[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CORNELL UNIVERSITY]

The Precursors of the Anthocyanins of Autumn Foliage

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Introduction

In a recent paper[1] it was shown that the precursors of the anthocyanins are distributed in plants in three ways. Some plants contained a flavone which was the probable parent substance from which the red color of the leaves developed. Leuco-anthocyanin was found to be the probable precursor in other plants. In the case of the Japanese Creeper both leuco-anthocyanin and flavone were found. Between them Combes[2] and Jonesco[3] showed that the Virginia Creeper contains both flavone and leuco-anthocyanin. So the idea that some plants might contain both leuco-anthocyanin and flavone is not entirely new. Undoubtedly the Robinsons believe that flavones and leuco-anthocyanins co-exist in many plants.

Nobody appears to have determined the relative frequency of occurrence of these two parents of the anthocyanins in autumn foliage. Therefore it is not known whether most leaves form anthocyanin from flavone or from leuco-anthocyanin. The main object of this paper is to throw light on this question. To that end 86 species of plants belonging to 50 genera have been analyzed qualitatively for their flavone and leuco-anthocyanin contents. The results obtained necessarily apply only to Ithaca and vicinity and to the year 1938.

Burning Bush, *Euonymus alatus*, exhibits a brilliant red autumn color in Ithaca but in Colorado it becomes only slightly pink; likewise, *Forsythia suspensa* is not strongly colored in Colorado in the fall.[4] Some trees that usually exhibit brilliant autumn reds in many of the United States show little color in England in the fall.[5] There appear to be many other similar cases. These facts made it seem of interest to include in the present work a rough estimate of the 1938 autumn color of the leaves of the particular specimens of plants examined and to see whether the autumn colors correlate in any obvious way with the type or amount of precursor present. Inclusion of an approximate description of the autumn colors of the specimens that were examined provides a safeguard in that, for example, it may be found eventually that when the same species of plant exhibits greatly different autumn colors the precursors, or their relative amounts, may be different from those recorded here.

(1) Bancroft and Rutzler, THIS JOURNAL, **60**, 2738 (1938).

(2) Onslow, "The Anthocyanin Pigments of Plants," Cambridge University Press, 1925, p. 120.

(3) St. Jonesco, *Compt. rend. soc. biol.*, **97**, 975 (1927).

(4) Personal communication from Professor George Beach, of Colorado A. and M. College.

(5) Personal communication from Dr. H. T. Skinner of Cornell University.

Experimental Procedure

Green leaves were collected from plants in and about Ithaca in September and early October of 1938. At the time of collection all of the plants from which the green leaves were obtained showed autumn reds, purples, or oranges. The autumn colors of the various plants were noted at the time of collection. The green leaves were air-dried and analyzed for flavone and leuco-anthocyanin by means of the method of Bancroft and Rutzler.[6] The method was modified slightly in order to make it very roughly precise. The leaves were extracted with a 10% solution of formic acid for approximately eighteen hours at room temperature using throughout about the same ratio of weight of leaves to volume of acid solution. The formic acid extract was divided into two parts, one for use in analyzing for flavones and the other for testing for leuco-anthocyanin. In examining the extract for flavones by extracting it with ether the ratio of the volumes of extract and ether was not far different from test to test, nor was the ratio of volume of ether to volume of aqueous ammonia in the final operation. The same concentration of aqueous ammonia was used throughout.

In testing for leuco-anthocyanin a measured amount of the formic acid extract was made approximately 2% with respect to sulfuric acid by adding a measured amount of standard sulfuric acid to it. The solution then was heated in a bath of boiling water until there was no further visible change in color. This required less than half an hour in all cases in which leuco-anthocyanin was found. In cases in which owing to the dark color of the formic acid extract it was doubtful whether or not anthocyanin was formed by this procedure, the solution was cooled and brought back to its original volume and its color compared with the original formic acid extract. A modification of this procedure gave the same result in the case of the Yama Cherry, the formic acid extract of which was reddish brown due to the presence of anthocyanin in the green leaves. The modification consisted of making the formic acid extract strongly enough ammoniacal to turn the anthocyanin yellow, allowing to stand for about fifteen minutes, and then acidifying with sulfuric acid. The acidified solution also was yellow showing that the anthocyanin was partly decomposed by ammonia. The acidified solution then was heated, whereupon it turned reddish

(6) Bancroft and Rutzler, THIS JOURNAL, **60**, 2945 (1938).

brown. This shows that leuco-anthocyanin was present and that it was not decomposed by aqueous ammonia. The same modification of the test for leuco-anthocyanins was carried out on the pink formic acid extract of the Broadleaf Lilac; this extract was negative for leuco-anthocyanin both by the usual method of analysis and by the modified method. One other check on the reliability of the test for leuco-anthocyanins was made. Thinking that perhaps in some leaves which showed no leuco-anthocyanin the formic acid solution did not extract the leuco-anthocyanin effectively, the extract was heated in presence of some of the leaves in order to determine whether or not the test would then be positive in the leaf. This was done with Pin Oak, Dunbar Crab, *Forsythia viridissima koreana*, and *Malus ioensis fimbriata* and in no case did the leaves show any additional color after heating. So a 10% solution of formic acid appears to extract leuco-anthocyanins effectively.

Experimental Findings

The data that were obtained are given below and include, for completeness, the results reported previously.[1] Following the name of each plant the autumn color in Ithaca in 1938 is given and then two numbers which refer to the relative amounts of leuco-anthocyanin and flavone, respectively. When the tests failed to detect any of either precursor the amount present is denoted by 0; the number 1 means a very small amount was present, 2 means a small amount, 3 a medium amount, 4 a large amount, and 5 a very large amount. This classification results from a comparison by eye without a standard. Most of the names of plants used in reporting the present results follow "Standardized Plant Names" which is the official code of the American Nurserymen's Association.

A flavone is the probable precursor of the anthocyanin in the following plants: *Acer palmatum*, maroon, 2, 3; *Atropa belladonna*, dull blue, an unusual autumn color, 0, 3; *Berberis verruculosa*, red, 0, 1; *Cornus amomum*, dull purplish, 1, 3; *C. florida*, bright red, 0, 4; *C. paniculata*, dull purple, 0, 4; *Deutzia gracilis*, purplish red, 0, 3; *Diervilla florida*, dull orange, 1, 3; *D. lonicera*, red, 1, 4; *Forsythia intermedia*, purplish red, 0, 2; *F. intermedia primulina*, dull purplish, 1, 3; *F. ovata*, dull purple, 0, 3; *F. susp. sieboldi*, deep red, 0, 4; *F. viridissima*, dull purple, 1, 3; *F. viridissima koreana*, dull purplish, 1, 3; *Juniperus horizontalis*, rust, 0, 3; *Ligustrum amurense*, deep reddish brown, 2, 3; *L. ibota regelianum*, purplish red, 1, 3; *Lonicera tatarica hyb.*, deep red, 1, 4; *Malus glaucescens*, red, 0, 4; *M. ioensis fimbriata*, dull orange, 1, 4; *Quercus palustris*, purplish maroon, 1, 3; *Q. rubra*, orange, 1, 3; *Rhus copallina*, orange, 2, 3; *R. cotinus*, dull purplish, 0, 3; *R. typhina*, bright red, 1, 4; *Spiraea prunifolia plena*, maroon, 2, 3; *S. reevesiana*, dull purplish, 2, 3; *S. thunbergii*, maroon, 2, 3; *Syringa oblata dilitata*, purple, 0, 3; *Vaccinium macrocarpum*, purplish red, 2, 4; *Zanthoriza apifolia*, purplish red, 1, 3; Zinnia, red, 0, 4.

Either a flavone or a leuco-anthocyanin, or both, may be the precursor of the anthocyanin in the following plants: *Acer ginnala*, bright red, 3, 4; *A. rubrum*, deep purplish red, 3, 4; *Aronia arbutifolia*, maroon, 3, 3; *Calycanthus floridus*, red, 3, 3; *Cornus alba*, purplish, 4, 4; *Cotoneaster acutifolia*, bright red, 4, 3; *C. diviricata*, bright red, 4, 4; *Crataegus cordata*, deep brownish red, 5, 4; *Deutzia rosea conspicua*, dull orange, 4, 4; *D. scabra plena*, dull maroon, 3, 4; *D. wellsii*, dull purplish, 4, 4; *Diervilla biformis*, dull orange, 4, 4; *D. candida*, dull orange, 4, 3; *D. eva rathke*, dull orange, 4, 3; *D. floreal*, red, 4, 3; *D. hyb. desboisii*, red 4, 3; *Kolkwitzia amabilis*, dull orange, 3, 3; *Lonicera chrysantha*, purplish red, 4, 3; *L. morrowi*, purple, 3, 4; *Oxydendron arboreum*, dull maroon, 5, 4; *Parthenocissus tricuspidata*, red, 3, 3; Poinsettia, bright red, 4, 3; *Prunus canascens*, dull pink, 3, 4; *P. domestica* var. *Burbank*, dull purplish, 3, 4; *P. sargenti*, purplish red, 3, 3; *P. serrulata*, dull reddish, 3, 4; *P. subhirtella*, reddish rust, 4, 4; *P. yedoensis*, bright red, 4, 3; *Pyrus communis* var., orange and red, 4, 3; *Rhododendron catawbiense*, bright red, not an autumn color, plant unhealthy, 3, 3; *Rubus allegheniensis*, dark red, 4, 4; *Sorbraria sorbifolia*, dull orange, 3, 3; *Sorbus decora*, red, 4, 4; *Spiraea prunifolia*, dull orange, 3, 3; *S. tomentosa*, dull purple, 4, 4; *Taraxacum officinale*, purplish red, 3, 4; *Viburnum opulus*, maroon, 4, 3; *V. prunifolium*, purplish red, 3, 3; *V. wrightii*, dull red, 3, 3; *Weigela amabilis*, dull purplish, 4, 3; *W. hybrida*, dull orange, 3, 3.

A leuco-anthocyanin is the probable precursor of the anthocyanin in the following plants: *Acer saccharum*, red, 4, 1; *Amelanchier laevis*, maroon, 2, 1; *Ampelopsis quinquefolia*, deep red, 4, 1; *Euonymus alatus*, bright red, 3, 2; *E. bungeanus*, orange and red, 4, 1; *E. maackii*, rust, 4, 1; *Fraxinus americana*, dull purplish, 4, 1; *Quercus alba*, reddish brown, 3, 2; *Q. imbricaria*, orange and red, 2, 0; *Q. prinus*, rust, 3, 2; Seckel pear, skin, dull red, 3, 1; *Viburnum lentago*, red, 3, 2.

It should be emphasized again that the approximate autumn colors given here apply to the particular plant selected in Ithaca and its environs in 1938. In general, however, these colors are approximately the normal autumn colors in this vicinity.

The grouping with respect to precursors is arranged so that in the first group all plants contained much more flavone than leuco-anthocyanin. The plants in the second group contained considerable quantities of both parent substances; while leuco-anthocyanin predominated over flavone in the plants in the third group. Only in about 16% of the cases was one of the precursors present to the entire exclusion of the other.

The data show that in 38% of the species examined the probable precursor of the anthocyanin was a flavone; in 48% there was present enough of both flavone and leuco-anthocyanin so that, without additional work, it is not possible to tell from which precursor the anthocyanin was formed. Leuco-anthocyanin appears to be the precursor of the anthocyanin in 14% of the cases. It is hoped that either by comparing the absorption spectra of the anthocyanidins developed from the leuco-anthocyanins extracted from green leaves with those of the anthocyanins extracted from the cor-

responding red leaves, after converting them into anthocyanidins, or by determining which precursor is present in smaller quantities in red than in green leaves, or by both procedures, it will be possible to determine which is the probable precursor when both are present in quantity.

When it is indicated that leuco-anthocyanin is either the probable or a possible parent substance from which anthocyanin is formed, as in the second and third groups, an error may be made because in a few cases a phlobaphene may have formed from a phlobatannin as a result of heating the formic acid extract with sulfuric acid. Phlobaphenes and anthocyanins give much the same color reactions in acid and alkali, and have similar solubilities in water and amyl alcohol. Owing to the presence of catechin in the products of hydrolysis of the tannin from *Acer ginnala*, *Quercus alba*, and *Q. prinus*,[7] leuco-anthocyanins and phlobaphenes possibly may be confused in these cases. However, *Q. palustris*, which also contains a catechin-yielding tannin,[7] gave practically no test for leuco-anthocyanin.

It is of interest to examine the data that have been obtained to see to what extent the anthocyanin in all of the species of any given genus came from the same type of parent substance. All six species of *Prunus* as well as both species of *Cotoneaster* that were examined contained both flavone and leuco-anthocyanin in quantity. A flavone was the probable precursor of the autumn color in each of the six species of *Forsythia* and in all of the species of *Rhus*, *Ligustrum*, and *Malus* that were investigated. The anthocyanin of all species of *Euonymus* that were analyzed probably came from a leuco-anthocyanin. No other genus was homogeneous with regard to type of precursor.

When a flavone was the probable precursor of the anthocyanin, the data show a correlation between autumn color and amount of flavone present in the leaves. When the autumn color was purplish there was a medium amount of flavone and either no leuco-anthocyanin or only a very small amount in 17 out of the 19, or about 89%, of the cases. When the autumn color was red all six species contained a large amount of flavone and either no leuco-anthocyanin or only a very small amount. There appears to be no relationship between amount of precursor and autumn color when both parent substances are present nor when leuco-anthocyanin is the probable parent of the anthocyanin. Leuco-anthocyanin appears to give rise to distinct red coloration in the autumn more frequently than flavone because 51% of the leaves containing both precursors and 58% of those containing mainly leuco-anthocyanin showed distinct red autumn colors while only 18% of those leaves that contained mostly flavone exhibited distinct autumn reds. On the other hand, only 23% of the colors were purplish when a leuco-anthocyanin was a possible precursor while 45% were purplish when the probable precursor was a flavone. Thus, when the autumn color originates from a leuco-anthocyanin it is more likely to be red than purplish. The reverse tendency is found when the autumn color is formed from a flavone. The purplish colors observed when flavones were present in considerable quantity appear to be examples *in vivo* of the bluing or co-pigmenting effect of flavones on anthocyanins which has been observed frequently *in vitro* by Robinson.[8]

According to Mrs. Onslow,[9] Gertz found that the anthocyanin of *Quercus*, *Rhus*, *Acer*, *Ampelopsis*, *Prunus*, *Cornus*, and *Viburnum* is situated in the ground parenchyma but it is present in the epidermis in *Deutzia*, *Euonymus*, and *Spiraea*. There appears to be no correlation whatever between the anatomical location of the anthocyanin as reported by Gertz and the type of parent substance found in the present work.

Discussion

Owing to the fact that about 84% of the specimens examined in this study contained both leuco-anthocyanins and flavones, it is possible in only relatively few cases to say without fear of contradiction which type of precursor gives rise to the autumn color. At the same time, several interesting relationships between the behavior of the plant and the probable type of precursor of its anthocyanin appear to have been uncovered. It seems clear, for example, that the species of some genera are uniform with regard to the type of precursors present while some of the species of other genera contain mainly one type of precursor and other species of the same genera contain another type.

Since leuco-anthocyanins appear to be asso-

(7) Nierenstein, "The Natural Organic Tannins," J. and A. Churchhill, London, 1934, pp. 103, 241.

(8) Robinson and Robinson, *Biochem. J.*, **26**, 1663 (1932).

(9) Onslow, "The Anthocyanin Pigments of Plants," Cambridge University Press, 1925, pp. 42–45.

ciated most frequently with red autumn colors and flavones most frequently with purplish autumn colors, the methods of analysis employed here ought to be a valuable guide to the plant breeder. For example, *Acer rubrum* is frequently purplish red in Ithaca in the autumn and contains both precursors in quantity. By breeding out flavones it may be possible to produce a red variety containing no purple while by breeding out leuco-anthocyanin a purple variety may result. The starting point for the production of the red variety would be specimens containing smaller amounts of flavone. Specimens containing smaller amounts of leuco-anthocyanin would be selected in attempting to produce the purple variety. The data suggest many other breeding experiments such as the above. These possibilities make it desirable to increase further the precision of the analytical methods.

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Conclusions

The following conclusions are drawn from the results obtained.

1. Of 86 species of plants belonging to 50 genera which have been analyzed for leuco-anthocyanin and flavone, 84% contained demonstrable quantities of both precursors of the anthocyanins.

2. The probable precursor of the autumn color was found to be a flavone in 38% of the cases and a leuco-anthocyanin in 14% of the cases. Both precursors were present in quantity in 48% of the cases.

3. It is suggested that in a few cases a phlobaphene may be mistaken for a leuco-anthocyanin.

4. These results necessarily apply only to Ithaca and vicinity in the autumn of 1938.

5. In some cases all the species examined of a genus contained the same type of precursor of the autumn color. In other cases the genera were not homogeneous in this respect.

6. Leuco-anthocyanins appear to give rise to red autumn colors more frequently than purplish colors. The reverse appears to be the case when a flavone is the probable precursor because of a co-pigmenting effect of the flavone.

7. The apparent relationship between type of precursor and autumn color suggests a criterion based on analyses for flavone and leuco-anthocyanin for selecting plants from which to breed red or purple varieties.

8. This same method can be applied to fruits which develop anthocyanin pigments. The author hopes to be able to report this year on the precursors of the anthocyanins in strawberries, currants, cherries, barberries, raspberries, blackberries, peaches, apples, plums, grapes, etc.

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The Formation of Intermediate Compounds in Hydrocarbon Syntheses by the Friedel and Crafts Reaction, and the Preparation of Certain Symmetrical Trialkylbenzenes

BY JAMES F. NORRIS AND DAVID RUBINSTEIN[1]

In order to obtain more information in regard to the mechanism of the Friedel and Crafts reaction, certain of the substances described by Gustavson[2] as complexes of aluminum chloride and aromatic hydrocarbons have been studied. Menschutkin[3] showed earlier that aluminum bromide did not form a compound with toluene, but the fact is not significant in this connection, because the complexes described by Gustavson were formed in the presence of hydrogen chloride or hydrogen bromide.

In the study of the complexes aluminum bromide was used in most cases, because the compound is readily soluble in toluene, whereas aluminum chloride is insoluble or only slightly soluble. When dry hydrogen bromide was passed

(1) From the thesis of David Rubinstein submitted in partial fulfilment for the degree of Doctor of Philosophy, 1934.

(2) G. Gustavson, *Ber.*, **11**, II, 1841 and 2151 (1878); *ibid.*, **16**, 784 R (1883); *J. prakt. Chem.*, [2] **42**, 250 (1890); [2] **68**, 209 (1903); [2] **72**, 57 (1905).

(3) Menschutkin, *J. Russ. Phys.-Chem. Soc.*, **3**, 41, 1089 (1909); *Chem. Centr.*, **81**, I, 167 (1910); Kablukow and Saashanow, *Chem. Centr.*, **81**, I, 912 (1910).