SOME NEW CONSIDERATIONS IN THE BIOGENESIS OF SAP-SOLUBLE PIGMENTS

By T. R. Seshadri, F.A.Sc.

(From the Department of Chemistry, Delhi University)

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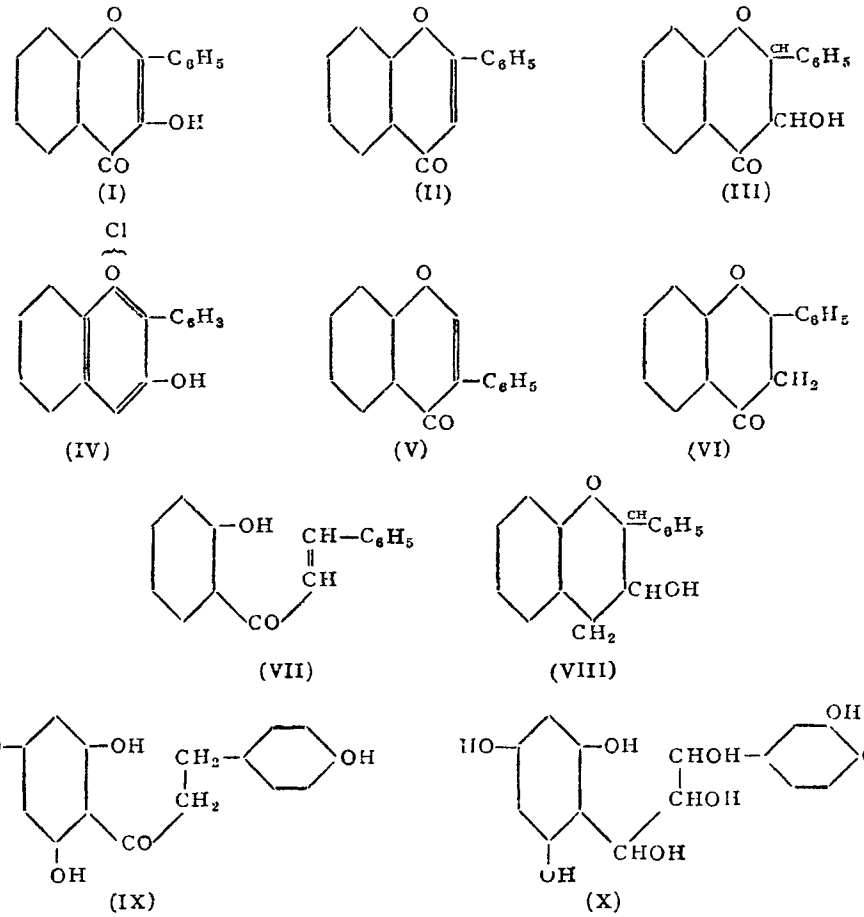
Just like the components of blood for animals, the sap-soluble pigments (anthoxanthins and anthocyanins) should be of importance for the physiology of plants though adequate information is not available about the part they play. Szent-Gyorgyi[1] considered that hydroxy-flavones play an important part as components of oxidation-reduction systems. Suggestions have also been made that the pigments control light absorption in the growing parts, leaves and flowers and thus regulate photosynthesis. That some of them have hormonal properties was shown by Kuhn and Low.[2] But it seems to be definitely established that they are closely connected with the individual characteristics of a plant and the occurrence and details of the constitution of the different pigments are genetically controlled. As far as human interest is concerned, the anthocyanins have an æsthetic appeal and are of value in horticulture whereas the anthoxanthins used to be valued as mordant dyes. With the advent of synthetic dyes this value of anthoxanthins has ceased to exist, but more recently they have been found to have definite physiological effects on the animal system and may continue to elicit interest for this reason. To the plant-breeder the study of these pigments provides great help in the study of genetics.

Among the sap-soluble pigments containing the C_{15} skeleton the following represents the arrangement of groups based on the state of oxidation of the oxygen ring: (1) flavonols (highest) (I); (2) flavones (II), flavanonols (III), anthocyanidins (IV), and isoflavones (V); (3) flavanones (VI), and chalkones (VII); (4) catechins (VIII) and phloretin (IX). Any general theory should embrace all these different but closely related groups.

In the study of the biogenesis of the sap-soluble pigments attention was given earliest to the association of flavonols and anthocyanins and it was considered that the latter were derived from the former; chemically a process of reduction was needed and it could be carried out *in vitro*. But this theory of sequential origin could not be supported because of the lack of correlation in the structures of the flavonols and anthocyanins occurring together in the same source. Further in some cases the accompanying anthoxanthins were not flavonols but flavones; for example in the flowers

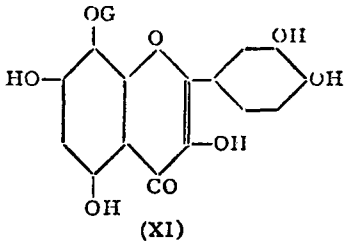


of *Antirrhinum majus* the flavones apigenin and luteolin accompany the anthocyanin, a cyanidin glycoside. The relation between flavones and anthocyanidins (3-hydroxy compounds) is not so direct and a number of intermediate stages will have to be postulated for the transformation of one into another. These difficulties were removed by the theory of parallel origin from a common precursor (X) as proposed by Robinson.[3] Some of the implications of the theory have been examined by making a survey of anthocyanins[4] and by a study of flavones and flavonols[5] and the results are confirmatory. Though the results of investigations in the field of genetics are in accord with this theory, the alternative of sequential evolution cannot be excluded. The position seems to be best summed up in the words of Stephens[6]: "Parallel syntheses of these structurally similar pigments would surely require that the genes controlling these syntheses should be extremely specific in their actions. Otherwise a gene responsible for a

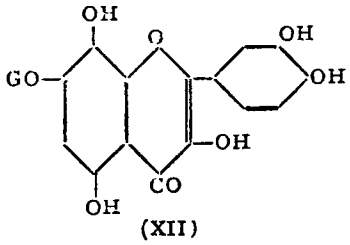
particular substitution in an anthocyanin molecule might be expected to bring about a similar substitution in the accompanying anthoxanthin. However if this situation is avoided by postulating extreme specificity of the genes, the whole argument in favour of parallel syntheses is much weakened. A situation involving sequential synthesis might be equally probable. For example, a certain genotype might contain specific genes for converting quercetin into cyanidin, and in addition a specific gene for methylating the cyanidin nucleus. In that case one would not necessarily expect quercetin to be accompanied by cyanidin in the same flower, since the cyanidin might easily be converted into a methylated form (that is, peonidin) as fast as it was formed. It seems therefore that a critical discrimination between the alternative hypotheses of parallel or sequential syntheses must await more information on the specificity of the genes concerned ".

As already mentioned these studies in biogenesis should be extended to embrace all the groups of sap soluble pigments in order to get a complete picture. The new considerations that are discussed in the present paper seem to indicate clearly that the process of sequential evolution does exist to a limited extent and should be considered as playing a part along with the process of parallel evolution. These relate to the occurrence of methyl ethers and glycosides of anthoxanthins. The position occupied by ether and glycoside groups varies and practically all positions are known to be capable of being involved. No precise information seems to be available as to why and how the choice of particular positions is made in nature in individual cases though the question is quite important. The following seem however to indicate the possible reasons: (1) solubility, (2) light absorption and (3) stability to oxidation. As an example gossypin[7] (XI) (8-glucoside of gossypetin) and gossypitrin[8] (7-glucoside) (XII) can be compared. The former is readily soluble in water whereas the latter is not. Further gossypin is more deeply yellow. It is also more stable to oxidation since the quinol structure is protected. Stability to oxidation is a particularly important consideration. Where there are a large number of phenolic hydroxyl groups especially in *ortho* or *para* orientation, oxidation can take place readily and this can be conrolled to a considerable extent by glycoside or ether formation. To these three reasons may be added a fourth. The physiological properties of the compounds seem to be also affected markedly by these modifications.

A question which has been raised in regard to them is whether glycoside formation and similarly ether formation represent final stages in evolution or whether they take place even earlier. These reactions are definitely genetically controlled and in most cases the glycosides and the aglucones



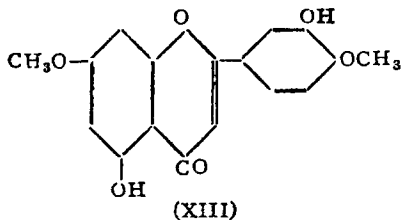
(XI)



(XII)

and similarly the methyl ethers and the parent hydroxy compounds occur together. Armstrong and Armstrong in their monograph on Glycosides came to the conclusion that glycoside formation is the final stage. Stephens[9] working from the side of genetics has also arrived at the same result. Even this seems to require some modification in the light of the chemical considerations discussed below.

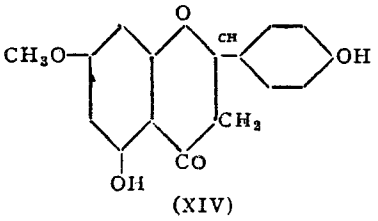
7-Methyl ethers of flavones and flavonols are of frequent occurrence and so also are 7-glycosides. This may indicate either that the 7-hydroxyl is markedly more reactive than the rest or that there is an efficient mechanism for protecting the other positions. Very little is known about the second alternative except that the 5-position is definitely protected by chelation with the neighbouring carbonyl. In the simple dihydroxy flavone, chrysin, the 7-hydroxyl is far more reactive than the 5- for this reason and partial methylation or glycosidation readily takes place. But as soon as fresh hydroxyls are introduced in the pyrone ring and side phenyl nucleus, the difference between the 7- and other positions becomes much less marked. Perkin and Horsfall[10] made attempts to prepare a monomethyl ether of luteolin by direct methylation and recorded that they could get only the 7:4'-dimethyl ether (XIII) in a small yield. It is therefore clear that direct partial methylation or glycoside formation of flavones and flavonols in the 7-position is not a successful one in the laboratory and cannot be expected to be successful even in the plant.



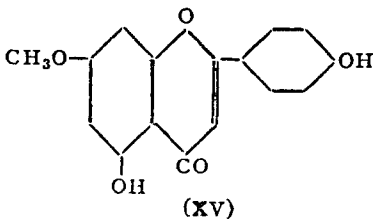
(XIII)

The possible explanation of the occurrence of 7-methyl ethers of hydroxy flavones is suggested by certain recent laboratory work. (1) The bark of *Prunus puddum*[11] contains three closely related 7-methyl ethers (i) sakuranetin (XIV), (ii) genkwanin (XV) and (iii) prunetin (XVI) which form a significant association. Sakuranetin and sakuranin have been known to occur

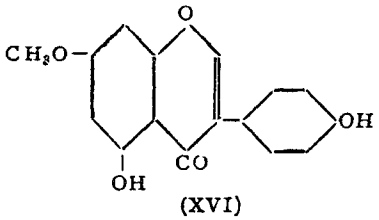
in various species of *Prunus* and so also prunetin and its glucoside. But the discovery of (i) and (iii) occurring together and along with genkwanin is new. (2) It has also been shown[12] that with hydroxy flavanones (*e.g.*, naringenin XVII) and isoflavones (*e.g.*, genistein XVIII) partial monomethylation of the 7-position can be carried out.



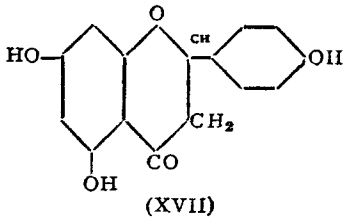
(XIV)



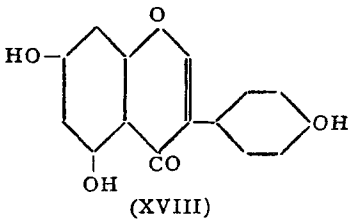
(XV)



(XVI)



(XVII)

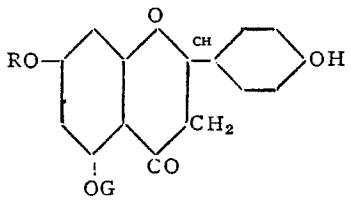


(XVIII)

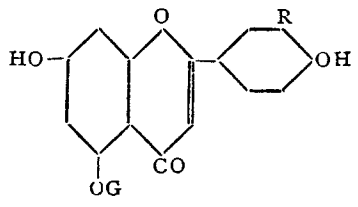
The markedly higher reactivity of the 7-hydroxyl as compared with the others is inherent in the structure of these flavanone and isoflavone molecules. The carbonyl group activates the 7-hydroxyl by electromeric polarisation and deactivates the 5-hydroxyl by chelation, the hydroxyls in the side phenyl nucleus not being affected. It is relevant to mention here that Zemplen, *et al.*[13] have successfully prepared hesperidin (7-glycoside) from the flavanone hesperetin and genistin (7-glucoside) from the isoflavone genistein. (3) A third useful observation is that flavanones can be readily oxidised by mild oxidising agents[14] (iodine in the presence of sodium acetate) to the corresponding flavones. Taking all the three together the correct explanation of the origin of genkwanin could be derived. It cannot be obtained by the direct methylation of apigenin, but can be prepared by the oxidation of sakuranetin along with which it occurs in the plant, and sakuranetin is the direct product of the monomethylation of naringenin. The scheme is as follows: XVII → XIV → XV. It would mean acceptance of the sequential evolution of 7-methoxy flavones from the corresponding flavanones and that methylation is not always the last stage in evolution.

The above mentioned occurrence of the isoflavone (prunetin) along with its isomeric flavone (genkwanin) is a rare example of such association. It is obviously a result of parallel evolution, sakuranetin forming an intermediate stage for genkwanin. The same genetic factor could then be responsible for the methylation in both cases since in regard to this reaction prunetin and sakuranetin are very similar.

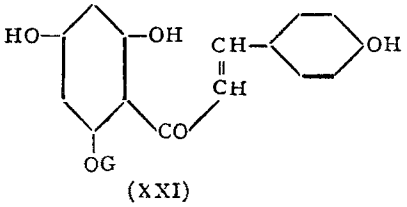
A similar position requiring special explanation is created by the discovery of glycosides with the sugar groups in the difficultly accessible 5-position of flavones and flavanones. Examples are: (1) salipurposide, 5-glucoside of naringenin[15] (XIX, R = H); (2) sakuranin, 5-glucoside of sakuranetin[16] (XIX, R = CH_3); (3) galuteolin,[17] luteolin-5-glucoside (XX, R = OH) and (4) apigenin-5-glucoside[18] (XX, R = H). It will be quite unacceptable to suggest direct entry of the sugar groups as the last stage in the formation of these compounds. A reasonably correct explanation is indicated by the presence of sugar groups in a corresponding position (6-position) in isosalipurposide (XXI),[19] phloridzin[20] (XXII, R = H) and asebotin[21] (XXII, R = CH_3). In the case of these compounds in which the oxygen ring has not been closed up, the 6-position can be reactive and direct glycoside formation can take place. This can happen even when there is a carbonyl group since it can deactivate only one *ortho*-hydroxyl group. It could therefore be suggested that glycoside formation in the 6-position takes place at an earlier stage and the flavanone ring is subsequently formed leading to the production of a 5-glycoside (XIX). In this connection the occurrence in *Salix purpurea* of isosalipurposide (XXI) is of some significance as it may indicate an intermediate stage in the formation of salipurposide in the same plant. But it may not be correct to say that glycoside formation takes place at the chalkone stage, because it has been shown[22] that chalkones with free



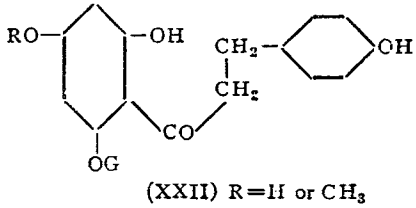
(XIX) R = H or CH_3 G = Sugar



(XX) R = H or OH



(XXI)



(XXII) R = H or CH_3

hydroxyls in the 2- and 6-positions have little stability and would immediately change into flavanones in which the 5-hydroxyl would become unreactive. Hence the sugar group should have entered in some as yet undetermined earlier stage. From the flavanone-5-glycoside to the flavone-5-glycoside is the next step involving oxidation or dehydrogenation. Zemplen and Meister[18] have been able to convert salipurposide (XIX, R = H) into the 5-glucoside of apigenin (XX, R = H). Hence in regard to the 5-glycosides also the ideas of glycoside formation at an earlier stage and sequential evolution have to be applied.

SUMMARY

The sap-soluble pigments of plants consist of anthocyanins and a number of related groups of anthoxanthins. Any comprehensive theory of biogenesis should embrace all these groups. The theories of sequential and parallel origin are discussed. Based on chemical considerations with reference to certain methyl ethers and glycosides of anthoxanthins it is concluded that sequential origin should be recognised as playing a definite part though parallel evolution may be the major factor. Further, glycoside and ether formation may not be the last stages in evolution in all cases.

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