

NUCLEAR OXIDATION IN THE FLAVONES AND RELATED COMPOUNDS

Part XIII. A Discussion of the Results

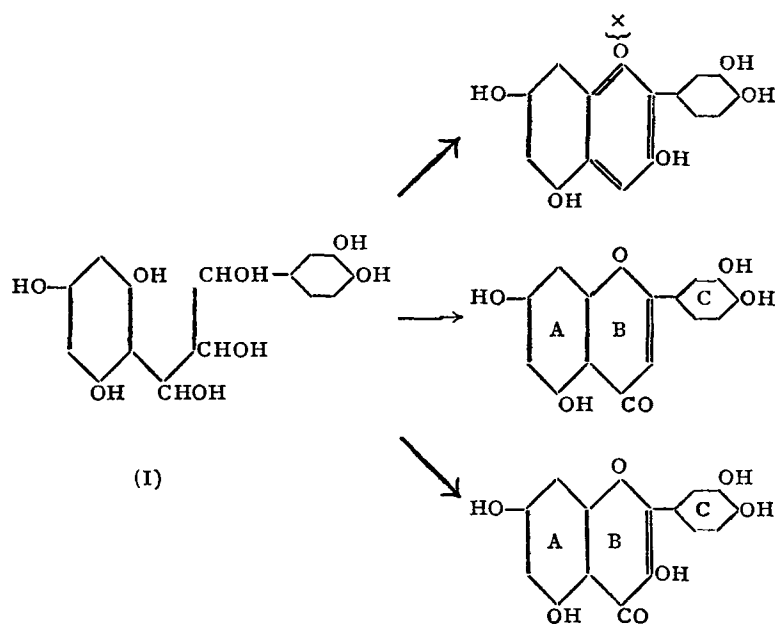
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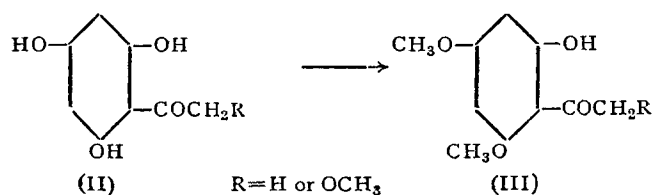
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I. BIOGENESIS

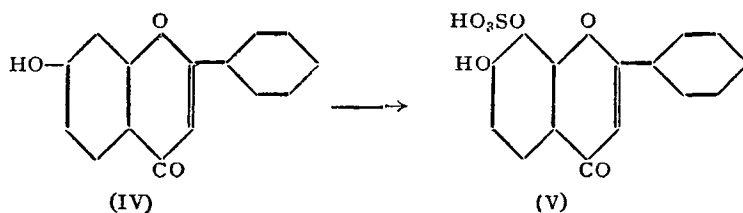
IN a paper by Rao and Seshadri¹ published in 1943 data bearing on the occurrence of anthoxanthins and their association in nature were presented and discussed. They were found to support the theory of Robinson regarding the origin of anthocyanins and anthoxanthins from a common precursor (I). The most outstanding structural feature of the anthoxanthins is the large number of states of oxidation in which ring (A) is capable of existing. The biological data could satisfactorily be explained on the basis that flavones and flavonols having two hydroxyl groups (5:7) in ring (A) represent primary stages in biogenesis and those having three hydroxyls (5:6:7 or 5:7:8) and four hydroxyls (5:6:7:8) involve additional stages of oxidation.



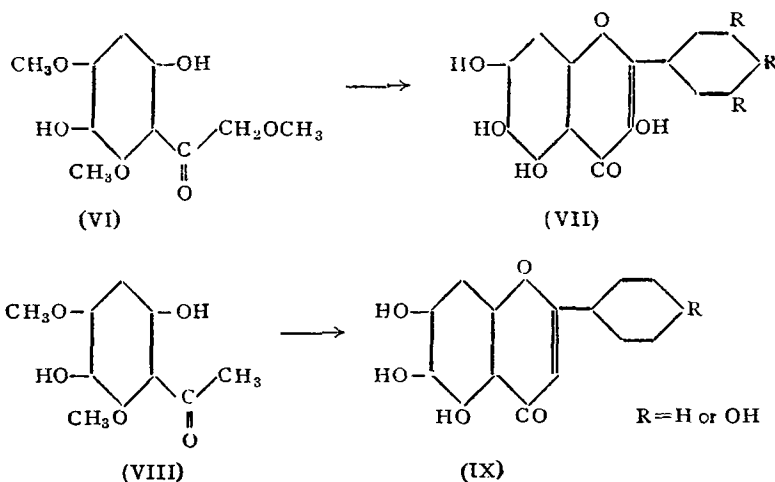
Laboratory experiments on nuclear oxidation in support of the above conclusions could not be carried out at that time owing to severe war conditions. They could be taken up more recently and the subject examined in detail. At the beginning it appeared that direct attack on the flavones themselves was not likely to be successful in view of the failures recorded in the past.^{2, 7} Consequently as the first stage in the development of the model experiments phloroglucinol derivatives were chosen. Phloroacetophenone and ω -methoxy-phloroacetophenone (II) and their derivatives had not been subjected to nuclear oxidation before. In order to prevent general oxidation of the molecules the 4:6-dimethyl ethers (III) were prepared leaving the hydroxyl in the 2-position alone free. The partial methylation is easily effected since this particular hydroxyl is definitely more resistant to methylation than the others.



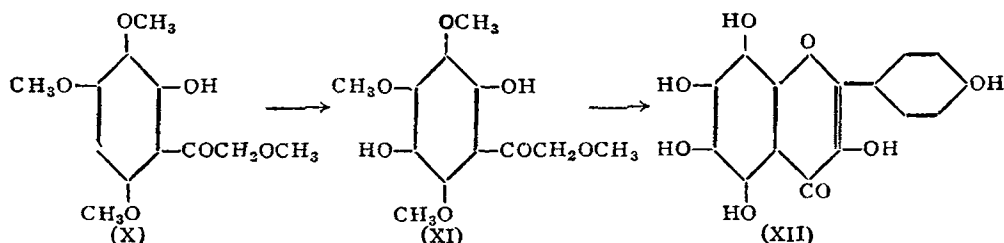
As the most suitable and direct oxidising agent alkaline potassium persulphate was chosen. It behaves as a kationoid (electrophilic) reagent and introduces a hydroxyl group in the position para to the activating group; ortho substitution is also possible as a second alternative though not so readily, if a para position is not available. As a convenient intermediate stage a sulphate ester is formed and this being soluble in water, separation of the unchanged original substance becomes easy. Subsequent hydrolysis of the ester yields the final product. In the course of our work on nuclear oxidation this intermediate sulphate could be isolated in one case³ as a crystalline solid and characterised.



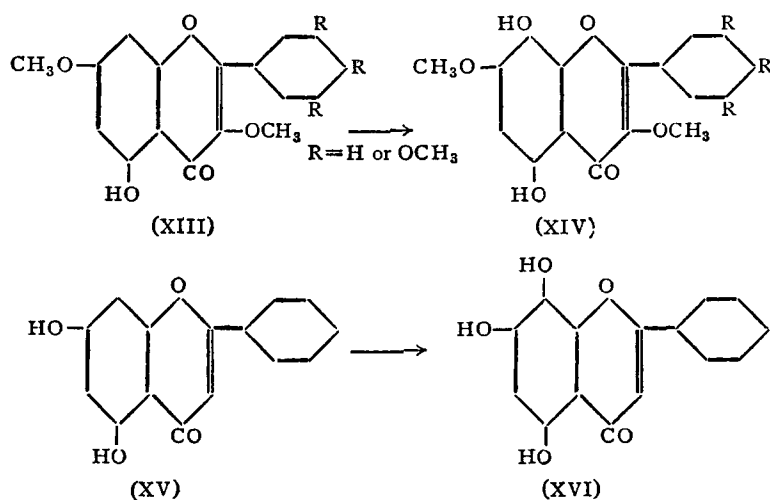
The oxidation products (VIII, VI) of phloroacetophenone and its ω -methoxy-derivative could then be subjected to the flavone condensation; thus all the four flavonols (VII) of the quercetagenin series⁴ (1) 6-hydroxygalangin, (2) nor-tangeretin, (3) quercetagenin and (4) 6-hydroxy myricetin were synthesised and so also the flavones (IX), baicalein and scutellarein.⁵



As the next higher stage, starting with gossypetol-tetramethyl-ether the flavonols of the calycopteretin series⁶ could be similarly synthesised.

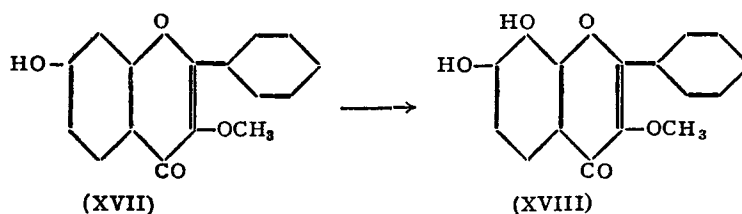


More recently the nuclear oxidation of typical 5:7-hydroxy flavones and flavonols could be effected. Though attempts made by earlier workers² had been unsuccessful the reaction was reinvestigated⁷ repeatedly since the possibility of such nuclear oxidation in the flavone series was so definitely indicated by evidences from other directions. Here too partial methylation had to be first done in order to protect against general oxidation. It was found to be quite easy to carry this out leaving the resistant 5-hydroxyl alone free. Alkaline persulphate proved again to be the most satisfactory oxidising agent. At first there was difficulty owing to the very sparing solubility of these 5-hydroxy compounds in aqueous alkali. This was subsequently got over by adding the required amount of pyridine in order to get a clear solution. Thus starting from the flavonols of the quercetin series have been synthesised in an easy manner the flavonols (XIV) of the gossypetin series⁷—8-hydroxy-galangin, herbacetin, gossypetin and hibiscetin—and the flavone (XVI), nor-wogonin⁸ from chrysin.



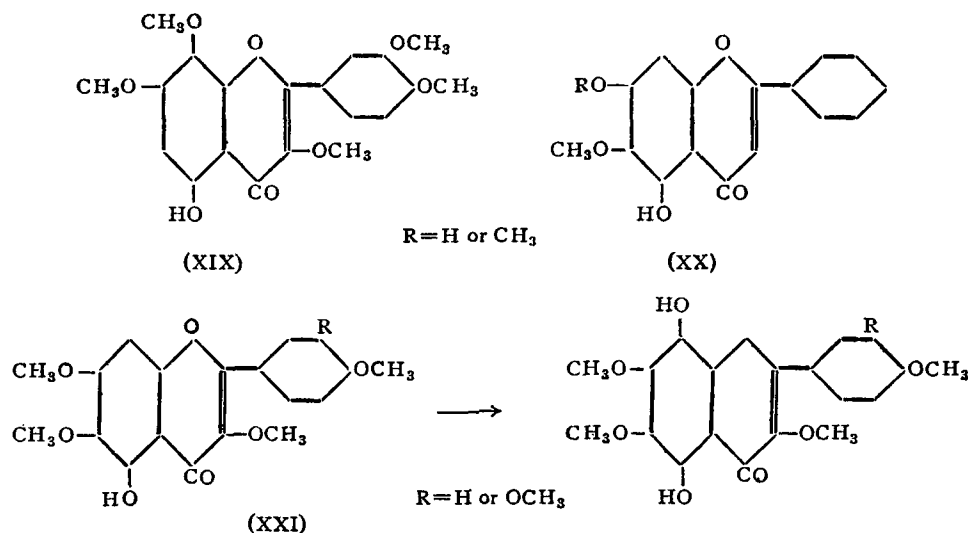
Later it was realised that it was not invariably necessary to protect the hydroxyl group in the 7-position. As a matter of fact it was an advantage to have it free in some cases since the compound was then soluble in aqueous alkali and gave rise to better yields of the oxidation product.

As shown above it is very easy to oxidise a 5:7-hydroxy flavone into a 5:7:8-hydroxy compound. There seemed to be no chance of preparing the 5:6:7-hydroxy compounds by this means. The hydroxyl group in the 5-position activates markedly the 8-position. The simplest case is the oxidation of 5-hydroxy flavone to primetin.⁹ When the 8-position is occupied as in gossypetin 3:7:8:3':4'-pentamethyl ether¹⁰ (XIX) no reaction takes place and the compound is recovered unchanged. As far as the hydroxyl in the 7-position is concerned it is generally known to activate position 8 in all reactions. The nuclear oxidation is no exception to this. Actually in some cases good yields of 7:8-hydroxy compounds³ (XVIII) could be obtained from 7-hydroxy compounds (XVII).

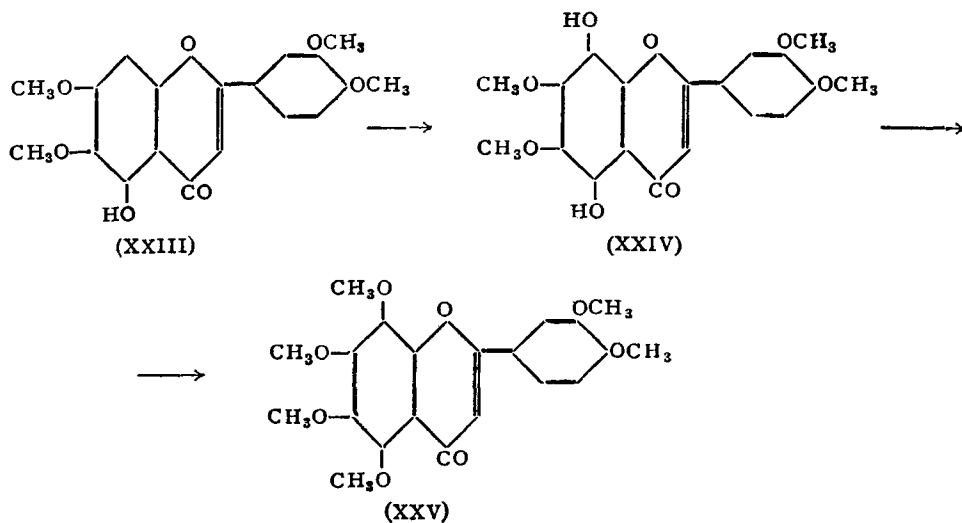


Since the free 6-position was found to be unreactive in gossypetin-pentamethyl ether (XIX), the 5:7:8-hydroxy flavones and flavonols cannot be used for obtaining the tetrahydroxy (5:6:7:8) compounds (XII). On the other hand it is quite easy to oxidise the 5:6:7-hydroxy compounds

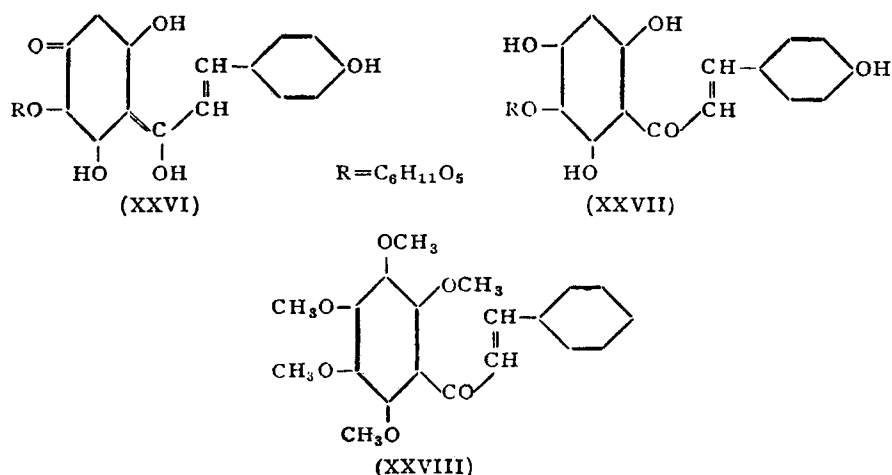
to the 5:6:7:8-hydroxy analogues employing the conditions already mentioned. In these cases it appeared to be necessary to protect even the 7-hydroxyl, since oroxylin-A (XX, R = H) did not give satisfactory results whereas baicalein 6:7-dimethyl ether (XX, R = CH₃) underwent the change smoothly.¹¹ From nortangeretin, calycopteretin and from quercetagenin, 6:8-dihydroxy quercetin have been prepared¹⁰ (XXI → XXII).



Similarly the synthesis has been carried through using the flavones, baicalein, scutellarein and 6-hydroxy-luteolin (XXIII) and members of the nobiletin (XXV) (nornobiletin) series obtained.^{11, 12}

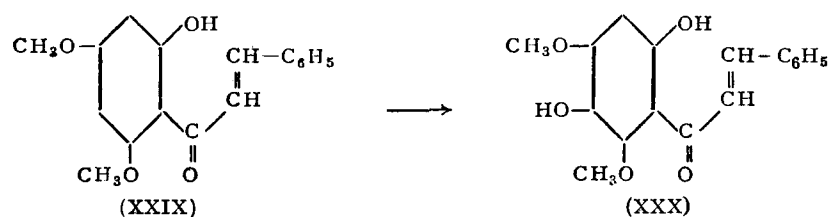


The experimental results described so far would lead to the following conclusions regarding the evolution in nature of compounds with three and four hydroxyl groups in ring (A). (1) 5:7-Hydroxy-flavones and flavonols as well as their analogues with ring (B) open are involved in the oxidation. Partial protection of the hydroxyl groups is effected whatever may be the mechanism adopted. (2) 5:7:8-Hydroxy-flavones and flavonols (gossypetin and norwogonin series) result from the corresponding 5:7-hydroxy-flavones and flavonols by nuclear oxidation in the 8-position. This takes place very readily in the laboratory. Though the alternative, that is, the nuclear oxidation of the open form and subsequent ring closure to yield this type could not be altogether excluded, it seems to be unlikely as will be discussed below. (3) For the formation of the 5:6:7-hydroxy-flavones and flavonols the oxidation should involve the open form and the ring closure should take place subsequently. It has already been mentioned that these compounds cannot be produced from the fully formed 5:7-hydroxy-flavones themselves since the nuclear oxidation of the 6-position does not take place particularly when the 8-position is unsubstituted. As satisfactory model experiments could be taken the oxidation of phloroacetophenone and ω -methoxyphloroacetophenone-dimethyl ethers already described (III \rightarrow VIII). Among compounds more closely related to flavones and with the ring (B) open, chalcones could be taken for experimentation. These occur in nature and examples with the ring (A) containing four and five hydroxyl groups (free or methylated) are found in carthamin¹³ (XXVI) and *iso*-carthamin (XXVII) and pedicin, pedicellin (XXVIII) and their allies.^{14, 15}

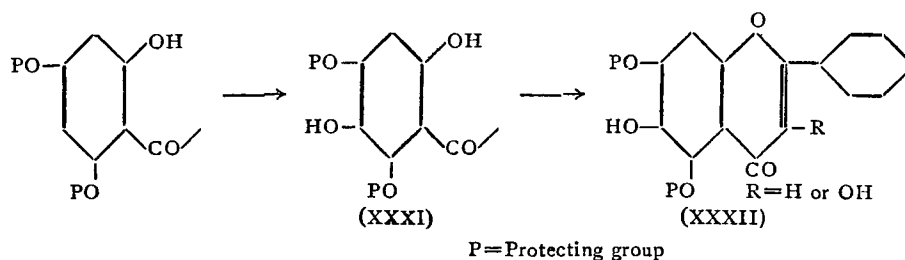


Experiments have now been carried out using 2-hydroxy-4-methoxy-chalkone and 2-hydroxy-4:6-dimethoxy chalkone¹⁶ (XXIX). They give rise to the

5-hydroxy compounds (XXX) under almost the same conditions as the simpler ketones.



Here it may be contended that in the plant after the oxidation of the open structure, ring closure could take place in either of two ways leading to the 5:6:7-hydroxy compounds or the 5:7:8-hydroxy compounds. The latter possibility seems to be very small from the following considerations. In laboratory experiments the 5:6:7-hydroxy flavones have greater stability and are formed in preference; in several cases 5:7:8-hydroxy flavones undergo isomeric change into the 5:6:7-type when boiled with hydriodic acid (see for discussion Sastri and Seshadri^{5, 17}). Further, since some kind of protection of the hydroxyl groups is employed as indicated below, the ring closure to the 5:6:7-hydroxy form (XXXII) will be the most direct.



(4) The 5:6:7:8-hydroxy-flavones and flavonols are produced by the nuclear oxidation of the corresponding 5:6:7-hydroxy compounds. This has been found to take place easily in the laboratory.^{10, 11, 12} The occurrence of tangeretin and nobiletin in the closely related species of citrus nobilis has been mentioned in this connection. The alternative formation from the oxidation of the appropriate open form analogous to gossypetol-tetramethyl ether⁶ (X) cannot be excluded though this process may be less direct. Actually pedicin, pedicellin and its associates are the results of such complete hydroxylation in the chalcone group (see Rao and Seshadri¹⁵). The case of pedicin and related compounds is discussed in detail later on.

II. PARTIAL PROTECTION

The necessity for the protection of the large number of hydroxyl groups in order to escape general oxidation has now to be emphasised. This also

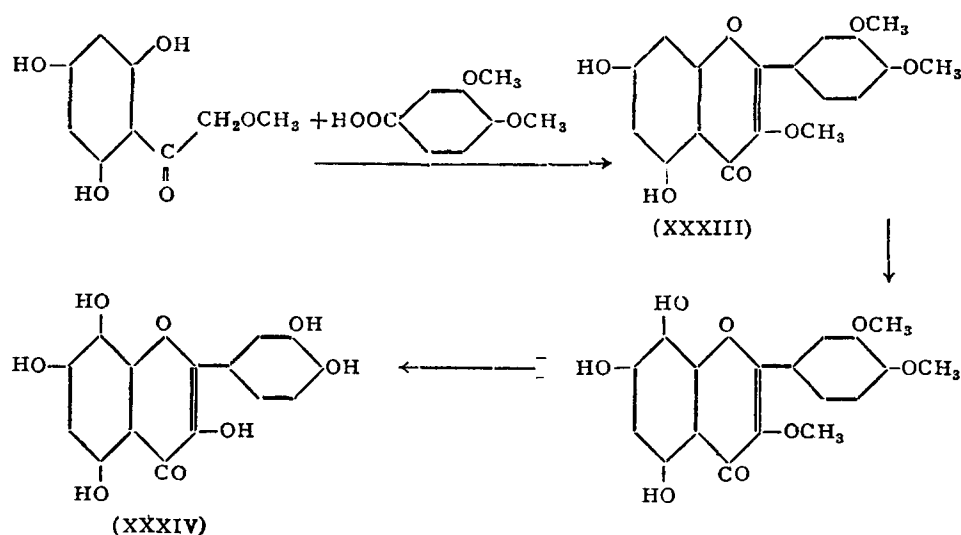
seems to direct the course of the nuclear oxidation. In the laboratory partial methylation can be conveniently adopted. Benzyl groups can also be used for this purpose and their more easy removability renders them handy in special cases though benzyl ethers are more difficult to prepare and to oxidise. In nature too protection of hydroxyl groups should be in operation whatever may be the protective mechanism involved. Probably the simplest suggestion would be that the protection is effected by a process of absorption or chemisorption on the enzymes required for biological oxidation. Such a mechanism has been suggested by Cook¹⁸ in connection with the hydroxylation of carcinogenic hydrocarbons in the animal body.

In the case of the flavones and flavonols the 5-hydroxyl is definitely more resistant than the others and hence is left conveniently out during partial protection. It is then free to activate the 8-position giving rise to the easy formation of the 5:7:8-hydroxy compounds. Regarding the related open forms (*e.g.*, chalcones) it is again interesting to note that only one orthohydroxyl is resistant to methylation or protection^{4, 5} (see also Robertson, Robinson and Struthers¹⁹ for explanation) and partial protection can therefore be conveniently provided leaving out this hydroxyl which can then activate the available para position. Hence the schemes given above based on partial protection of the phenolic hydroxyl groups become valid.

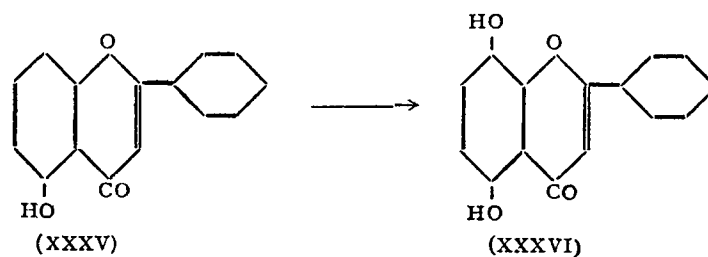
III. SIMPLIFICATION OF THE SYNTHESIS OF NATURALLY OCCURRING FLAVONES

It is not infrequent that efforts to understand the methods of Nature lead to considerable simplification of laboratory methods of synthesis. The classical example of Robinson's synthesis of tropinone is well known. As a result of the experiments on nuclear oxidation a large number of naturally occurring flavones and flavonols have now been synthesised by methods which are direct and simple and mean considerable improvement over older methods. The new syntheses of the flavonols of the quercetagenin series⁴ and of baicalein and scutellarein⁵ have already been mentioned. These involve the oxidation of the partial methyl ethers of phloroacetophenone and ω -methoxy phloroacetophenone. Nuclear oxidation of the flavones themselves have led to more important results. Thus gossypetin, herbacetin and hibiscetin⁷ are readily prepared from quercetin, kaempferol and myricetin. From the point of view of total synthesis the discovery that partial methyl ethers (XXXIII) with both 5 and 7-hydroxyl groups free, undergo oxidation more readily than the 5-hydroxy compounds is a definite advance, because these dihydroxy-compounds are the immediate products of Allan-Robinson

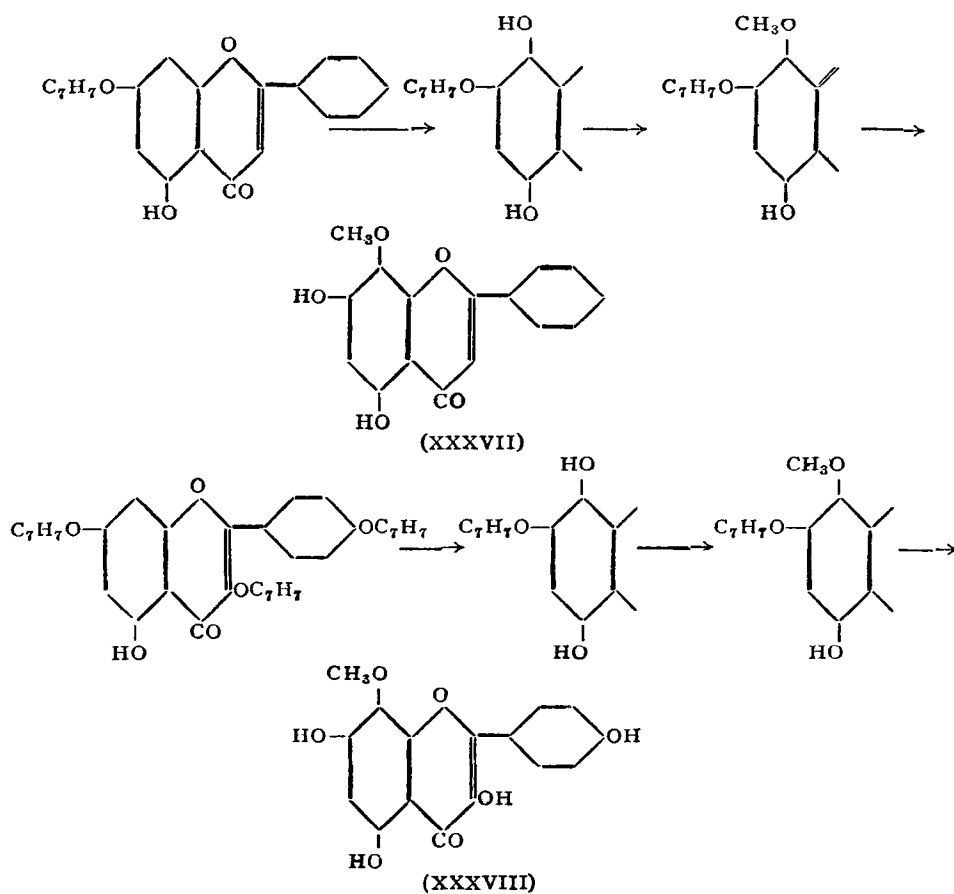
synthesis. The following illustration takes the case of gossypetin (XXXIV) synthesised from quercetin trimethyl ether.



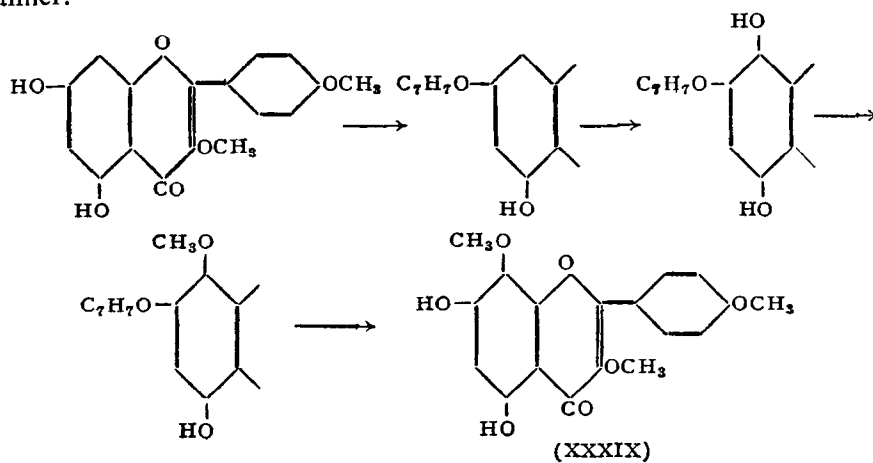
More complex examples are the synthesis of calycopterin and its higher analogue¹⁰ from nortangertin and quercetagetin and of nobiletin¹² and its group¹¹ from the flavones of the baicalein series. Norwogonin and primetin are simpler compounds, but there was originally considerable difficulty in their synthesis. Besides the usual difficulties of the preparation of flavones as compared with flavonols, such as poor yields and 3-acylation, in these cases there was the additional difficulty arising from isomeric change during demethylation. However nuclear oxidation of chrysin (XV) and 5-hydroxy flavone (XXXV) takes place smoothly and norwogonin⁸ (XVI) and primetin⁹ (XXXVI) can be obtained pure with great ease.



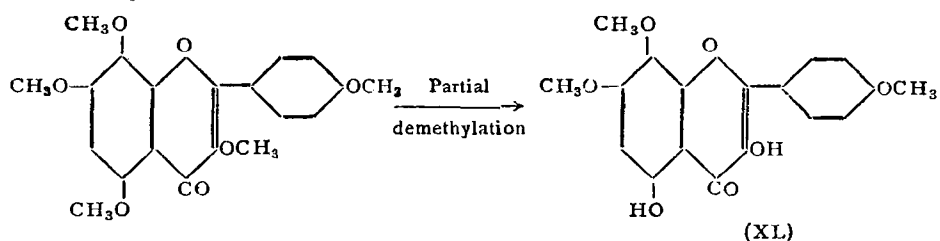
Another interesting application of nuclear oxidation is in the synthesis of the partial methyl ethers, wogonin²⁰ (XXXVII) and tambuletin²¹ (XXXVIII). These involve initial protection of some hydroxyl groups by benzylation and removal of the benzyl groups finally.



Tambulin was originally considered to be 3:8:4'-trimethyl ether of herbacetin (XXXIX). This partial ether was prepared in the following manner.²²



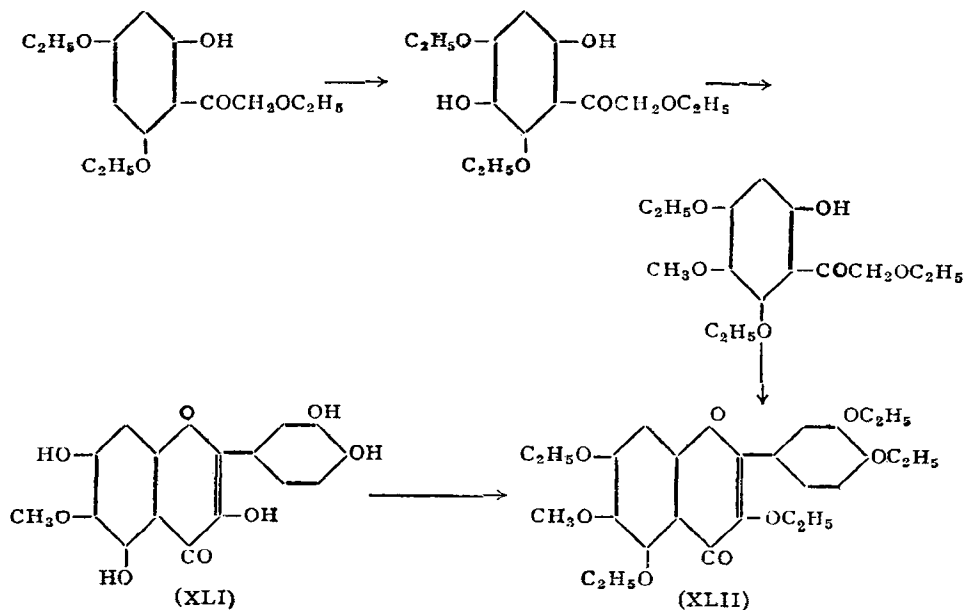
The product was however found to be different from tambulin. Examining other possibilities it has been shown that it should have the constitution of 7:8:4'-trimethyl ether of herbacetin (XL).²³ This ether is conveniently prepared by the partial demethylation of pentamethyl herbacetin using aluminium chloride.



IV. STUDY OF CHEMICAL CONSTITUTION

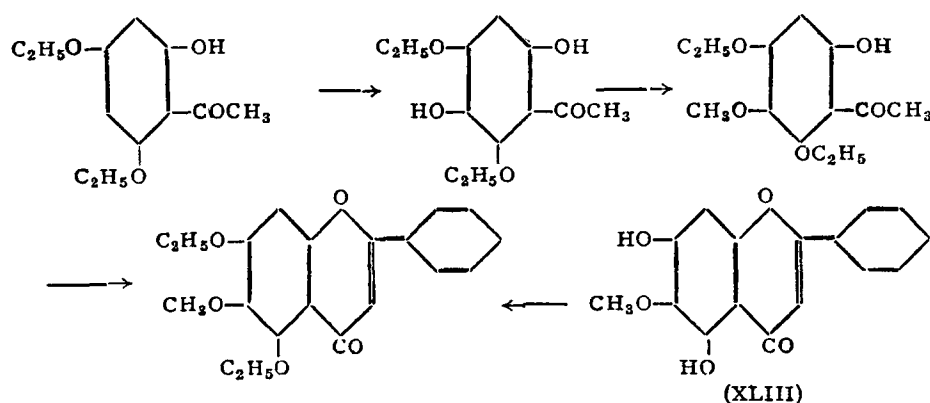
The method of nuclear oxidation is particularly useful in the study of the chemical constitution of glycosides and partial methyl ethers of anthoxanthins having three and four hydroxyls in ring (A). It has been successfully applied to a number of cases and has resulted in considerable simplification of the work and definiteness of the conclusions.

(a) *Partial methyl ethers*.—The earliest example was patuletin (XLI). That it is the 6-methyl ether of quercetagenin²⁴ was surmised from a comparison of its colour reactions with those of suitably chosen related compounds. This was then confirmed²⁵ by the preparation of pentaethyl patuletin (XLII) and its synthesis from ω -ethoxy phloroacetophenone. The

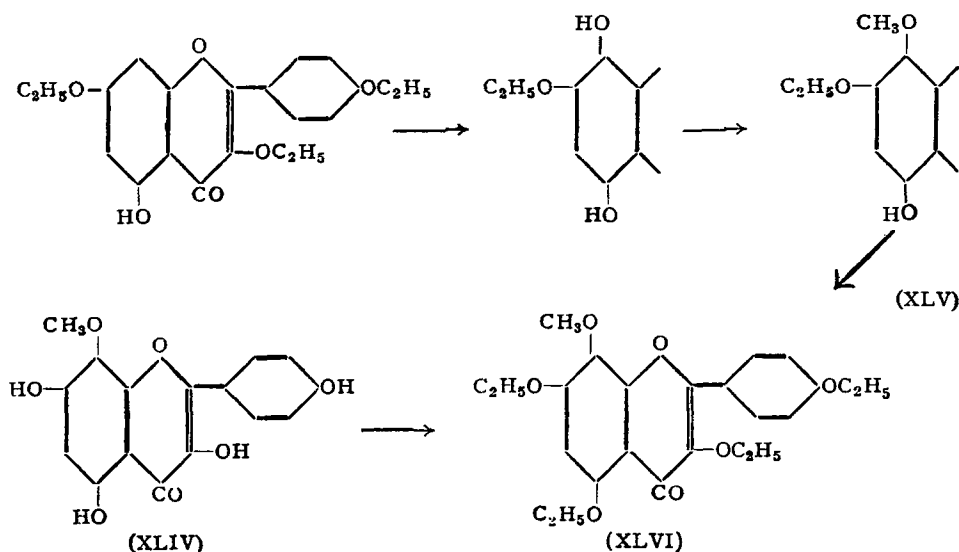


use of ethylation for this purpose and the study of the mixed ethers is a marked improvement in this type of work. The presence of free hydroxyl groups in these partial ethers is a disadvantage in both degradation and synthetic work. The difficulty is removed by ethylation and hence it has been adopted regularly in this laboratory.

Oroxylin-A (XLIII) is a similar case. It is the 6-methyl ether of baicalein and its constitution was also established in an analogous manner by the synthesis of its diethyl ether starting from phloroacetophenone.²⁶

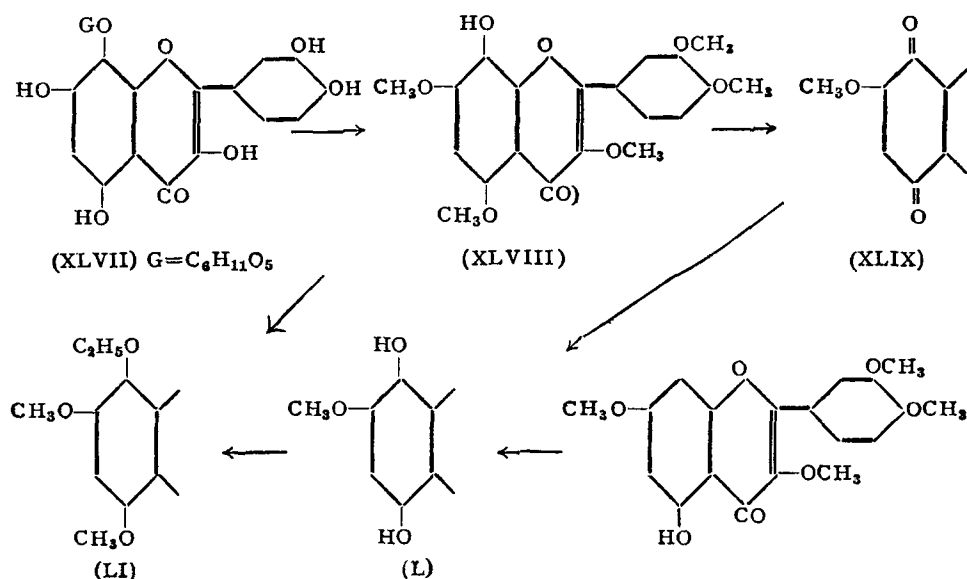


An example where the nuclear oxidation of a flavone itself has been employed is that of tambuletin²⁷ (XLIV). Its constitution as the 8-methyl-ether of herbacetin was inferred from its properties and colour reactions. This was confirmed by the preparation of its triethyl (XLV) and tetraethyl (XLVI) ethers and their synthesis²⁸ as follows:

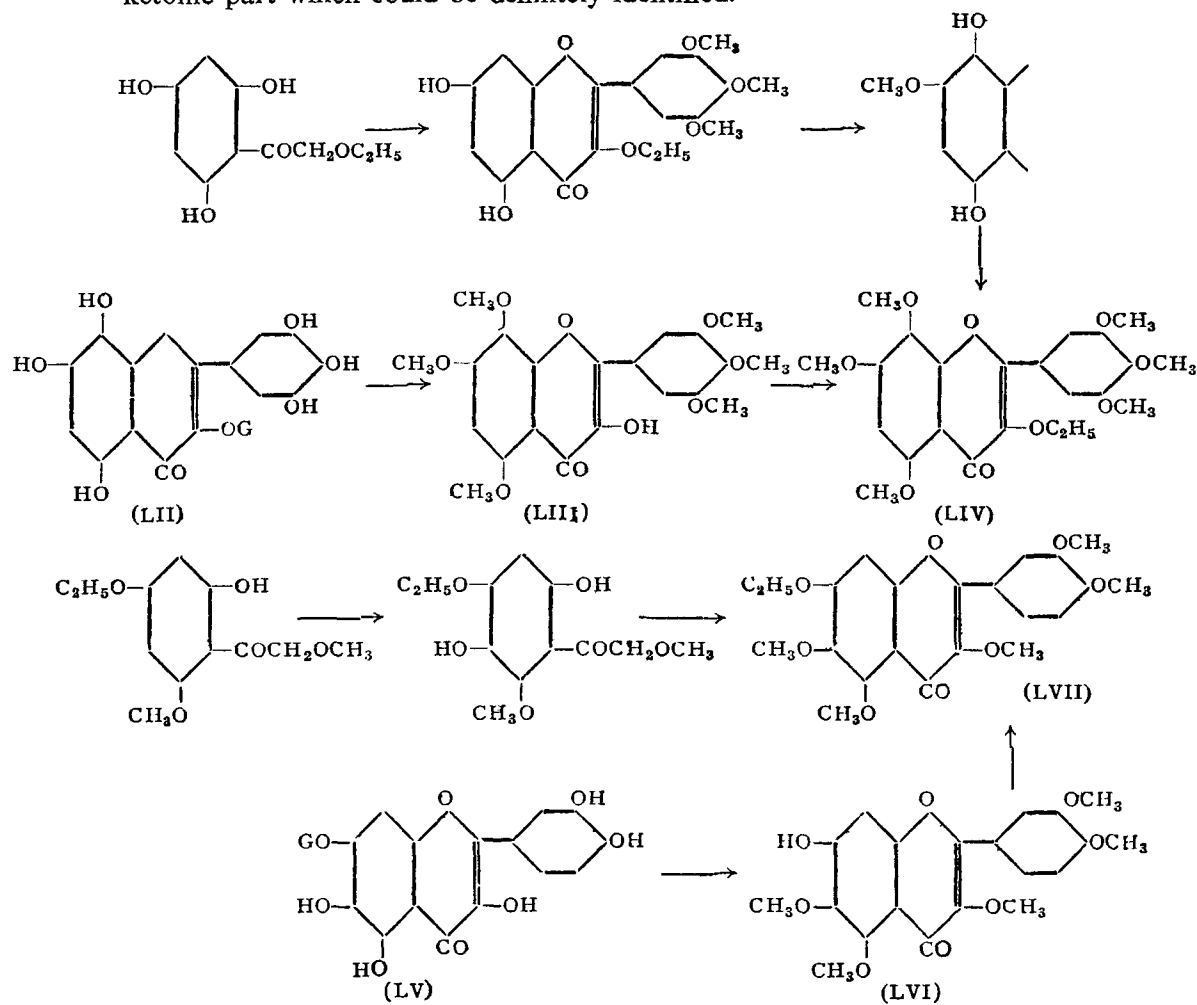


Later tambuletin itself was synthesised as already mentioned. This technique of nuclear oxidation along with ethylation has also been employed in the study of the constitution of tambulin.²²

(b) *Anthoxanthin glycosides*.—The application of this method of nuclear oxidation to the study of glycosides can be illustrated using gossypin and hibiscitrin. The former is 8-mono-glucoside of gossypetin (XLVII) and belongs to an unusual type. This conclusion was first arrived at from a consideration of its properties and reactions.²⁹ When fully methylated and the product hydrolysed a partial methyl ether with only one hydroxyl left out was obtained. It did not undergo fission with alkali satisfactorily; but veratric acid could be isolated as one of the products and this indicated that the free hydroxyl was in the benzopyrone part. The location of the hydroxyl in the 8-position (XLVIII) was first established by eliminating other possibilities using synthetic samples. This was supported by the oxidative demethylation³⁰ of this partial methyl ether yielding flavoquinone (XLIX) which could be reduced to the flavoquinol (L). This flavoquinol could be readily prepared, as already mentioned by the persulphate oxidation of tetramethyl-queracetin.⁷ The conclusive stage was the ethylation of the partial methyl ether (XLVIII). The mixed ether (LI) not only underwent smooth alkali fission, but it could be easily synthesised by the stepwise ethylation and methylation of the flavoquinol (L) thus fixing the position of the ethoxyl and hence of the original glucose group unequivocally in the 8-position.



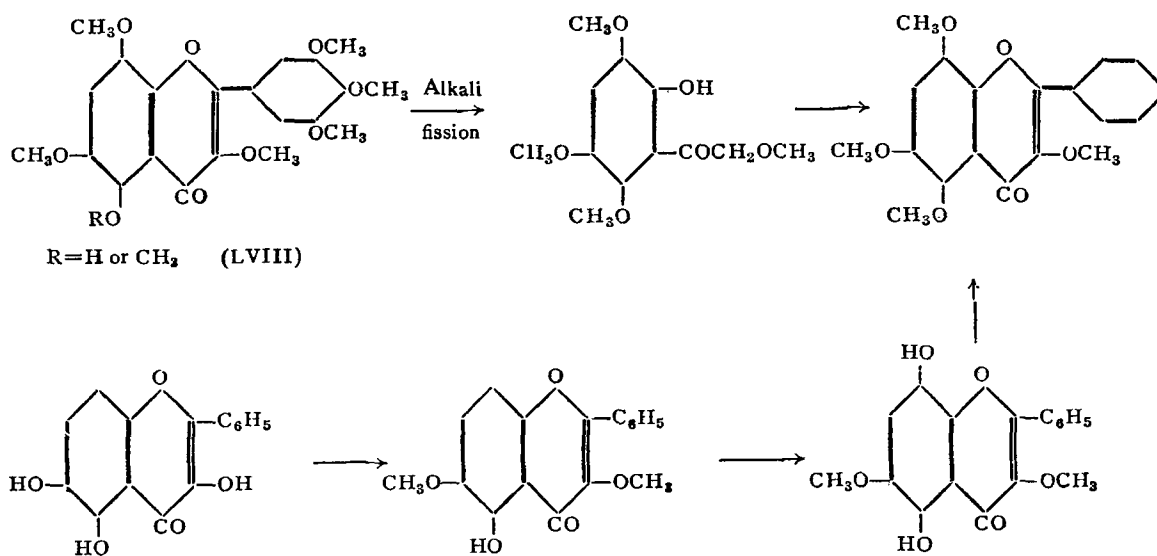
Hibiscitrin³¹ is a monoglucoside of hibiscetin and belongs to a different type. On methylation and hydrolysis it yielded a hexa-methyl ether having one free hydroxyl group. Here again alkali fission yielded only the acid part as trimethyl gallic acid; the free hydroxyl was therefore in the benzo-pyrone half of the molecule. From a consideration of the properties of the glucoside and the degradation product the free hydroxyl was located to be in the 3-position (LIII) and hence hibiscitrin was a 3-glucoside (LII). The other alternative positions were also eliminated by the synthesis of suitable reference compounds for comparison. The method of nuclear oxidation made this work considerably easy. Final confirmation was obtained by ethylating (LIII) and synthesising the mixed ether (LIV) as follows. This compound further underwent smooth degradation with alkali to yield a ketonic part which could be definitely identified.



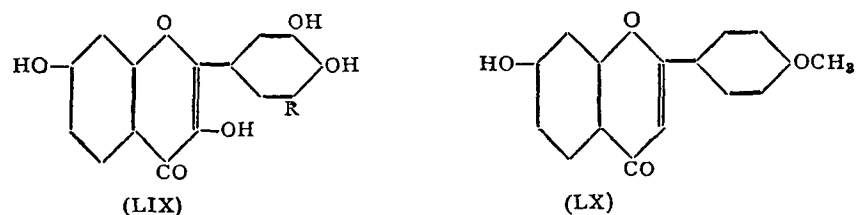
Quercetagitrin (LV) the monoglucoside of quercetagenin, forms a third typical example. After methylation and hydrolysis it yielded a pentamethyl ether of quercetagenin. Its properties and reactions and particularly the behaviour of the allyl ether indicated that the free hydroxyl was in the 7-position (LVI).³² This has now been conclusively established by preparing its ethyl ether (LVII) and synthesising it in the manner³³ shown in page 14.

V. GARDENIN

Gardenin³⁴ (LVIII, R = H) is a partial methyl ether of a flavonol belonging to a novel type not known before. That it has the 5:6:8-arrangement of substituent groups was arrived at by the elimination of all other possibilities. Definite confirmation of this constitution has recently been provided by Balakrishna and Seshadri³⁵ by synthetic experiments using nuclear oxidation as indicated below:



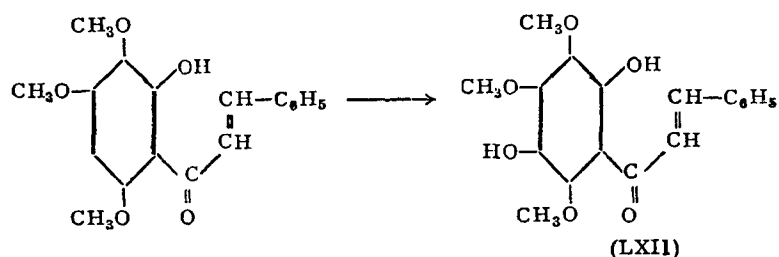
Flavones without the hydroxyl in the 7-position are rare in nature. Besides gardenin only primetin and 5-hydroxy flavone occur. They seem to result from the selective removal (reduction) of this hydroxyl at some stage of evolution. On the other hand fisetin (LIX, R = H) and robinetin (LIX, R = OH) are two flavonols devoid of the 5-hydroxyl group and pratol is a flavone (LX) of this type; again there are indications of the occurrence of 6:7:8-hydroxy-flavonols in the plant kingdom. Simple flavone which occurs as dust on various species of primula is an extreme case and lacks both these hydroxyl groups.



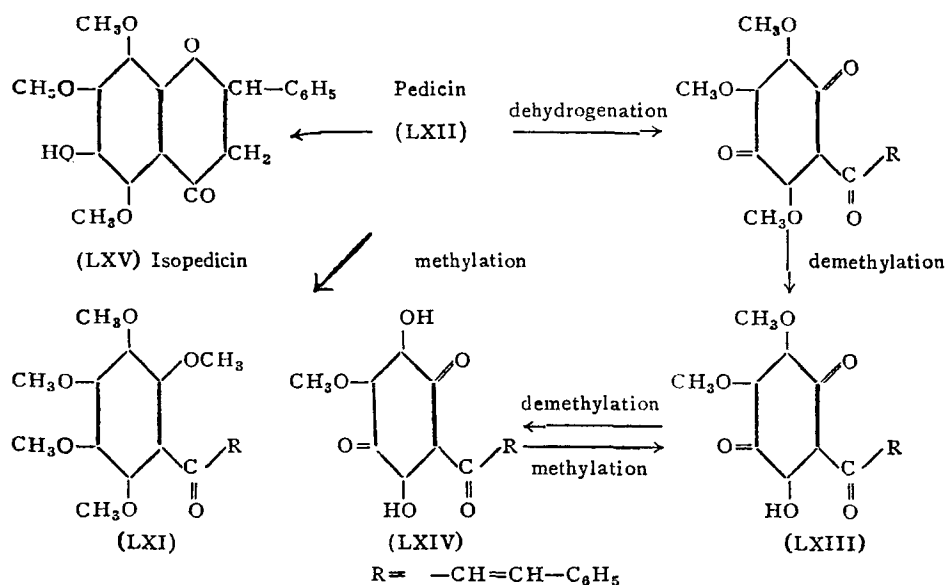
VI. PEDICIN AND ITS DERIVATIVES

Mention has already been made that pedicin, pedicellin and their allies are examples of chalcones in which ring (A) is completely substituted. An interesting application has been made of nuclear oxidation in the recent study of this group of compounds. It is based on the easy formation by this method of *p*-dihydroxy chalcones which are otherwise not easily accessible.

The main crystalline components isolated by Siddiqui³⁶ from the leaves of *Didymocarpus pedicellata* are (1) pedicin, (2) isopedicin, (3) pedicellin and (4) pedicinin. The constitution of pedicellin was readily established as the fully methoxylated chalcone (LXI)¹⁴ and its synthesis carried out by Baker.³⁷ There was some difficulty regarding the structure of pedicinin because that of pedicin was not correctly given; Sharma and Siddiqui¹⁴ considered the latter to be an ortho-dihydroxy compound. From biogenetic considerations and from its reactions it appeared to us that this required revision and that pedicin should be a paradihydroxy compound (LXII). It has been shown to be so¹⁵ and the new constitution has been confirmed by synthesis as follows:



The correct constitution of pedicin has thrown fresh light on the formula of pedicinin (LXIV) and on the biogenesis of the various compounds including methyl-pedicinin (LXIII) which has also been isolated from the leaves in this laboratory. Pedicin (LXII) which is a major component of the leaves, is considered to be the primary member formed according to the above synthetic process. The evolution of the others is indicated in the scheme given below and all the stages have been proved experimentally.



SUMMARY

The results contained in the recent publications from this laboratory on the subject of nuclear oxidation in the flavones and related compounds are summed up. Originally the main interest was in the theory of biogenesis of anthoxanthins; the bearing of the results on the evolution of the various types of flavones and flavonols is therefore discussed in detail. This study has led to the development of considerably simplified methods of synthesis of all the naturally occurring substances with three and four hydroxyl groups in ring A and of the special though simpler compounds like primetin. The application of this oxidation method along with ethylation for the establishment of the structure of partial methyl ethers as well as glycosides is illustrated by a number of typical examples. Special mention is made of the interesting and novel cases like gardenin and pedicin.

Note added in proof. In the above discussion of the persulphate oxidation and of the applicability of the results to the biogenesis of anthoxanthins, the benzopyrone part alone has been considered in detail. This is because the outcome of laboratory experiments regarding the side phenyl nucleus continued to be inconclusive. However, since submitting this paper for publication more definite results have been obtained and they will be published in a forthcoming paper.

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