

ANNIVERSARY ADDRESS

COUMARINS AS NATURAL PRODUCTS, A FIELD OF PERENNIAL INTEREST

by T. R. SESHADRI, F.N.I., *President, National Institute of Sciences of India*

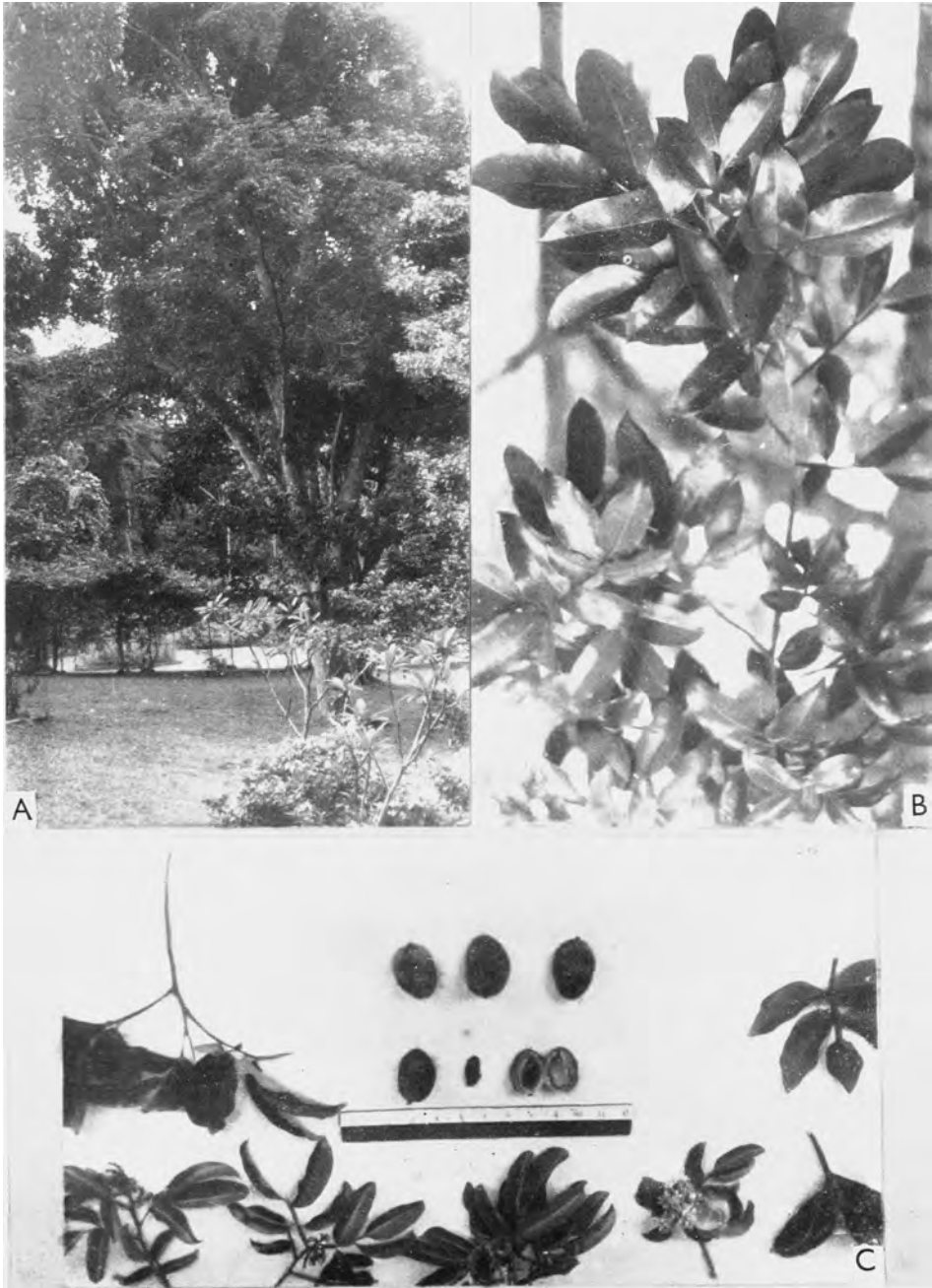
(*Delivered on January 2, 1968*)

In the progress of the chemistry of natural products during the past century and a half, coumarins have played an important part. We have had a large number of occasions to deal with them in the course of our own investigations. I propose to deal with some of the more important of these subjects.

Early studies:

The parent compound of this large group of natural products is a comparatively simple substance and was discovered by Vogel in 1820. He isolated it from tonka beans, seeds of *Dipterix odorata*, a plant (Fig. 1A-C) found in South America, and called it tonka benzoic acid. Actually crystals of the substance (Fig. 2) could be found inside the cut bean. Gerhard and Dumas determined its molecular formula correctly as $C_9H_6O_2$ in 1846. Later in 1868, its constitution was established by its synthesis (Chart 1a) by W. H. Perkin (Sr.). In essence this continues to be the method of commercial synthesis but there were great difficulties of obtaining good yields. It involved the condensation of salicylic aldehyde with sodium acetate and acetic anhydride and was the first example of the well-known 'Perkin's reaction'. Generally it gives poor yields and is temperamental, being markedly influenced by the purity of the reagents and the reaction conditions. The by-products which may become considerable are acetylcoumaric acid and salicylaldehyde triacetate. The addition of catalysts like iodine and zinc chloride has been reported to increase the yield of coumarin. Salicylaldehyde as starting material is also quite costly. Improvements have been made in its preparation (Chart 1b) from orthoeresol and condensation with cyanoacetic acid yielding coumarin-3-carboxylic acid which undergoes decarboxylation to yield coumarin.

Coumarin is one of the most important aromatic compounds, and its annual production is very large. It is used extensively in perfumery because of its characteristic pleasant odour and to a greater extent for flavouring purposes, e.g. artificial vanilla compositions. Large quantities of coumarin are consumed by the bakery industry to impart a pleasant and palatable vanilla-butter note to cakes and biscuits. The beverage industry is another user of



{By the courtesy of Dr. L. B. Singh, Director, National Botanical Gardens, Lucknow, and of the Director, Royal Botanical Garden, Peradeniya, Ceylon

FIG. 1A-C. A, *Dipteris odorata* (planted in 1881); B, close-up to illustrate leaf characteristics; C, seeds.

coumarin and many soap perfume formulae contain a small percentage of coumarin; it is also added to smoking tobacco in order to impart a pleasing aroma. More recently, coumarin is being used in ever-increasing quantities as a neutralizer of disagreeable odours of many industrial products such as



FIG. 2. Crystals of coumarin.

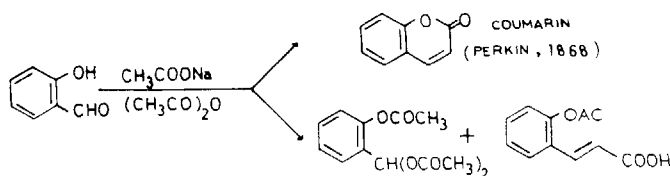


CHART 1a. Perkin's coumarin synthesis.

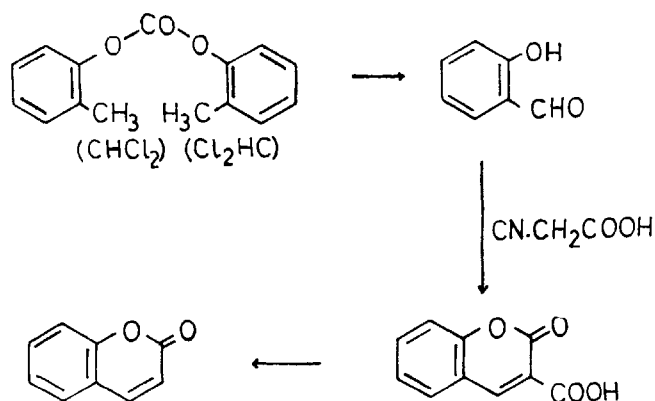


CHART 1b. Improvements in coumarin synthesis.

rubber and plastic materials, as well as household articles such as curtains and aprons and even in paints and sprays. Coumarin also possesses several useful physiological properties; it has antifungal action and controls seed germination and root growth.

In the closing years of the last century and the early years of this century, a large amount of work was done on the physical properties and chemical reactivity of the coumarin molecule, and the preparation and reactions of substituted coumarins. Substitutions readily took place in the positions 6, 8 and 3 and many derivatives were obtained. The marked stability of the coumarin structure and its properties were considered to indicate that it has an aromatic structure as shown in the following formulae (Chart 2). It satisfies Huckel's rule for aromaticity. In some respects, however, it can be compared to phenyl cinnamate with only one benzene ring.

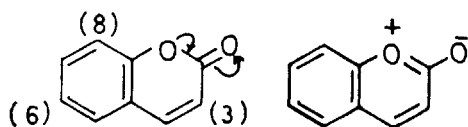


CHART 2. Ordinary and polarized forms of coumarin.

About the same time, several methods of synthesis of coumarin and substituted coumarins were studied. Besides Perkin's reaction the well-known Pechmann's method and its various modifications were developed and used. Some of the products occurred in nature, e.g. umbelliferone, aesculetin, daphnetin, limettin and bergapten and some of them were found to be useful as drugs, e.g. derivatives of coumarin-3-carboxylic acid. A large volume of data was collected about them.

The double bond in coumarin is a centre of reactivity; besides inorganic compounds like sodium bisulphite and mercuric chloride, compounds with reactive methylene groups readily add to it, and produce a variety of compounds. The reactivity is considerably controlled by substitution in the various positions. Another study is the geometrical inversion in the *o*-hydroxy cinnamic acids derived from the coumarins (Chart 3). It is markedly catalysed by mercury compounds and influenced by light. A typical example is the complete conversion of coumarin into coumaric acid (trans) when shaken with dil. sodium hydroxide and mercuric oxide for ten minutes. The reverse change of coumaric acid or ester into coumarin takes place fast with mercuric chloride in acid medium or when exposed to sunlight. Analogous process of catalysed ring closure from trans acid to coumarin was suggested for its biosynthesis in plants. This has been realized in recent years. The origin of coumarin seems to be cinnamic acid which undergoes oxidation and glucoside formation to yield O-glucoside of coumaric acid that has been found to occur

in plants. By enzyme hydrolysis of the glucoside and ring closure either involving illumination or catalyst, coumarin is produced (Chart 3).

Photodynamic coumarins:

During the thirties, active work was done on the chemical components of plant drugs which had been for a long time used for the cure of skin diseases, like psoriasis and leucoderma. The seed of a leguminous plant commonly growing in various parts of India, called *Psoralea corylifolia* (Fig. 3), was being

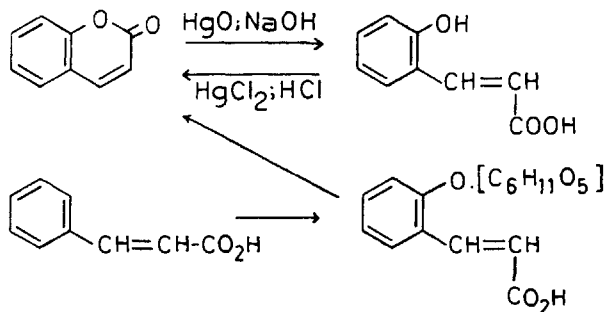


CHART 3. Geometrical inversions and biogenesis of coumarin.



FIG. 3. *Psoralea corylifolia*.

used in this way. It was found to contain, as main components, psoralen and isopsoralen (Chart 4a). Their constitutions were established as isomeric furanocoumarins. Further, the seeds of *Ammi majus*, largely grown in Egypt and also to some extent in Indian gardens as an ornamental plant and used for a similar purpose, were found to contain xanthotoxin, imperatorin and bergapten. Later, these compounds were found in a number of other sources also. The widespread occurrence of such compounds like bergapten and xanthotoxin explains the frequent incidence of photodermatitis of vegetable origin; for example, celery and parsley cause dermatitis in the hands of people who handle them and this has been traced to the presence of bergapten in them.

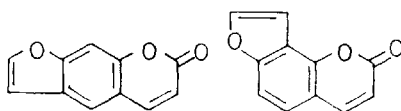


CHART 4a. Components of *Psoralea* seed; psoralen and isopsoralen.

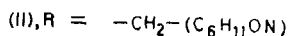
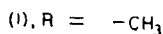
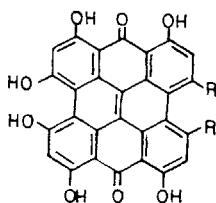


CHART 4b. Hypericin and fagopyrin.

The photodynamic property of the above coumarins has been studied in detail in recent years and is found to differ markedly from the activity of hypericin and fagopyrin (Chart 4b) which are present in certain plants used as cattle fodder and cause undesirable photosensitiveness to animals. The coumarin group can be used as drugs. Therefore effective methods of preparing these compounds from the plant sources have been satisfactorily developed. Special mention may be made of psoralen-isopsoralen mixture from *Psoralea* seeds in India and xanthotoxin and related compounds from *Ammi majus* seeds in Egypt. There were difficulties in their synthesis by the methods known earlier. Most of them were tedious and gave poor yields. We have recently described new procedures based on considerations of possible biosynthesis of these compounds in plants. Psoralen and xanthotoxin can be made conveniently in less number of steps and in good yields (Chart 5). The main intermediates are C-allyl compounds which are subjected to oxidation and ring closure.

Dicoumarol group:

A very important discovery was made in 1940 by Link and his co-workers. They studied the cause of the haemorrhagic disease of cows that had been fed on spoilt clover. The toxic chemical substance was isolated and named dicoumarol. Its constitution was given as in Chart 6 ($R=H$). It had already been synthesized by Anschutz as early as 1909 in the course of his study of the properties of 4-hydroxycoumarin called also as benzotetronic acid. It condensed with formaldehyde to yield dicoumarol. Link *et al.* considerably

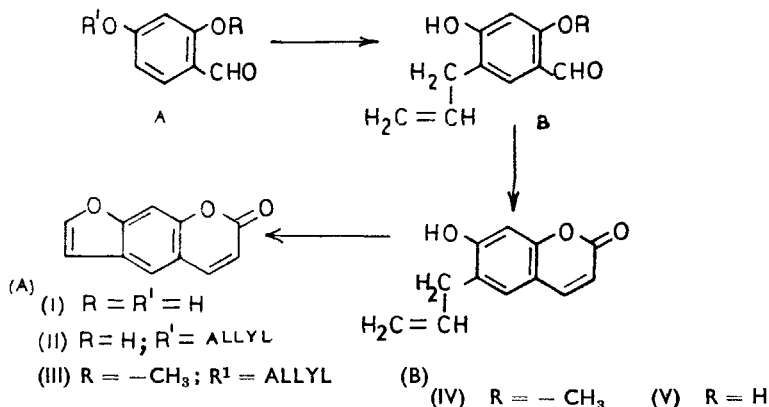
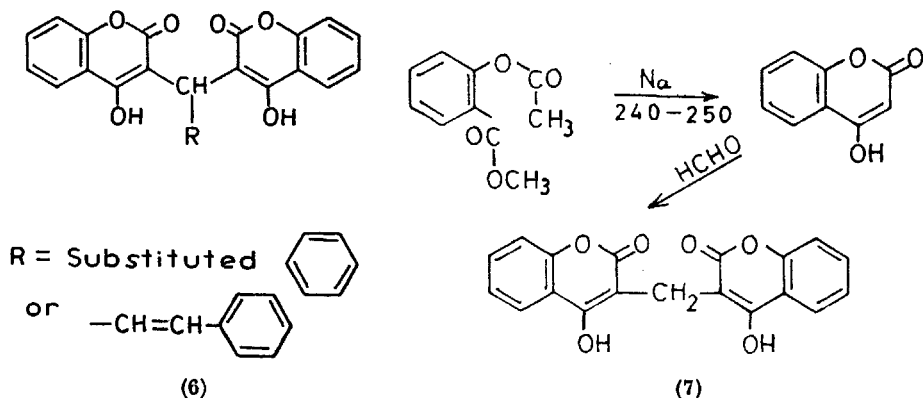


CHART 5. Synthesis of psoralen.

improved the synthesis (Chart 7). It was extraordinary that clover hay which normally contains coumarin had developed the new compound under improper conditions of storage when the clover got spoiled. Its formation was considered to arise as shown in the formulae (Chart 8) and involved a stage of oxidation.

The interesting result is that this toxic compound has turned out to be a very useful drug; it functions as anticoagulant and is thus used. A large amount of research has been done to prepare derivatives with better properties.



CHARTS 6 and 7. 6, dicoumarol and its derivatives; 7, synthesis of dicoumarol.

'Syntrome' is one such. By a deviation in the synthesis can be prepared a useful rodenticide which goes by the name wharfarin.

In order to learn more about the mode of action of the coumarin anticoagulants from structure-activity correlation studies, we investigated dicoumarol derivatives having a phenyl group on the bridge methylene unsubstituted as well as variously substituted. The prevailing theories that anticoagulants have anti-vitamin K action and that hemi-ketal formation is a key step in their functioning do not seem to be adequate to explain all observations. Though the introduction of a phenyl group on the bridge in dicoumarol considerably brings down the activity, it could be stepped up to varying

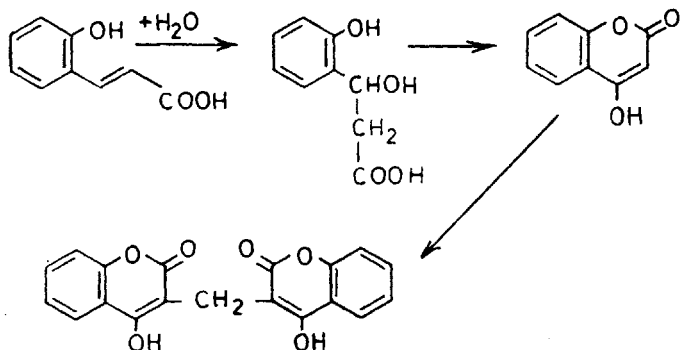


CHART 8. Biogenesis of dicoumarol.

degrees by introducing various substituents on the phenyl, e.g. a methoxyl at the 4'-position which seems to produce some other desirable properties also; similar is a styryl derivative obtained by the action of cinnamic aldehyde on 4-hydroxy coumarin (Chart 6).

3-Substituted umbelliferones:

Earlier work had shown that several coumarins exhibit considerable fish-toxicity; 3-phenyl umbelliferone derivatives were specially good. There was an example of picrotoxin that was a fish poison which also possessed anti-veratrinic and antiarrhythmic activities. Based on this analogy coumarins of

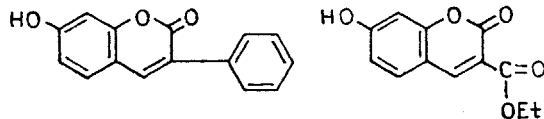


CHART 9. 7-hydroxy coumarin (umbelliferone) derivatives.

different types were prepared and tested for antagonizing the veratrine response in the muscle of frogs. The best among these were 7-methoxy-3-phenyl coumarin and 7-methoxy-4-phenyl coumarin, each of which was three times as active as quinine.

Coumarin derivatives have marked fluorescence and some of them give bright fluorescence not only in UV but also in ordinary daylight. Coumarin itself fluoresces in conc. sulphuric acid; umbelliferone is much better. A detailed study of a large number of coumarins was made by us during the last war. The influence of substituent groups and solvents were traced to their effect on the resonance of the molecular structure; the double bond in the pyrone ring is essential for this purpose. A hydroxyl or methoxyl in the 7-position and an electron sink in the 3-position considerably enhance the fluorescence. Among them 3-phenyl and 3-carbethoxy umbelliferone (Chart 9) and their methyl ethers are very powerful. They fluoresce in neutral alcoholic solution and even in the solid state. Some of these may be useful against sunburn and for radiation protection. Experiments have been made by Baker and Haksar incorporating these structures in other compounds for improving chromatographic separation of colourless compounds; 7-methoxy coumarin-3-carboxylic acid is one of the most useful for this purpose.

Coumarins of Derris species :

During the war years there was great interest in the study of Indian Derris species because owing to the occupation of Indonesia and Malaysia by Japanese forces, the supply of Derris as a safe and powerful insecticide was cut off. At this time it was well established that rotenone (Fig. 5 and Chart 10) and closely related rotenoids are the active components of *D. elliptica* (Fig. 4) and *D. malaccensis*. Among Indian Derris species, the roots of



FIG. 4. *Derris elliptica* roots.



FIG. 5. Crystals of rotenone.

D. ferruginea from Assam contain rotenone (4.3%) and are important. But there is a variety of this species that is devoid of rotenone. *Derris elliptica* was also grown in suitable localities. However, the roots of *D. scandens*

(Fig. 6) and *D. robusta* (Fig. 7) did not yield rotenoids but yielded members of the scandenin group which now number fairly large. They exhibit strong acid properties and are fairly stable in alkali. They have considerable toxicity though not so high as the rotenoids. Their full chemistry has been understood

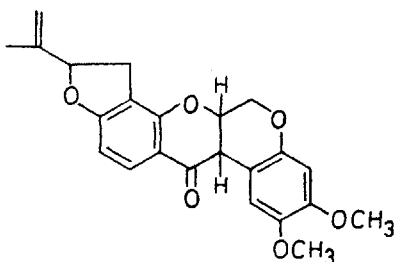


CHART 10. Structure of rotenone.



FIG. 6. *Derris scandens*.

FIG. 7. *Derris robusta*.

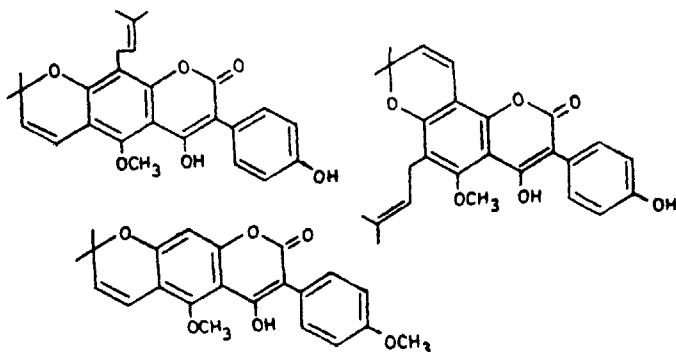


CHART 11. Lonchocarpic acid, scandenin and robustic acid.

only recently and they are now known as derivatives of 3-phenyl-4-hydroxy coumarin having one or more C_5H_8 units. Some of the typical members are given in the formulae (Chart 11).

Coumestrol group :

These are also derivatives of 3-phenyl 4-OH coumarin but this hydroxyl group is involved in the formation of a fourth ring. The earliest member,



FIG. 8. *Wedelia calendulacea*.



FIG. 9. *Eclipta alba*.

wedelolactone, was isolated from *Wedelia calendulacea* (Fig. 8) by Govindachari *et al.* in 1956 and later from *Eclipta alba* (Fig. 9) also. These are well-known vegetable drugs used for curing certain types of jaundice. Besides this, *E. alba* also contains nor-wedelolactone and trithienyl carbinol

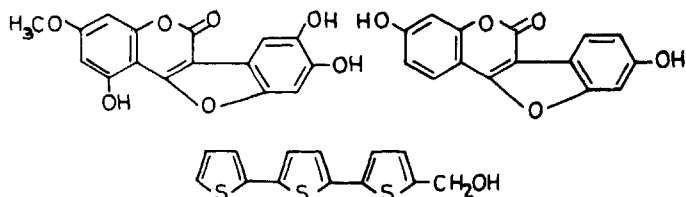


CHART 12. Wedelolactone, coumestrol and trithienyl carbinol.

(Chart 12) and sulphur containing amino-acids. Possibly all these play some part in the curative action. Later the simpler compound coumestrol was found in clover and it has marked oestrogenic properties. Many more members of this group have recently been isolated and several of them have been synthesized by convenient methods.

Novobiocin :

This is an antibiotic produced by some *Streptomyces* species and has special value because it controls penicillin resistant organisms, particularly

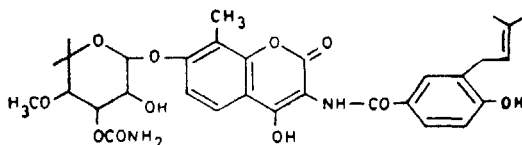


CHART 13. Novobiocin.

Staphylococci. Its molecule consists of three parts, a central 4,7(OH)₂ coumarin ring with the sugar unit linked in the 7-position and a para hydroxybenzoylamide linked in the 3-position (Chart 13); it has been synthesized. Many similar coumarins of simpler type have been prepared by N. V. Subba Rao *et al.* of Osmania University and tested for antibiotic activity.

Neoflavonoids :

These are 4-phenyl coumarins. The first members, dalbergin and methyl dalbergin, were isolated from *Dalbergia sissoo* about the year 1950. Dalbergin has also been found in *D. latifolia*. Along with it, related quinone and quinol derivatives also occur, e.g. dalbergenone and latifolin (Chart 14). The neo-

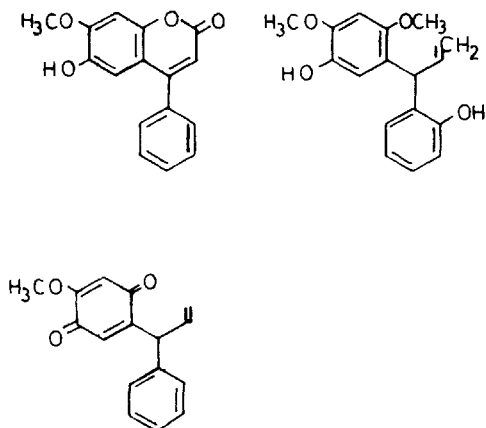


CHART 14. Dalbergin, latifolin and dalbergenone.

flavonoid group has also increased in number in recent years. It may be mentioned that brazilin and haematoxylin which have been known as components of dyewoods for over 100 years and have been studied by Perkin and Robinson are now recognized as neoflavonoids.

In 1916 Simonis wrote his well-known book on the Chemistry of Coumarins. He listed only a few members of this group as occurring in nature. Many more have been discovered later and some of them have been mentioned earlier in this lecture. The family *Umbelliferae* (the common *coriander* is a member of this family) is very prolific in the production of a large number of coumarin compounds. They are mainly derivatives of umbelliferone and 5-hydroxy umbelliferone with substituents in the 6- and 8-positions. Isoprene units are commonly present either as a chain or in the form of a ring involving the neighbouring hydroxyl groups. Even the 2-carbons present in simple benzofuran rings are considered to arise from the modification of the C₅ units and this idea has been used for the convenient synthesis of these compounds, e.g. psoralen and xanthotoxin. Recently a very large number of new types have been isolated and studied in detail. As representative examples I propose to give the results of our study of two important plants of this family occurring in India.

Selinum vaginatum :

More than 10 years ago there was great interest in the properties and components of Jatamansi (*Nardostachys jatamansi*) and we were supplied with samples of roots which were considered to be belonging to this plant. Since they did not yield jatamansone and were found to be different from the genuine root, we did not continue the investigation then, but more recently when more supply of the plant became available from Jammu and Kashmir we have made detailed study of it. It resembles Jatamansi only in general form and its colour is grey, whereas Jatamansi is brown. Its microscopic characters are very different. It is now quite easy to differentiate between the two by carrying out the TLC of the petroleum ether extracts. *Selinum vaginatum* (Fig. 10) contains a number of coumarins (Chart 15) that are not present in Jatamansi and is lacking in the ketone jatamansone which can be extracted easily from Jatamansi and tested. There is another marked distinction. An alcoholic extract of *S. vaginatum* gives a deep red colour with magnesium and hydrochloric acid indicating the presence of a flavonoid, whereas Jatamansi does not give this test. The flavonoid compound is new and named selenone. So far the association of coumarins with flavonoids has been considered to be rare.

From the petroleum ether extracts five coumarins could be isolated. Three of them are already known ones, angelicin, oroselin and lomatin (Chart 15). The other two, selinidin and vaginidin, are new compounds. The former is

a major component and readily crystallizes out of the petroleum ether extract.

Selinidin is a highly crystalline compound melting at 97-98°, has the molecular formula $C_{19}H_{20}O_5$ and shows the characteristic properties of coumarins. Comparison of its spectra with those of known coumarins provided strong evidence for the presence of 7-oxygenated coumarin chromophore. It underwent hydrogenation to yield a tetrahydro derivative. IR spectral studies showed that selinidin was an ester of a hydroxy coumarin with an



FIG. 10. *Selinum vaginatum*. a, aerial portions; b, roots; c, fruit.

unsaturated acid. Though alkali hydrolysis gave tiglic acid, it was clear that originally angelic acid was present from the NMR spectral data; the change from angelic to tiglic is particularly facile. Important evidence for the structure of the hydroxy coumarin part (selenetin) was obtained by the dehydra-

tion of the hydroxy coumarin by acid, the product being identified as dihydro-orselone (Chart 16). This left four possibilities for the constitution of selenetin; however, from other reactions and consideration the correct choice was made; it was clear that during dehydration of selenetin there was ring contraction also involving an interesting molecular rearrangement. About the same time as our work, Soine *et al.* obtained it as a free alcohol from another source and named it lomatin. Using this name selinidin will be lomatin angelate.

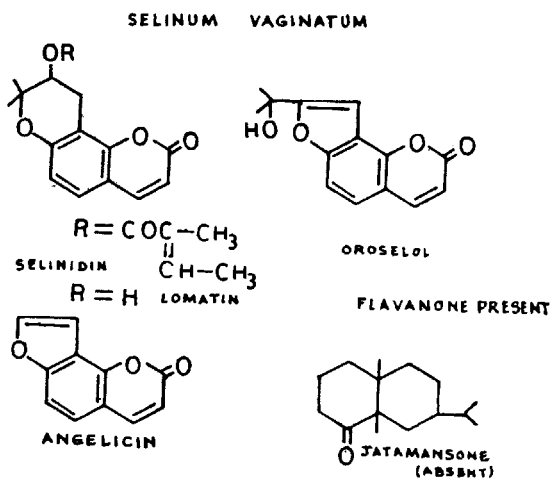


CHART 15. Components of *Selinum vaginatum*.

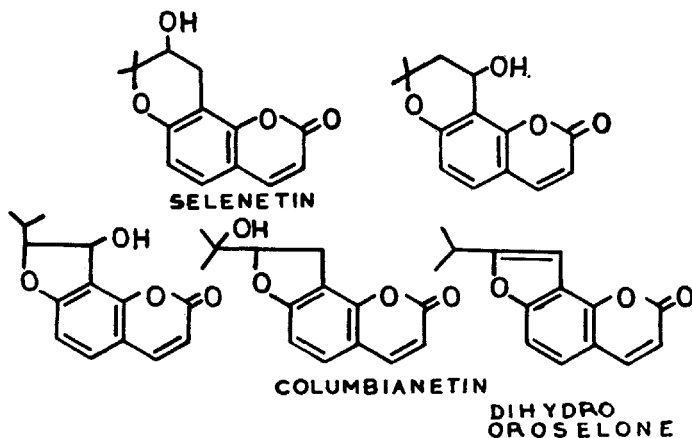


CHART 16. Structure of selinidin.

Vaginidin:

The second new coumarin, vaginidin, present in *S. vaginatum*, is also a highly crystalline compound melting at 133-34° but it has been far more difficult to isolate and required elaborate column chromatography. Its molecular

formula is $C_{19}H_{22}O_6$, its spectral data and colour reactions suggested that it had the same chromophore as selinidin and was also an ester. It was therefore subjected to alkaline as well as acid hydrolysis and in both cases it yielded angelicin and isovaleric acid. Obviously besides ester hydrolysis there were other changes involving loss of groups. These observations gave rise to four possibilities for the structure of vaginidin. However, by the study of hydrogenation and NMR spectrum the correct choice has been made as shown in the formula (Chart 17). It is clear that during the hydrolysis retro-aldol cleavage takes place followed by loss of water.

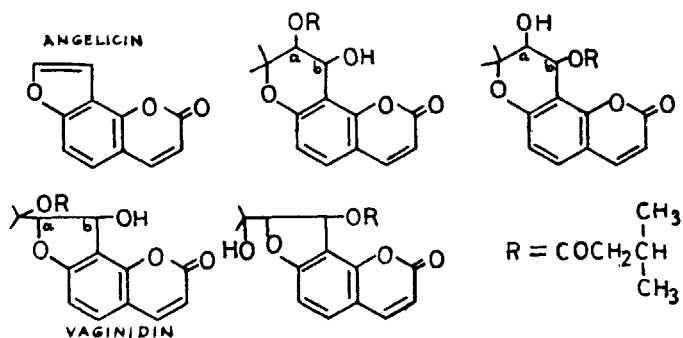


CHART 17. Structure of vaginidin.

Absolute configuration:

In recent years a large volume of effort has been devoted to the establishment of absolute configuration of optically active compounds. In many cases the work has been rendered easy by the availability of reference compounds whose configurations have been definitely established and the new ones are studied by determining relationship with the known. The absolute configuration of selinidin and vaginidin has been settled in the following manner:

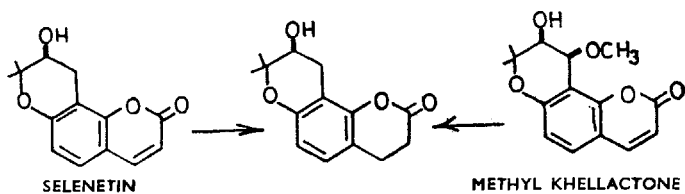


CHART 18. Absolute configuration of selinidin.

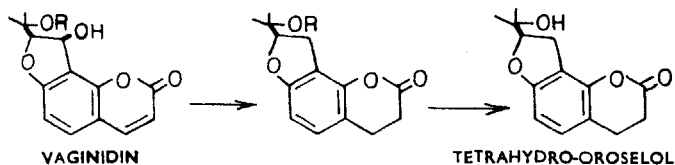


CHART 19. Absolute configuration of vaginidin.

The absolute configuration at the 3'-position of selinidin and selenetin was established as (S) by correlation with *cis*-methyl khellactone (Chart 18). In vaginidin there are two asymmetric positions, 2' and 3'. Since the compound could be converted into tetrahydro-oroselol by catalytic hydrogenation and ester hydrolysis, the configuration at 2' was concluded to be (S) (Chart 19). From its properties and NMR spectrum vaginidin should have *cis*-configuration in 2'- and 3'-positions. Consequently it should have 2'(S), 3'(S) configuration. These studies seem to throw useful light in regard to the biogenesis of these compounds in plants.

Considerable interest has been shown on the biogenesis of these compounds. About 10 years ago we collected and correlated the structures of compounds containing the C_5H_8 units attached to coumarins and other types of compounds. These units derived from mevalonic acid enter reactive nuclear positions yielding first dimethyl-allyl derivatives (Chart 20). Subsequent transformations are indicated by the following series of formulae:

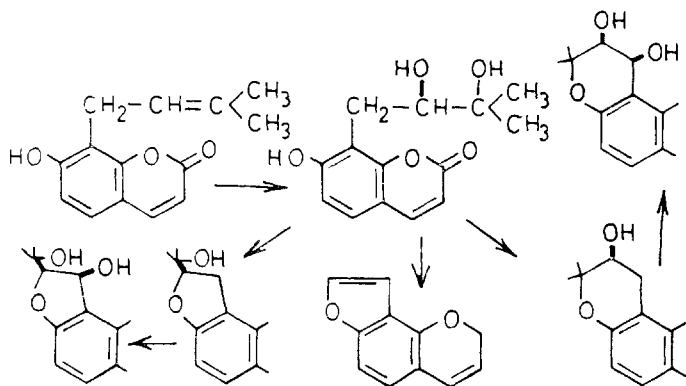


CHART 20. Biogenesis of coumarins in *Selinum vaginatum*.

In some compounds there are two hydroxyls; the second ones in the benzylic position seem to arise by stereospecific oxidation. Angelicin represents a type in which oxidative cyclization involves loss of three carbon atoms.

Seseli sibiricum (Fig. 11) is another plant belonging to the Umbelliferae and occurring mainly in Kashmir. It is used for blending beverage and as medicine for livestock. The umbels of this plant have yielded, besides the well-known compounds—osthol, imperatorin and bergapten (Chart 21), a new member, sibiricin, which is a representative of an important type. It has the molecular formula $C_{16}H_{18}O_5$ and has the chemical and spectral properties of a 5, 7-oxygenated coumarin with an epoxide unit. Two important reactions which established its structure are: (1) conversion into a glycol (sibiricin glycol) when treated with 1 per cent oxalic acid and (2) formation of sibiricic



FIG. 11. *Seseli sibiricum*. a, aerial portions; b, root; c, fruit.

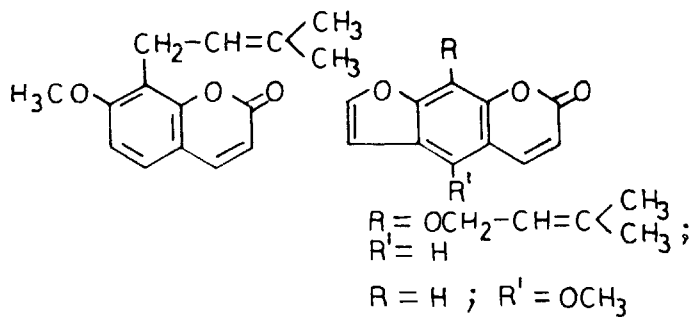


CHART 21. Components of *Seseli sibiricum*, osthol, imperatorin and bergapten.

acid by alkali hydrolysis involving a rearrangement (Chart 22). A significant observation is that the above glycol is optically active and appears to be identical with mexotycin recently isolated from *Murraya exotica* (Chart 24).

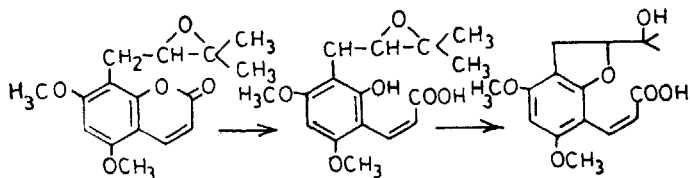


CHART 22. Structure of sibiricin.

Epoxide formation is the result of easy oxidation of unsaturated compounds in plants and epoxides occur frequently. The above structure of sibiricin has been confirmed by synthesis (Chart 23). Synthesis of such types is somewhat difficult and has been engaging our attention. A direct synthesis would be by the introduction of isoprene unit in 5-methoxy-7-hydroxy coumarin. But this does not take place in a satisfactory manner and has therefore been done in an earlier stage (salicylaldehyde stage) and coumarin ring closed and epoxidation effected finally.

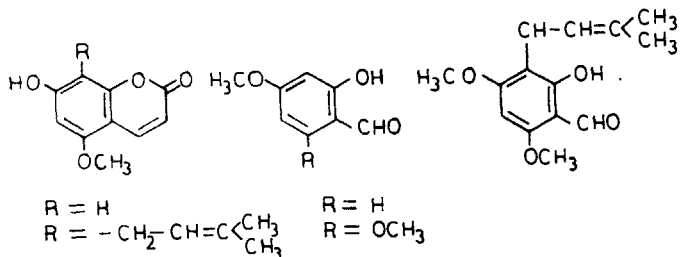


CHART 23. Synthesis of sibiricin.

The dimethyl-allyl coumarin, prepared as an intermediate in the synthesis of sibiricin, has been very recently found to occur as a natural product in the ripe fruits of *Murraya paniculata* and has been named coumarrayanin (Chart 24). The components of *Murraya* species indicate paths of biogenesis.

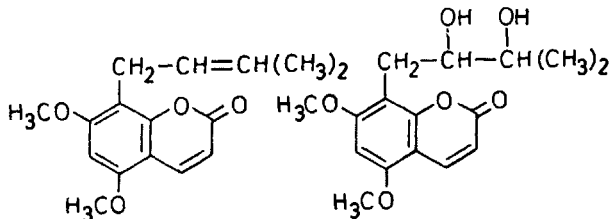


CHART 24. Coumarrayanin and mexotycin.

SUMMARY

In the progress of the chemistry of natural products during the past century and a half, coumarins have played an important part. We have had a large number of occasions to deal with them in the course of our own investigations.

The parent compound coumarin was isolated by Vogel in 1820 from tonka beans. Its synthesis was effected by W. H. Perkin in 1868 and his method is even now adopted with some essential improvements. It has become one of the most important and useful among aromatic compounds and its annual production is very large. Towards the end of the last century and early this century considerable work was done on the properties and reactions of coumarins and on the methods of their syntheses. The additive reactivity of the double bond in coumarins and the geometrical isomerism in acids derived from them led eventually to an understanding of the biogenesis of the naturally occurring members.

The study of Indian and Egyptian drugs used for skin diseases that began with the thirties of this century led to detailed investigations on the furano-coumarins and their photodynamic properties and to their easy syntheses. The next decade witnessed the discovery of dicoumarol and its important anti-coagulant property and the development of other drugs in this line. Some coumarins have anti-veratrinic and anti-arrhythmic action and some are capable of being used against radiation damage.

Many species of *Derris* do not contain rotenoids but members of the scandenin group which are 3-phenyl-4-hydroxy coumarins having one or more isoprene units. A related group consists of derivatives of coumestrol having important physiological properties. Novobiocin is an important antibiotic derived from 4-hydroxycoumarin. The discovery of dalbergin, a 4-phenyl coumarin in the woods of *Dalbergia* spp., initiated the study of a new group of compounds called 'neo-flavonoids'. In recent years the study of the plants of the family Umbelliferae has led to the isolation of a large number of coumarins with isoprene units disposed in various ways. *Selinum vaginatum* and *Seseli sibiricum* contain a number of these compounds in interesting association. Selinidin, vaginidin and sibiricin are important new members that are representatives of different structural types.

ACKNOWLEDGEMENT

I wish to express my thanks to Dr. P. Srinivasa Rao for help in the preparation of the manuscript.