation. We have an example, then, of interacting factors, neither of which, alone, produces color, but which produce brown by interaction when both are present together. The symbols for these factors are B and Pₐ. The two different green types mentioned above contain B and Pₐ, respectively, each alone. A third factor is called A, which in the presence of B and Pₐ produces purple. With B alone the presence of A produces the type known as sun red, for the reason that the red color appears only if the plants are exposed to direct sunlight. With Pₐ alone, A produces a dilute purple color. A, then, is a factor for the production of some type

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of anthocyanin, the nature of which is determined by the other factors present. If A is present at all, the plant color in Emerson's material has always been purple or red. If the plant color is to be brown, A must be absent, but both B and Pl must be present. This interesting series of relations must have a discoverable chemical basis. To find such a basis has been the motive for the present paper, which is to be followed by others as the examination of the several genetical types proceeds.

Thus far we have worked mainly upon the brown type, since it seemed to provide the most tangible point of departure.

It might have seemed more logical to begin with one of the green varieties, of which there are three in Emerson's series, their formulas being aabbPlPl, aaBBPlPl, and aabbPlPl. The first two are the ones that give the brown type when crossed.

Using the same reasoning which has been advanced in the past, one might expect to find that these three green varieties would be characterized as follows: (a) one containing a chromogen, i.e. some colorless or relatively colorless mother substance from which flavonol or anthocyanin may be produced, but lacking the enzyme which accelerates the reaction, (b) a second variety containing the enzyme, but not the chromogen, and, (c) the ultimate Mendelian recessive, a variety containing neither chromogen nor enzyme. Lacking a clue to the nature of the hypothetical chromogen or mother substance of the pigment, however, and expecting only one of the three green genotypes to contain such a chromogen, it seemed best to work first with the brown type, upon the supposition that it would be found to contain a flavonol. This supposition was confirmed by the isolation and identification of quercetin, derived from an unidentified glucoside.

Preparation of Isoquercitrin.

The quercetin glucoside of the brown-husked maize was originally obtained in too small a quantity for thorough study by methods outlined in our former paper. A much larger quantity was obtained subsequently by the following procedure. The ground husks were extracted for 2 or 3 weeks with 95 per cent alcohol, and the extract was evaporated under reduced pressure. The syrupy residue was taken up with boiling water and filtered by means of a hot water funnel. The filtrate was allowed
to cool and was then shaken with ether to remove free quercetin and other substances. The quercetin glucoside was then removed from the aqueous solution by repeated shakings with ethyl acetate. The ethyl acetate extracts were then evaporated in a distilling flask, and as tarry substances separated on the bottom of the flask they were eliminated by changing the flasks. As the process of concentration proceeded, this deposit became lighter in color, and was then filtered off and added to the residue obtained by complete distillation. The procedure affected a mechanical separation of the phlobaphene-like substances, which separated in a tarry condition and could easily be removed from the portions richer in the unidentified glucoside. The crude glucoside was purified by solution in successive small amounts of boiling water, followed by repeated crystallization from relatively large amounts of boiling water, aqueous pyridine, dilute acetic acid (1 per cent), and finally from boiling water. In the presence of contaminating substances, the quercetin glucoside was found to be easily soluble in boiling water. As it becomes purer the solubility decreases. Approximately 5 liters of boiling water were required to dissolve 11 gm. of air-dried glucoside, although this relation does not accurately represent the solubility ratio. A quantitative approximation of the glucosidal content of air-dried brown maize husks was determined by extracting 1,000 gm. with 95 per cent alcohol for 3 weeks. This extract was freed of alcohol by vacuum distillation and the residue taken up in boiling water as before. After filtering, cooling, and removing ether-soluble substances, the aqueous solution was shaken with ethyl acetate until the final extracts no longer gave a test for flavonol. Evaporation of acetic ether left a residue, which after initial separation from tarry substances and final purification by repeated crystallization from large volumes of boiling water, amounted to 4 gm. in the air-dried state. For carrying through much of the tedious work of extracting the glucoside our thanks are due to Mr. Paul Williams.

The corn glucoside, in its final state of purity, forms a felty mass of primrose-yellow needle-like plates, which are often branched as shown in Fig. 1. In our former paper the color of this glucoside is reported as lemon-yellow and the melting point as 220–222°C. It is obvious that the small amount obtained for the previous investigation precluded the possibility of getting it pure and this
accounts for its deeper color and lower melting point. The pure glucoside melts at 220–222.5°C. An aqueous solution of this compound, using the filtrate from which most of the pigment had separated on cooling, gives with a few drops of ferric chloride, a pale olive-green, and with excess of the reagent, an intense olive-green color. Addition of sodium carbonate or dilute ammonia to the pigment solution intensifies the original yellow color. Lead acetate added to the cold aqueous solution gives a yellow precipitate which becomes more voluminous on the addition of a trace of ammonia. When an aqueous solution is reduced with magnesium ribbon and hydrochloric acid a clear pale rose-red color is produced. Probably the spectral transmission curve
Fig. 2. The spectral transmittance, throughout the visible spectrum and ultra-violet, of quercetin and maize isoquercitrin in alcoholic solution. For methods and apparatus refer to Bureau of Standards, Pub. No. 440, 1922.
affords as useful a criterion of the identity of a pigment as any that can be obtained. To facilitate future comparisons of unknown glucosides with the flavonol glucoside isolated from brown corn, the U. S. Bureau of Standards has very kindly prepared comparable spectral transmission curves (Fig. 2) of our maize glucoside and the quercetin prepared from it. In order to assure comparability the two pigments were dissolved in absolute alcohol and were made of the same molecular concentration (0.906 cg. of quercetin per liter; 1.392 cg. of isoquercitrin per liter, that is, solutions of m/300,000 concentration). The measurements in the visible part of the spectrum were made by Dr. M. K. Frehafer, and those in the ultra-violet by Mr. H. J. McNicholas, to whom we wish to express our thanks.

The spectral transmission of the glucosides of the flavone and flavonol derivatives will doubtless afford important evidence as to the position of attachment of the sugar residues, since the removal of a particular hydroxyl group by condensation with sugar might be expected to give a glucoside with approximately the same transmission as the non-glucoside with an H instead of an OH in the same position. Since the phenols and phenol acids formed as cleavage products of the flavones and flavonols (by fusion with alkali) indicate the position of the hydroxyl groups, it follows that the point of condensation in a glucoside would be indicated by a close similarity in optical properties between a glucoside of a given flavonol A, and a free flavonol B, containing one less OH group than A. Thus, if a monoglucoside of quercetin were closely similar in spectral transmission to kaempferol, it would indicate that the point of attachment of the sugar residue would be the particular hydroxyl which differentiates quercetin from kaempferol. The establishment of homologies between glucosides of the flavonol and anthocyanidin series is going to require the points of attachment to be known. It is therefore suggested that in future the measurement of spectral transmission be made a part of the routine examination of these glucosides, and that each curve be plotted against the free flavonol, in the same molecular concentration.
Identification of Isoquercitrin.

In our former paper we stated that the glucoside of the brown maize was very similar to one isolated by Heyl from the pollen of the ragweed, *Ambrosia artemisiifolia* L. A search of the literature showed that the latter was similar in many ways to the isoquercitrin described by Perkin from cotton flowers. Heyl very kindly placed at our disposal the small sample of his compound which remained and Perkin likewise sent a liberal sample of authentic isoquercitrin. As a result of a careful comparison of the three preparations, we conclude that the corn glucoside is identical with isoquercitrin. We are not so certain about the identity of Heyl’s compound with isoquercitrin, for the reason that the small amount available (90 mg.) was insufficient for complete identification. However, several points of identification have been made. All are monoglucosides. The products of hydrolysis in each instance are quercetin and glucose. All crystallize in the same form (see Figs. 1, 3, and 4). The melted glucoside in each case is a cherry-red oily liquid. Identical reactions are obtained when solutions of the three glucosides are reduced with magnesium and hydrochloric acid, and when they are treated with lead acetate, ferric chloride, sodium carbonate, and hydrochloric acid. Before we received samples of Perkin’s isoquercitrin and Heyl’s ragweed glucoside, a determination of the melting point of the corn glucoside led us to believe that all three compounds were different isomeric monoglucosides, since the melting point which we obtained for the corn glucoside differed from that of isoquercitrin, reported as 217–219°C, as well as from that of Heyl’s glucoside one sample of which melted at 224–226°C and the other at 228–229°C. Since receiving these samples, however, we have found that the rapidity with which the heating is carried out in the melting point determination influences the melting point. When the compounds are placed in the bath at 200°C, and the heating is so regulated that there is a rise of 1° in 1 minute, the melting points are as follows: corn glucoside, 210–211°; Perkin’s cotton isoquercitrin, 208.5–209.5°; Heyl’s ragweed glucoside, 215.5–217.5°. Under similar circumstances, except that heating was at the rate of about 7.5° per minute, the melting

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points were 220–222.5°, 218–220°, and 224–226.5°, respectively. Heyl’s compound mixed in an agate mortar with an equal amount of the corn glucoside did not show the depression in the melting point which would be expected if these two substances were different. The melting point of the mixture was slightly higher than that of the corn compound alone. Perkin’s isoquercitrin mixed with the corn glucoside changed the melting point of the latter only slightly, the difference being too small to be of any significance. Finally a mixture of all three glucosides was found to have a melting point identical with that of the corn glucoside. From the above results one is led to suspect that these three compounds will be found to be identical when larger amounts are available for examination.
One observation was made which may account for the difference in melting point between Heyl's glucoside and the other two. In crystallizing the three compounds under the same conditions, 0.034 gm. of air-dried pigment in each case dissolved in 5 cc. of boiling water, filtered hot and set aside to crystallize, we noticed that in each instance the crystalline precipitate was admixed with a small amount of droplets (amorphous spherules). Heyl's glucoside showed the least of this non-crystalline admixture and this may explain why his compound melted at a higher temperature than the other two. The amorphous spherules, according to this interpretation, represent some slight contamination, from which Heyl's compound is nearly free.
When deposited from aqueous solution, the corn glucoside contains water of crystallization, which is liberated at 160°C. The air-dried substance was, therefore, heated at this temperature to determine the loss of weight.

1.1554 gm. lost 0.0467 gm. H$_2$O. Found. H$_2$O 4.04.

In order to eliminate the error caused by the presence of hygroscopic moisture, the three following determinations were carried out using material which had been vacuum-dried at room temperature until it reached constant weight.

- 0.5229 gm. lost 0.0201 gm. H$_2$O. Found. H$_2$O 3.84.
- 0.5980 gm. lost 0.0239 gm. H$_2$O. Found. H$_2$O 3.99.
- 0.5229 gm. lost 0.0201 gm. H$_2$O. Found. H$_2$O 3.84.

$C_{31}H_{24}O_{12}$. H$_2$O requires: H$_2$O 3.73.

Analyses of the vacuum-dried (a) and anhydrous (b, c, d) glucosides are here given:

(a) 0.1302 gm.: 0.2491 gm. CO$_2$ and 0.0537 gm. H$_2$O. Found. C 52.18, H 4.02.

$C_{31}H_{24}O_{12}$. H$_2$O requires: C 52.26, H 4.59.

(b) 0.1229 gm.: 0.2441 gm. CO$_2$ and 0.0480 gm. H$_2$O. Found. C 54.17, H 4.38.

(c) 0.1043 gm.: 0.2057 gm. CO$_2$ and 0.0414 gm. H$_2$O. Found. C 53.79, H 4.45.

(d) 0.1824 gm.: 0.3623 gm. CO$_2$ and 0.0732 gm. H$_2$O. Found. C 54.16, H 4.49.

$C_{31}H_{24}O_{12}$ requires: C 54.29, H 4.34.

Isoquercitrin upon hydrolysis is resolved into glucose and quercetin in molecular proportions. Our sample of corn glucoside gave upon hydrolysis glucose and quercetin. For the quantitative determinations anhydrous material was used. The yield of quercetin accorded satisfactorily with that calculated from the equation:

\[ C_{31}H_{16}O_{12} + H_2O = C_{31}H_{19}O_7 + C_6H_6O_4. \]

0.5424 gm.: 0.3521 gm. quercetin. Found. Quercetin 64.91.

0.5638 gm.: 0.3650 gm. quercetin. Found. Quercetin 64.74.

$C_{31}H_{16}O_{12}$ requires: Quercetin 65.08.

The above determinations were carried out by hydrolyzing the samples (oven-dried at 160°C.) by boiling with 100 cc. of approx-
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imately 4 per cent H₂SO₄ for 1 hour and then placing on a boiling water bath for another hour. The mixture was cooled over night, filtered, and the residue thoroughly washed with cold water and dried to constant weight at 130°C. The acid filtrates gave positive rotation indicating the presence of a dextro-rotatory sugar. Excess of sodium acetate and phenylhydrazine was then added and the solution heated on a boiling water bath until a yellow crystalline osazone separated. This was recrystallized from water containing 5 per cent pyridine, then dissolved in a small amount of pyridine to which were then added hot alcohol and a little hot water. The characteristic glucosazone, melting at 205–205.5°, crystallized out.

The quercetin obtained from the glucoside by hydrolysis was identified by all the usual tests and by combustions of both hydrated (a) and anhydrous (b, dried at 130°C.) samples. A determination of the water of crystallization in a sample of quercetin exposed to the air for 1 month and the combustion results are as follows:

0.6220 gm. lost, at 130°C., 0.0656 gm. H₂O. Found. H₂O 10.54.
C₁₅H₁₀O₇.2H₂O requires: H₂O 10.65.
(a) 0.1652 gm.: 0.3229 gm. CO₂ and 0.0630 gm. H₂O. Found. C 53.30, H 4.27.
C₁₅H₁₀O₇.2H₂O requires: C 53.24, H 4.18.
(b) 0.1890 gm.: 0.4121 gm. CO₂ and 0.0680 gm. H₂O. Found. C 59.46, H 3.43.
C₁₅H₁₀O₇ requires: C 59.50, H 3.34.

Combustions of acetyl quercetin, dried at 160°C., gave the following figures:

0.1301 gm. gave 0.2794 gm. CO₂ and 0.0480 gm. H₂O. Found. C 58.57, H 3.13.
0.1277 gm. gave 0.2744 gm. CO₂ and 0.0440 gm. H₂O. Found. C 58.59, H 3.86.
C₁₅H₆O₇(C₆H₅O)₅ requires: C 58.57, H 3.93.

The hydrolysis of the penta-acetylquercetin carried out in glacial acetic acid by means of hydrochloric acid gave the following results:

0.0408 gm.: 0.3753 gm. quercetin (dried at 130°C.). Found. Quercetin 58.65.
1.0578 gm.: 0.6204 gm. quercetin (dried at 130°C.). Found. Quercetin 58.56.
C₁₅H₆O₇(C₆H₅O)₅ requires: Quercetin 58.98.
SUMMARY AND CONCLUSIONS.

1. A series of color types in maize involving pigments of the flavonol and anthocyanidin groups, has been genetically analyzed by Emerson. The known genetic constitution of these color types makes it very desirable to conduct parallel chemical investigation with them, in order to understand the operation of the Mendelian factors involved.

2. As a beginning in this work, we have isolated a flavonol glucoside from brown-husked maize, one member of Emerson's series and the lowest one showing the presence of a pigment of the groups under consideration. This glucoside is found to be the same as Perkin's isoquercitrin, originally isolated from cotton, and probably the same as an unnamed glucoside isolated by Heyl from pollen of ragweed. It is a monoglucoside yielding only glucose and quercetin on hydrolysis. The large yield of this glucoside obtained from corn husks made it possible to add to the characterization of isoquercitrin by Perkin who had only a limited amount at his disposal. As a contribution to the more accurate identification of such pigments, the spectral transmission has been determined by McNicholas and Frehafer of the U. S. Bureau of Standards. Their data are published in Fig. 2.

3. It is suggested that spectrophotometric and spectrographic comparison of the pigments of the flavones, flavonols, and anthocyanidins may possibly afford the best evidence as to the points of attachment of the sugar residues in the glucosides.
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