

SOME ASPECTS OF THE BIOGENESIS OF ANTHOXANTHINS

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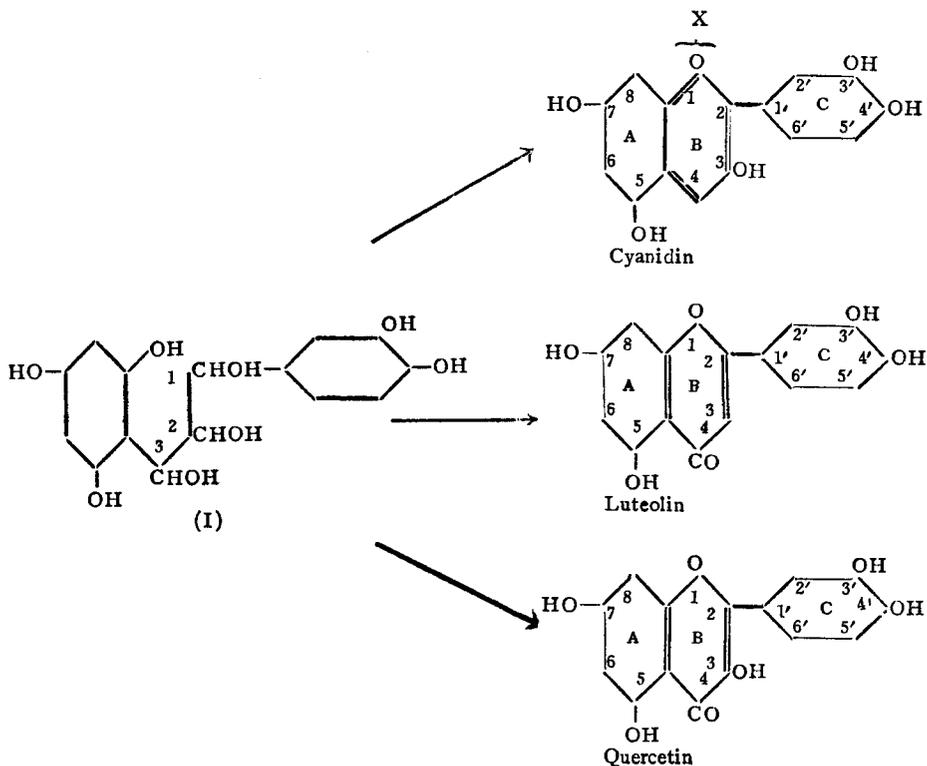
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IN a recent publication was given a brief account of our knowledge about naturally occurring flavones and flavonols whose constitutions have so far been definitely known.¹ They were arranged into different groups depending upon the number and the orientation of the hydroxyl groups in the benzopyrone part of the molecules. Their occurrence and their characteristic properties were also recorded. From the data thus presented it seemed to be possible to obtain information regarding the manner of their evolution in the plant kingdom. Possibility of success in this venture appeared to be greater particularly in view of the valuable conclusions which Robinson and his collaborators have recently arrived at in regard to the biogenesis of the anthocyanins.²

Anthocyanins and anthoxanthins are two groups of closely related sap soluble pigments. They are built upon the same pattern having 15 carbon atoms and 3 rings. Even before their chemical constitutions were definitely known, biologists were impressed by their association and they considered that anthocyanins were generated from the anthoxanthins by a process of oxidation taking place in the plant.³ In support of this idea they could mention the comparatively later appearance of the bright red and blue colours due to anthocyanins and under conditions favourable to oxidation. When, as a result of the important work of Kostanecki, Perkin and others on the anthoxanthins and of Willstätter and his co-workers on anthocyanins, the chemistry of these two groups was made clear, the close chemical relation between them was established, but the anthocyanins represented a lower state of oxidation. Consequently the suggestion was made that anthocyanins were really products of reduction of flavones and flavonols, and no oxidation was involved.³ On either of these two ideas which mean sequential evolution of the pigments, a correlation could be expected between the individuals of the different groups occurring in the same parts of the plants. For example, pairs like pelargonidin and k ampferol, cyanidin and quercetin, delphinidin and myricetin, should occur. In the cases examined in this connection this correlation could not be found. Further, the presence

of pelargonidin with myricetin, delphinidin with k ampferol was not uncommon. In the few cases where correlation was found, cyanidin and quercetin were involved and this could be considered as incidental to their prolific occurrence in the plant kingdom.

The difficulties were satisfactorily explained by Robinson who developed the idea of parallel origin of these groups of compounds from a common source²; different degrees of oxidation are involved in the production of anthocyanins, flavones and flavonols. According to his scheme no correlation is required between the members of the different groups occurring together. On the supposition that anthocyanins and other related substances are built up from simple carbohydrates through a series of aldol condensations, the hypothetical intermediate (I) was suggested as the basis from which all these compounds are derived. The intermediate can be constructed from two hexose units and one triose, and is in the same state of oxidation as a carbohydrate. The central three-carbon fragment of (I) may be modified in several ways to give different end products. For example, oxidation at C_1 leads to the formation of cyanidin, at C_3 to the flavone, luteolin and at both C_2 and C_3 or at C_1 and C_3 to the formation of the flavonol quercetin.



During the past several years Robinson and his collaborators have been engaged in a survey of anthocyanins occurring in nature. Making use of a quick method of analysis requiring small quantities of plant material they have been able to study a very large number of samples and thus provide sufficient material for statistical analysis. It has been found by them that the basic structures concerned are remarkably small in number being pelargonidin, cyanidin and delphinidin. Only a few rare cases correspond to flavones, *i.e.*, they lack in a hydroxyl group in the 3 position; gesnerin and carajurin are the rare examples. Further from their data it could be concluded that cyanidin is the primary member of the anthocyanidins and the production of delphinidin (oxidation) and pelargonidin (reduction) involve one stage more in evolution. These are remarkable results and give great support in favour of structure (I) as the common precursor.

The study of the biogenesis of anthoxanthins is more difficult for several reasons. In contrast to the few basic types found in the anthocyanins the variations in flavones and flavonols are quite numerous. There are at least 18 flavonols and 12 flavones of established constitutions. Quick methods of analysis of plant materials for anthoxanthins have not yet been developed and hence it has not been possible to analyse such large number of samples as for anthocyanins. Further, whereas anthocyanins usually occur single and in some cases with one more as minor component, anthoxanthin mixtures are more frequently complex. Consequently the number of cases of complete analysis are limited and deductions cannot therefore be comprehensive. However, as stated at the beginning it seems to be possible to arrive at some conclusions based on the following considerations :—(1) since anthocyanins and anthoxanthins are closely related, the results obtained from the more comprehensive study of the former could be accepted as applicable for the latter also, (2) the association of compounds in the same part of a plant, in different parts of the same plant and in closely related plants could be utilised as evidence of biogenetic relationship. In regard to alkaloids and other well-defined groups of naturally occurring compounds it is quite well known that plants of the same family contain the same compounds or closely related compounds. The differences amongst them are only in finer details and not in fundamentals. The minor changes mainly due to oxidation, reduction and dehydration could be brought about differently or to different stages in different members of a group of plants, in different parts of the same plant or even in the subdivisions of the parts. But the main framework may be said to be evolved in the same way.

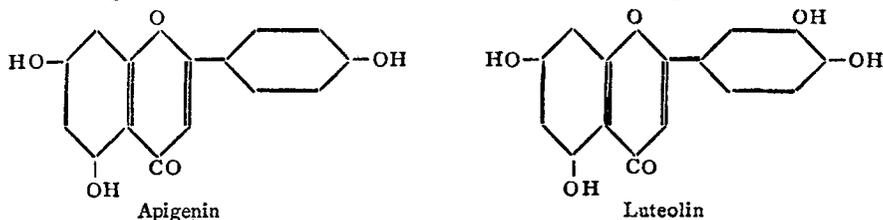
From consideration (1) given above, it follows that quercetin should be considered to be the simplest of the flavonols and luteolin the simplest of the

flavones and that the others require more stages for their evolution. This is also supported by the abundance of these two representatives in nature. Quercetin is the most frequently encountered anthoxanthin. Gisvold and Rogers⁴ give figures showing that out of 268 cases where anthoxanthins have been identified, 220 have the 3':4'-dihydroxy orientation in the substituted phenyl group. It could further be stated in general terms that as in the case of the anthocyanins, here also variations in the number of hydroxyl groups in the side phenyl nucleus is possible by the introduction of one stage more of oxidation or reduction, the positions 3' and 5' being involved. Support for the above statement is available from the association of compounds as they occur in nature and it is given later in this paper. The state of complete reduction leaving no hydroxyl in the side phenyl nucleus is common in the anthoxanthins and is a distinctive feature. But a more prominent characteristic of these compounds which does not find a parallel in the anthocyanins is the large variation in the state of oxidation of the benzene ring (A). In the latter group of pigments, two hydroxyls are invariably present in the 5:7-positions. Resoanthocyanins have not been found in nature. Carajurin represents an exceptionally rare case; but its occurrence indicates that the possibility of modification of ring (A) even in the anthocyanins cannot be excluded, though it may be uncommon. But in the anthoxanthins the variation in the number of hydroxyls is from 0 to 4. An explanation of this could probably be found in the marked difference in reactivity of the nuclear positions of anthoxanthins and anthocyanins after the structures have been formed from the common precursor, the modifications not taking place earlier. If quercetin and luteolin should be the simplest of the flavonols and flavones (containing hydroxyls in 5:7 positions) the biogenesis of the others should involve further stages of oxidation and reduction. How far this conclusion is justified and in what manner the variations arise can be indicated in a general way by a study of the pigment associations made below. Though in most of the cases considered the pigments occur as glycosides, only the aglycones are mentioned and used in the discussion. The nature of the sugars involved and the positions they occupy do not seem to be relevant for the present purpose. Similar considerations are applicable to the methyl ethers also. Further the list is not claimed to be comprehensive; typical cases alone are taken up and attention is particularly focussed on the evolution of special structures found only in the anthoxanthins and not in the anthocyanins.

I. Some associations of flavones and flavonols in which the side phenyl nucleus has different number of hydroxyl groups are given below. It could be noticed that compounds with one hydroxyl (4'-position) or three

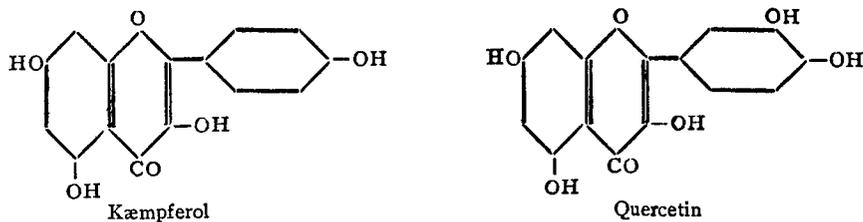
hydroxyls (3', 4' and 5') are accompanied by those with two hydroxyls (3' and 4'), indicating the evolution of the first two from the third. There seems to exist no definite cases where types with one and three hydroxyls in the side phenyl nucleus occur together, particularly to the exclusion of the 3': 4' dihydroxy compounds.

(i) The combination of apigenin and luteolin is frequently met with in different parts of the same plant or in plants of allied species and varieties.



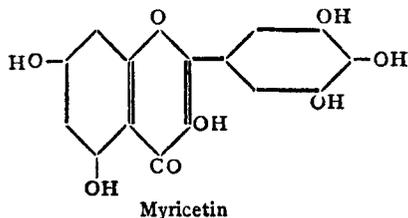
Apigenin occurs in the seeds of parsley, while in the stem and the leaves luteolin (as its monomethyl ether) is also present along with it.⁵ Both of them are found in the yellow variety of *Antirrhinum majus*⁶ and also in *Reseda luteola* (Weld.).⁷ The two varieties of *Chrysanthemum* yield the two related pigments; *C. indicum* contains luteolin and *C. leucanthemum* apigenin.⁸

(ii) Kæmpferol is known to be found along with quercetin or its methyl ethers in several sources.

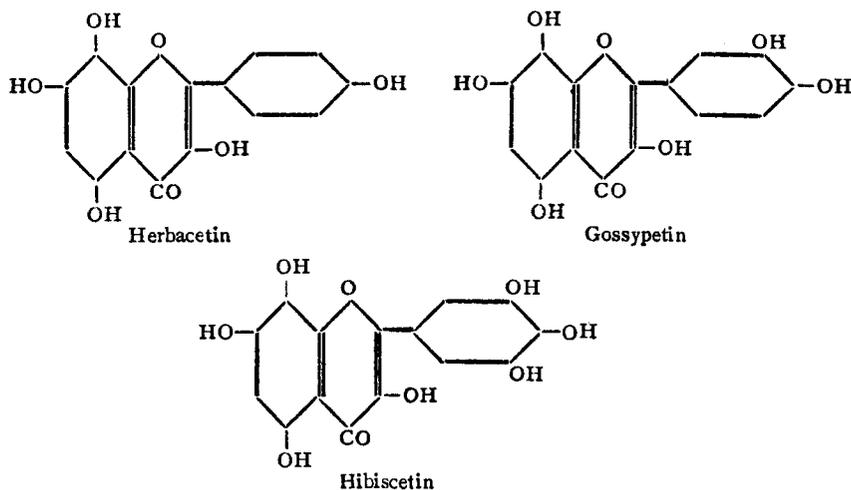


The stems of *Equisetum arvense* contain both these pigments, besides luteolin.¹⁰ In senna leaves kæmpferol and isorhamnetin (3'-methyl quercetin) are present.⁹ The same combination occurs in the flowers of *Delphinium Zalil* (Asbarg).¹¹ Kæmpferol and isorhamnetin along with kæmpferol monomethyl ether are found in *Rhamnus catharticus*.⁹

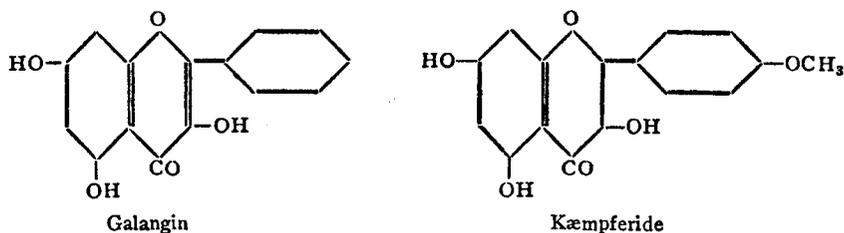
(iii) Quercetin and myricetin are both present in *Myrica nagi* and *Coriaria myrtifolia*.⁹



(iv) Indian cotton flowers (*Gossypium herbaceum* and *indicum*) contain gossypetin and herbacetin,¹² and in *Hibiscus sabdariffa* gossypetin and hibiscetin occur, the latter being the major component.¹³



(v) Galangin, its monomethyl ether and k ampferide occur together in the galanga root.⁹ This example could be taken as indicating the sequence from the di- and monohydroxy side-phenyl nucleus to one devoid of hydroxyl groups.



II. In some plants quercetin occurs along with other compounds which contain one more hydroxyl group in ring (A).

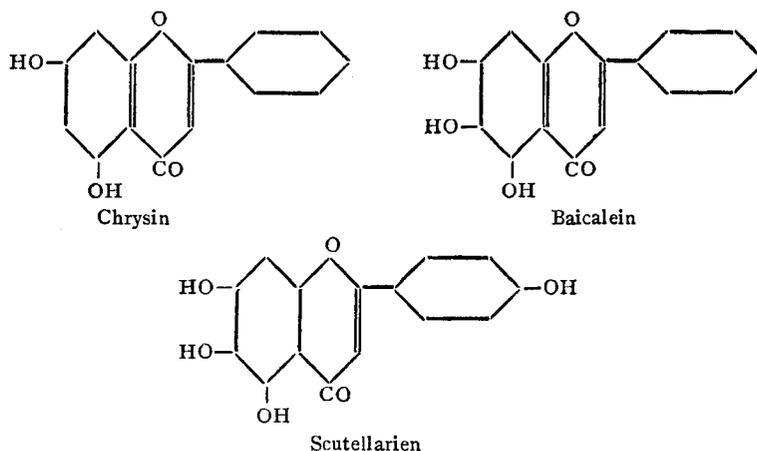
(i) The American cotton flowers, *G. hirsutum* contain as the sole component quercetin,¹⁴ whereas the Indian varieties *G. herbaceum* and *G. indicum* contain gossypetin and herbacetin besides quercetin, gossypetin being the major component.¹²

(ii) The flowers of two species of *Thespesia* have so far been examined. *T. lampas* was studied by Perkin and was found to contain quercetin.¹⁵ *T. populnea* has been shown by Rao and Reddy¹⁶ to contain herbacetin besides populnetin (a flavone whose constitution is not yet settled^{16a}).

From the above data, it seems to be reasonable to infer that quercetin is the primary stage, and gossypetin indicates a higher stage of evolution involving oxidation which leads to the formation of the hydroxyl group in position 8. Herbacetin is a subsequent stage of reduction resulting in the disappearance of the hydroxyl group in 3'-position in ring (C).

III. 5:6:7 Combination of three hydroxyl groups in ring (A) occurs fairly frequently. This also seems to be an evolution from the more fundamental 5:7-dihydroxy types involving oxidation.

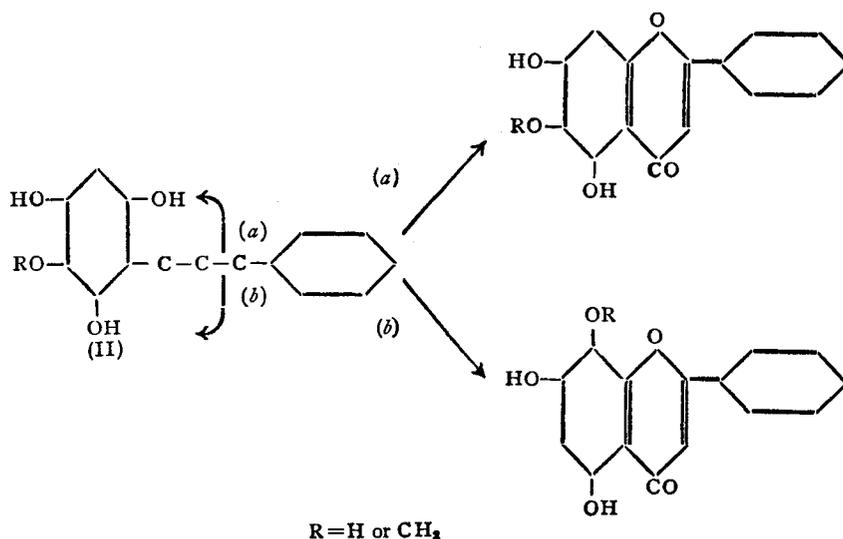
(i) The stem and root barks of *Oroxylum indicum* contain baicalein and its 6-methyl ether, oroxylin-A. Besides these two, the stem bark contains chrysin.¹⁷ Thus the relation between the 5:7-dihydroxy and the 5:6:7-trihydroxy compounds is indicated.



(ii) Regarding the existence of stages in the reduction of ring (C) the composition of the parts of different species of *Scutellaria* is useful. The leaves and flowers of *S. baicalensis* and *S. altissima* contain scutellarein¹⁸ whereas the roots of *S. baicalensis* contain baicalein.¹⁹

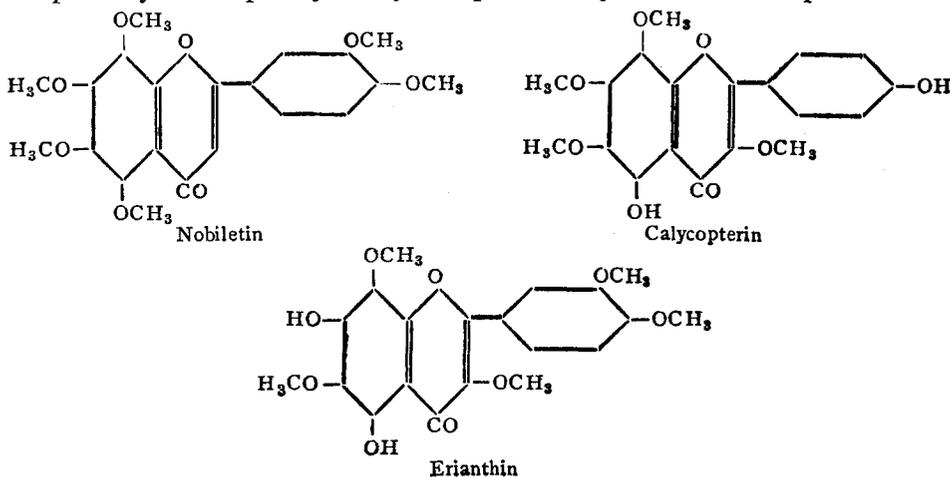
(iii) Another interesting point that should be noted here is that in the roots of *S. baicalensis*, wogonin, the 5:7:8 analogue of oroxylin-A is also found to be present¹⁹ and the ether group is in the 8-position. There are two ways of explaining this phenomenon:

(a) the 5:6:7 and the 5:7:8 combinations arise independently from the 5:7-dihydroxy compound by oxidation affecting position 6 or position 8 independently; (b) the oxidation takes place at some stage prior to the closure of the pyrone ring and the ring closure takes place in two different ways, one leading to the 5:6:7 and the other to the 5:7:8-configuration:



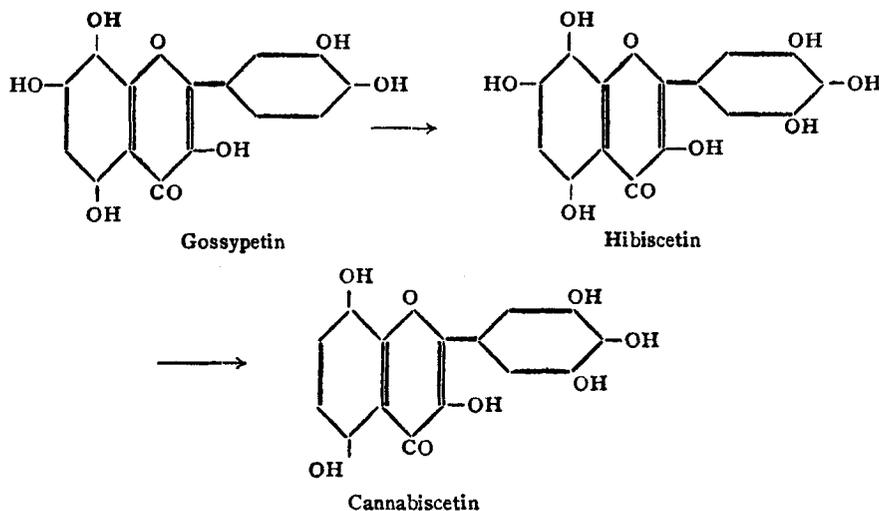
The existence of the second possibility is indicated by some laboratory experiments. But they are not numerous enough to be conclusive. The association of 5:6:7 and 5:7:8 types is found so far in one case only. It may not be, therefore, possible to say definitely which of the above two alternatives is more plausible. The first is probably more simple and also avoids certain other difficulties. It is, however, more definite that they are both derived from the 5:7-dihydroxy precursor.

IV. So far cases of one stage oxidation of the benzene ring (A) have been discussed. The idea could be extended to complete oxidation resulting in tetrahydroxy benzopyrone systems. Several of this type have been recently discovered. It is noteworthy that all these, nobiletin, calycopterin and erianthin are partially or completely methylated presumably in order to escape oxidation.



V. The following associations in closely related species indicate the existence of a stage of reduction of the hydroxyl group in position 7.

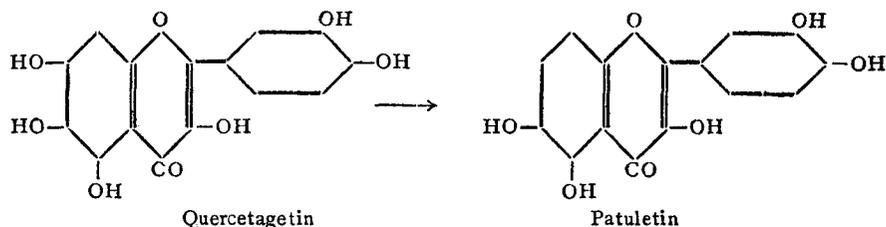
(i) *Hibiscus sabdariffa* contains hibiscetin as the main component and gossypetin and sabdaretin as the minor portion,¹³ whereas the closely related species, *H. cannabinus* contains cannabiscetin as the sole component.²⁰ The constitution of sabdaretin is not known yet and hence it is omitted from consideration. The relationship between the others can be expressed by the following formulæ and it seems to be reasonable to conclude that cannabiscetin is derived from hibiscetin by a stage involving reduction affecting position 7.



It may be mentioned here that in one experiment a small amount of cannabiscetin was found in *Hibiscus sabdariffa*; but it could not be isolated from other samples.

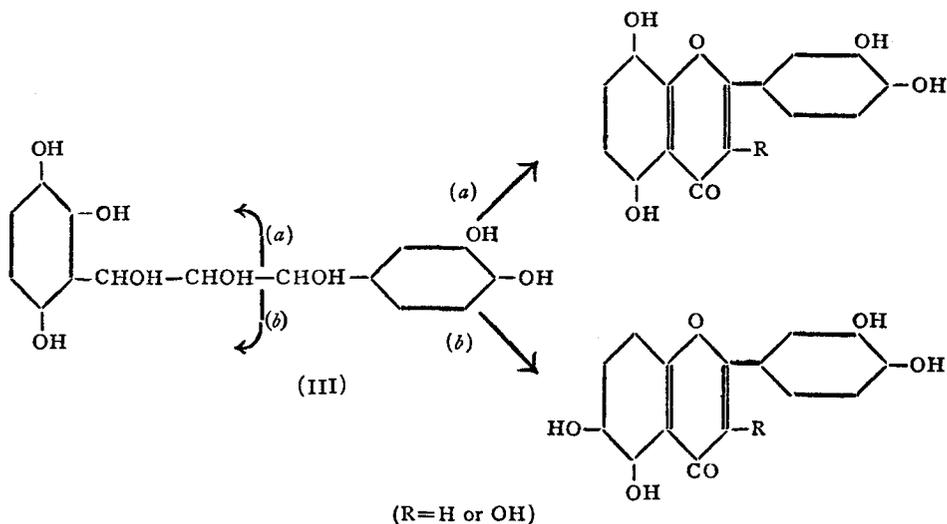
(ii) In this connection may be considered the components of the species of *Primula*. Primetin, 5:8-dihydroxy flavone, is obtained from the leaves of *P. modesta*.²¹ It is known that the flowers of *P. sinensis* contain pelargonidin or delphinidin glycosides depending on the variety.²² Obviously the compounds containing the 5:7 combination of hydroxyls are common in these plants and the evolution of the 5:8 type through the intermediate 5:7:8 is possible.

(iii) A very similar case arises in the two closely related flowers of the compositæ, *Tagetes erecta* and *T. patula*. The former contains quercetagetin and the latter patuletin in which the hydroxyl in the 7-position is missing.²³



The occurrence of the above-mentioned 5:8 and 5:6 combinations of hydroxyl groups which are not so common seems to be due to a stage involving reduction of 5:7:8 or 5:6:7 combinations whereby the 7-hydroxyl is lost. This process is obviously very rare but seems to exist definitely.

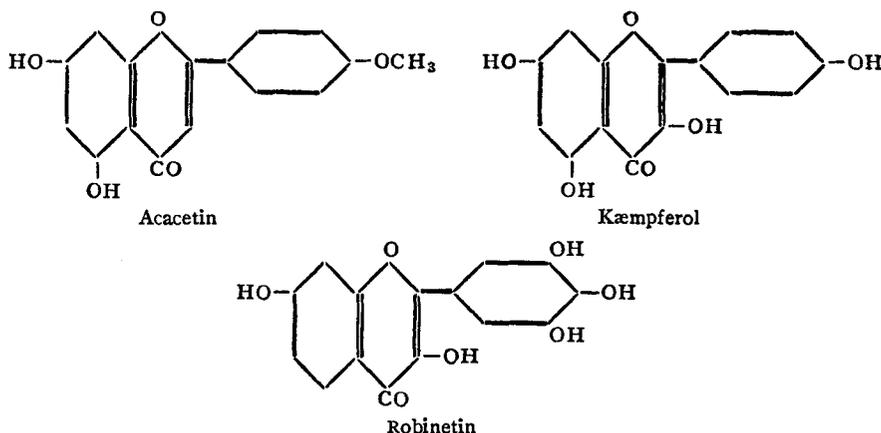
It could be suggested that the 5:6- and the 5:8-dihydroxy compounds are evolved from hydroxy quinol as shown below (III) instead of phloroglucinol present as part of the precursor (I).



But this idea is neither supported by the considerations of associations and plant relations discussed above nor by the occurrence of hydroxyquinol nucleus in other groups of compounds found in nature nor by the reactivity of this nucleus.

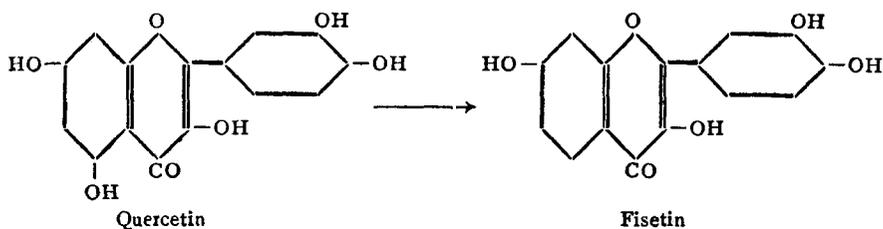
VI. There are two cases of compounds, fisetin and robinetin in which there is no hydroxyl group in the 5-position, only one being left in the 7th position. Are they evolved quite independently of the fundamental 5:7 compounds or are they derived from them? The second alternative is supported by the following considerations:—

(i) The leaves and bark of *Robinia pseudacacia* contain acacetin,²⁴ the flowers contain kæmpferol,²⁵ whereas the wood (dye wood) contains robinetin.²⁶



The association of 5:7 combinations with a compound containing only 7-hydroxyl group leads to the impression that it is derived from the fundamental dihydroxy compounds by a stage of reduction involving the hydroxyl in the 5-position. The possibility of oxidation or reduction in the side phenyl nucleus should be taken independently.

(ii) Other examples which support the above idea are obtained from "young fustic" and yellow cedar both of which are dye woods. The stem and branches of *Rhus cotinus* constituting "young fustic" contain fisetin, whereas the leaves of the plant (Venetian sumach) yield myricetin. In yellow cedar, fisetin is present in the stem and quercetin in the leaves.

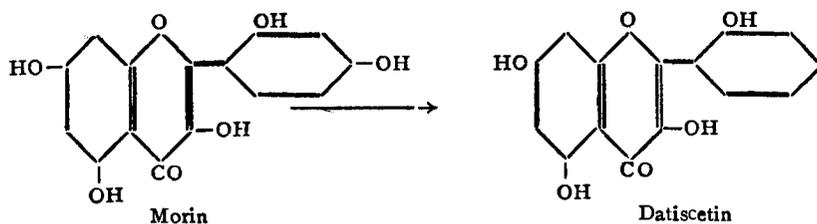


It is not unreasonable to consider fisetin as representing a later stage involving reduction resulting in the disappearance of the 5-hydroxyl found in quercetin. Myricetin, of course, is related to quercetin in a different way.

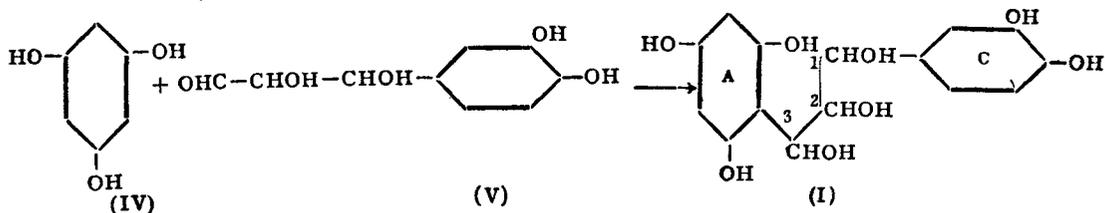
(iii) As a further example of this type may be mentioned pratol (7-hydroxy-4'-methoxy flavone) isolated by Power and Salway from the flowers of *Trifolium pratense*²⁷ and later by Rogerson from other species²⁸; it occurs in these along with quercetin and isorhamnetin.

From the data presented above the following conclusions could be arrived at. (1) The theory of Robinson regarding the origin of anthocyanins and anthoxanthins from a common precursor is supported. (2) The conclusion of Robinson and his collaborators regarding the stage of oxidation of ring (C) in anthocyanins could be applied to anthoxanthins also, *i.e.*, 3':4' combination of hydroxyls is the simplest and 3' and 3':4':5' combinations involve more stages. In the anthoxanthins complete reduction of the hydroxyls in ring (C) is represented by a large number of examples. (3) Superimposed on the changes brought about in ring (C), ring (A) is quite easily susceptible to changes involving oxidation and reduction of nuclear positions. 5:7 Combination of hydroxyl groups seems to be the primary state; 5:6:7- and 5:7:8-trihydroxybenzene structures involve a stage of oxidation and 5:6:7:8-tetrahydroxybenzene nucleus results from a further stage of the same nature. 5:8- and 5:6-dihydroxy types seem to result from trihydroxy types involving a stage of reduction leading to the disappearance of the 7-hydroxyl. Similar reduction seems to produce fisetin and robinetin in which the hydroxyl in the 5-position is lacking.

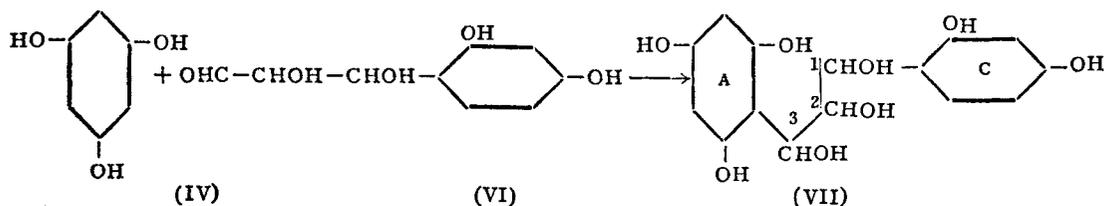
There is one more point that requires consideration. It relates to the occurrence of the two rare cases of flavonols morin and datiscetin. The former is found in "old fustic" (wood of *Chlorophora tinctoria*) and in jak wood whereas the latter is present in the leaves and roots of the bastard hemp. In the roots datiscetin is accompanied by galangin.²⁹



They have one hydroxyl group in the 2'-position. Obviously they belong to a rare type, but their occurrence may be taken as indicating the existence of a small variation from the fundamental precursor proposed by Robinson. This precursor is considered to be obtained from the union of two parts (IV) and (V), (IV) being phloroglucinol representing a hexose unit and (V) a nine carbon system derived from a hexose and a triose.



If the possibility of a variation in part (V) could be conceded as shown in (VI), then an alternative precursor (VII) having a resorcinol structure in the side phenyl nucleus instead of the catechol structure becomes available.



By the subsequent oxidation at C_1 and C_3 or at C_2 and C_3 morin could be obtained and from it involving a stage of reduction datiscetin could be evolved. But the occurrence of these types does not seem to be more common. The association of galangin with datiscetin is noteworthy. It could probably be derived from both catechol and resorcinol types of the side phenyl nucleus by complete reduction of the hydroxyl groups present in this part.

Summary

Based on the theory of Robinson *et al.* regarding the biogenesis of anthocyanins and on a detailed consideration of the occurrence of anthoxanthins (flavones and flavonols) in plants, a scheme of evolution of the various types of anthoxanthins is developed and discussed. Besides the existence of stages of oxidation and reduction of the side phenyl nucleus, similar modifications by oxidation and reduction of the benzopyrone part is involved. The possibility of a variation in ring (C) of the common precursor (I) as shown in structure (VII) leading to certain rare cases having hydroxyl groups in 2'- and 2':4'-positions is suggested.

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