T R Seshadri's Contributions to the Chemistry of Natural Products

Some Illustrative Examples

N R Krishnaswamy

N R Krishnaswamy was initiated into the world of natural products by T R Seshadri at University of Delhi and has carried on the glorious traditions of his mentor. He has taught at Bangalore University, Calicut University and Sri Sathya Sai Institute of Higher Learning. Generations of students would vouch for the fact that he has the uncanny ability to present the chemistry of natural products logically and with feeling.

Keywords
Flavonoids, lichen metabolite, methylation, Elbs-Seshadri oxidation, structure elucidation, natural products synthesis.

Seshadri developed many reagents and synthetic procedures which he used for elucidation and synthesis of natural products, in particular flavonoids.

Introduction

It is by no means easy to bring under the confines of an article the research output of someone like T R Seshadri whose scientific studies spanned nearly half a century and were prolific. To choose just a few examples from as many as a thousand and odd papers is a Herculean task and I am aware that my selections will not be free from subjective considerations. One or two cases have been chosen only because I can write about them with familiarity on account of my own personal involvement in them. Through these examples I can, therefore, convey the manner in which he trained his students in research methods. His approach towards elucidating the structure of a new naturally occurring compound was comprehensive and classical in the sense that it involved analytical, degradative as well as synthetic experiments. In later years, spectroscopic data were also extensively used in addition to conventional chemical methods. At Andhra University, he focused his attention almost exclusively on the flavonoids. At Delhi, while building up on the foundations laid at Waltair, he also considerably widened the scope of his investigations and several other types of secondary metabolites, such as the different types of terpenoids, seneceo alkaloids, quinonoids and lichen metabolites (such as depsides, depsidones, etc.), were isolated and their structures elucidated. However, his main forte remained the chemistry of oxygen heterocyclics, which he enriched in no small measure. What follows is a very brief account of some of his achievements in this area.

Synthetic Methods

Methylation

It is a little known fact that the technique of O-methylation of phenols using dimethyl sulphate, which is widely used these days, was developed by Seshadri and his students during World War II. At that time, the most commonly used methylating agent was methyl iodide in the presence of silver oxide or silver carbonate. These reagents being expensive, there was a need for cheaper but equally effective substitutes. Seshadri found that calculated quantities of dimethyl sulphate brought about quantitative methylation of phenols in acetone medium in the presence of anhydrous potassium carbonate. Thus, for example, using 1.1 molar proportion of dimethyl sulphate, it was possible to convert 2,4-dihydroxyacetophenone (resacetophenone) into 2-hydroxy-4-methoxyacetophenone by refluxing a solution of the substrate and the reagent in 'dry' acetone in the presence of freshly ignited potassium carbonate for about 4 to 8 hours. When the amount of the methylating agent used was doubled and the reaction time increased to 20 hours, it was possible to obtain the dimethyl ether. In general, to bring about the methylation of intramolecularly hydrogen-bonded phenolic hydroxyls it was necessary to increase the duration of the reaction to 20 hours or more. On the other hand, hydroxyl groups conjugated with a carbonyl function underwent ready methylation. The work-up of the reaction mixture was also easy. After filtering off the potassium salts and washing them with hot acetone, the solvent was removed from the filtrate to get the crude product. Later, it was found that for partial methylations replacing acetone as the solvent by benzene or acetonitrile yielded better results. The method was also used very effectively for the complete methylation of polyphenolic glycosides. The only drawback is that reaction times are long.

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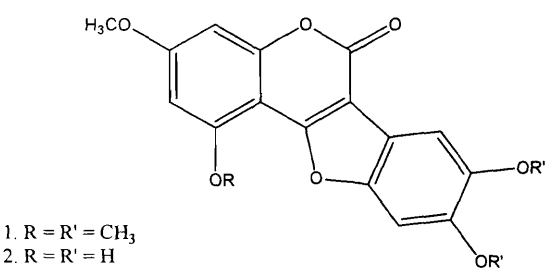
Demethylation

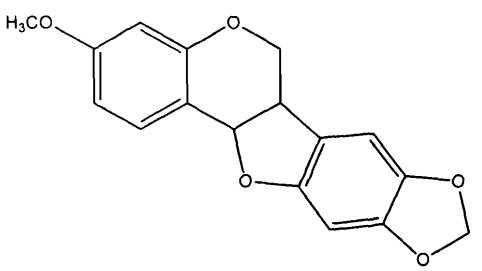
Demethylation, particularly selective demethylation, is also an important technique in the study of polyphenolic compounds

Apart from being a useful preparative method, demethylation reaction as well as the partial methylation reaction are able to differentiate between different methoxyl and hydroxyl groups on the basis of their differing Lewis basicities and acidities.

several of which occur in nature as partial methyl ethers. In the Seshadri school the most often used reagent for complete demethylation was 57% hydriodic acid and the reaction had to be carried out at 180 °C for 2 hours. However, if the reaction is done at a lower temperature (120 °C) for about half an hour it is possible to bring about selective demethylation. For example, using this method wedelolactone tri-O-methyl ether (**1**) was converted into wedelolactone (**2**). In this case, the methoxyl at position 7, which is the one that is retained in the product, is the least basic being in conjugation with the lactone carbonyl. The methoxyl at position 5, which is also conjugated with the same carbonyl group, however, undergoes demethylation presumably because the liberated hydroxyl group is stabilized by hydrogen bonding with the neighbouring oxyen atom. Indeed, if the demethylation reaction is carried out at a still lower temperature (100 °C) the product obtained is desmethylwedelolactone dimethyl ether (**3**). Apart from being a useful preparative method, this reaction as well as the partial methylation reaction are able to differentiate between different methoxyl and hydroxyl groups on the basis of their differing Lewis basicities and acidities. Seshadri and his students have also used hydrobromic acid and hydrochloric acid to bring about selective demethylations in certain cases. Another reagent useful for the selective demethylation of methoxyl groups ortho to a carbonyl function is anhydrous aluminium choride in acetonitile. Using this reagent it is possible, for example, to get pure 2-hydroxy-4-methoxyacetophenone from 2,4-dimethoxy-acetophenone. This reaction was also used in the selective demethylation of the 2' methoxyl group in isoflavones and was a key step in the synthesis of pterocarpin (**4**).



1. R = R' = CH_3
2. R = R' = H
3. R = CH_3 , R' = H



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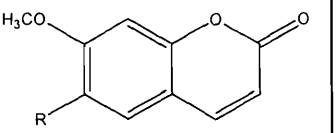
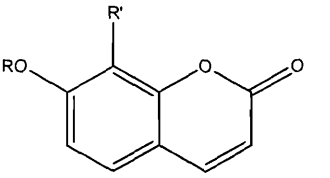
Elbs-Seshadri Oxidation



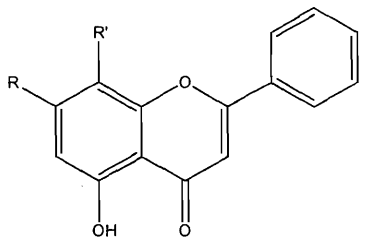
5. R = OH
6. R = H



7. R = $COCH_3$; R' = H
8. R = H ; R' = $COCH_3$
9. R= H ; R' = OH

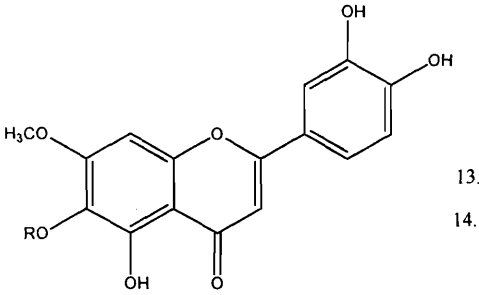
Polyhydroxy flavonoids and their partial methyl ethers occur widely in nature. For their synthesis it is more convenient to build up the flavonoid structure with some hydroxyl groups already in place and to introduce one or more later by a reaction termed by Seshadri as 'nuclear oxidation'. To introduce a hydroxyl para to an existing hydroxyl the most convenient method is that of oxidation using persulphate. This reaction first discovered by Elbs is now often referred to as Elbs-Seshadri oxidation. The hydroxylation can be done either before building up the final skeletal structure or after. For example, persulphate oxidation of 2-hydroxy-4-methoxybenzaldehyde yields 2,5-dihydroxy-4-methoxybenzaldehyde, which can be converted by Perkin reaction into 6-hydroxy-7-methoxy-coumarin (**5**). Compound 5 can also be prepared from 7-methoxy-coumarin (**6**) by persulphate oxidation. For the introduction of a hydroxyl ortho to an existing hydroxyl, Seshadri adopted a different strategy. For example, 7-acetoxycoumarin (**7**) was subjected to a Fries rearrangement and the resulting 7-hydroxy-8-acetylcoumarin (**8**) oxidized by the Duff method to obtain 7,8-dihydroxycoumarin (**9**).

Seshadri also developed a process, which he called 'nuclear reduction' by means of which a hydroxyl group could be eliminated. This could be done by converting the hydroxyl to its tosyl derivative and then subjecting the product to hydrogenolysis with the help of Raney nickel. Thus, the monotosyl derivative of 5,7-dihydroxyflavone (chrysin) (**10**) could be converted into 5-hydroxyflavone (**11**). The latter when subjected to Elbs-Seshadri oxidation yielded 5,8-dihydroxyflavone (primetin) (**12**), which occurs in *Primula* species.



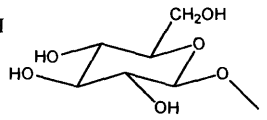
10. R = OH ; R' = H
11. R = R' = H
12. R= H ; R' = OH

An example of a total synthesis which incorporates some of the above mentioned reactions is that of pedalitin (**13**), whose 6-O-glucoside (**14**) occurs in the leaves of

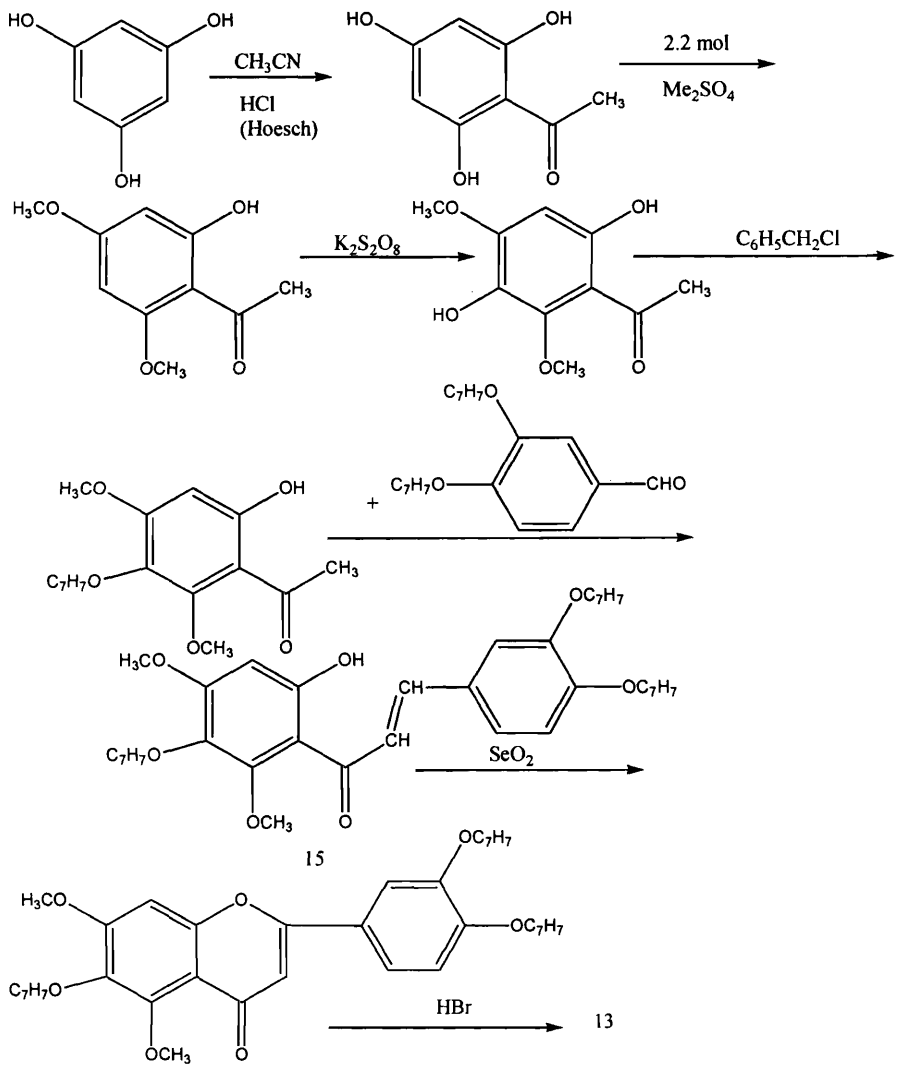


13. R= H

14. R=



Scheme 1.

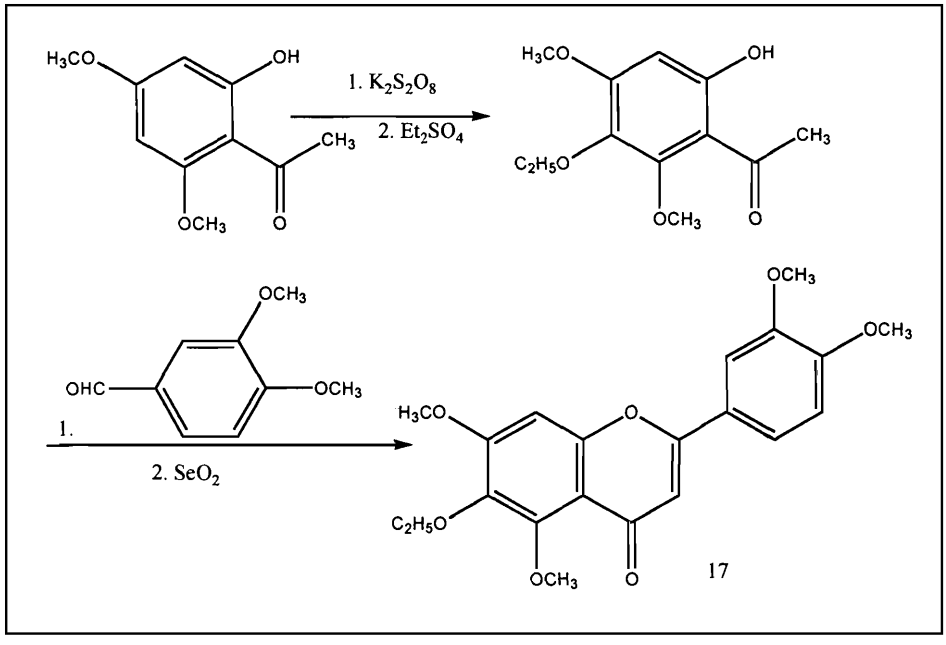


Sesamum indicum. The vari-ous steps beginning with phloroglucinol are shown in *Scheme* 1. As can be seen from it the oxygenation pattern of the A ring of the final product was created using a sequence of reactions which included Hoesch C-acylation followed by selective di-O-methylation, Elbs-Seshadri oxidation and O-benzylation. Subsequent condensation of the resulting acetophenone with 3,4-di-benzyloxybenzaldehyde yielded the chalcone **15**, which on oxida-tion with selenium dioxide gave the flavone **16**. The final deben-zylation and partial demethylation to obtain pedalitin could be achieved with the use of hydrochloric acid in acetic acid.

Location of Sugar Residue

In the same context, it is relevant to describe a related strategy used by Seshadri and his co-workers to locate the position of attachment of the sugar residue in pedaliin. The permethyl ether of the compound was hydrolysed and the product ethylated to get **17**. The structure of the latter was confirmed by a total synthesis as shown in *Scheme* 2, thus proving that the glucose moiety should be attached to position 6. In this synthesis, 2-

Scheme 2.



The location of the methoxyl group at position 6 of the aglucone nepetin (**19**) was also rigorously established by ethylating it to obtain **20**, whose structure was then confirmed by synthesis (*Scheme* 3).

hydroxy-4,6-dimethoxyacetophenone was converted into 2-hydroxy-4,6-dimethoxy-5-ethoxyacetophenone, which on condensation with 3,4-dimethoxybenzaldehyde followed by oxidative cyclisation of the resulting chalcone with selenium dioxide yielded **17**.

Structure of Nepitrin

Nepitrin (**18**), isolated from the leaves of *Nepeta hindustana*, is a related compound, which differs from pedaliin only in the positions of attachment of the methoxyl group and the sugar residue. Zeisel estimation showed the presence of a methoxyl group. Molisch test indicated the presence of a sugar unit which was identified as glucose by both acidic and enzymatic hydrolysis; the linkage was deduced to be beta as emulsin brought about the cleavage of the sugar unit. The location of the methoxyl group at position 6 of the aglucone nepetin (**19**) was also rigorously established by ethylating it to obtain **20**, whose structure was then confirmed by synthesis (*Scheme* 3). In this synthesis, 2-hydroxy-4,6-diethoxyacetophenone was the starting material. It was subjected to Elbs–Seshadri oxidation followed by methylation to obtain 2-hydroxy-4,6-diethoxy-5-methoxyacetophenone. Subsequent condensation with 3,4-diethoxybenzaldehyde and oxidative cyclisation with selenium dioxide gave **20**; identity between the natural product and the synthetic compound was established by mixed melting point determination and comparison of infrared spectra. The structure was also well supported by spectral data. For example, the absorption maximum in the UV spectrum of nepetin at 275 nm was the same as that seen in the spectrum of pectolinarigenin (**21**). On the other hand, the corresponding band in the spectrum of pedalitin (**13**) was observed at 285 nm, the same as in the case of scutellarein 7, 4'-di-O-methyl ether (**22**). The use of shift reagents such as aluminium chloride with and without the addition of hydrochloric acid and sodium acetate-boric acid revealed the presence of free hydroxyls at positions 5, 3' and 4' in nepetin and nepitrin.

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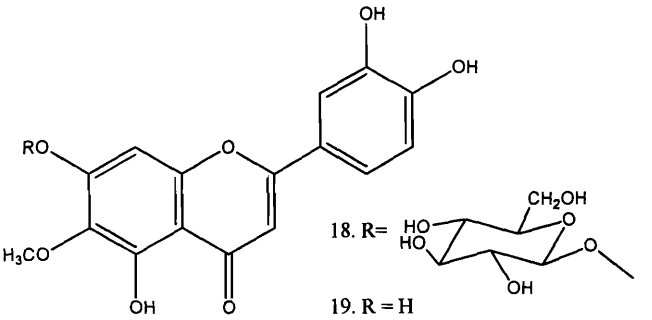
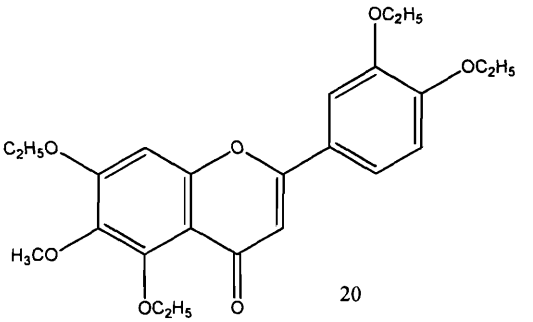
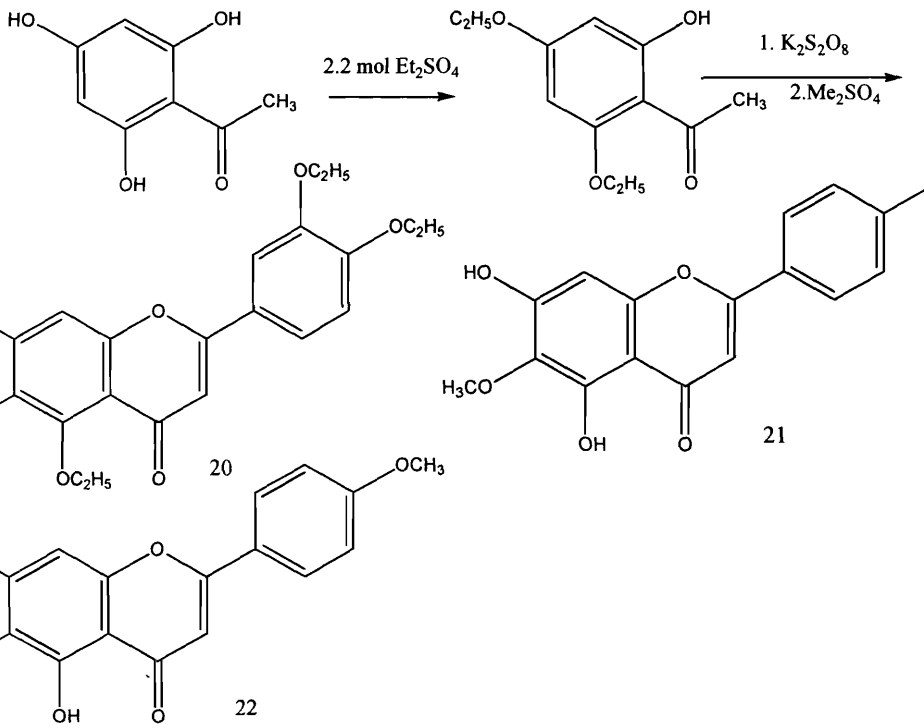

OH
OH
RO
O
H3CO
OH
O
18. R=
CH2OH
HO
HO
O
OH
19. R = H


OC2H5
OC2H5
C2H5O
O
H3CO
OC2H5
O

20

Scheme 3.


HO
OH
CH3
OH
O
2.2 mol Et2SO4
C2H5O
OH
CH3
OC2H5
O
1. K2S2O8
2.Me2SO4
OC2H5
OC2H5
C2H5O
O
H3CO
OC2H5
O
20
OCH3
HO
O
H3CO
OH
O
21
H3CO
O
OCH3
HO
OH
O
22

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Synthesis of a Rotenoid Munduserone

The synthesis of rotenone 23 and related compounds remained an unsolved problem for several years after its structure had been elucidated. Miyano and Matsui achieved the first synthesis of rotenone followed by a few others including the one described in *Scheme* 4 of munduserone (24), which has the same skeletal structure as rotenone, by Seshadri and coworkers. In this synthesis, the starting material was 7,2',4',5'-tetramethoxyisoflavone (25) which was subjected to partial demethylation with aluminium chloride in acetonitrile when the 2' methoxyl group alone suffered demethylation to yield 26. The 2' hydroxyl thus liberated was alkylated with methyl chloro-acetate and the resulting 27 was hydrolysed with alkali. The product (28), a desoxybenzoin, was then converted into the isoflavone, dehydromunduserone (29) by refluxing with acetic anhydride in the presence of sodium acetate. This compound had earlier been converted into munduserone by a two-step reaction sequence involving reduction by sodium borohydride followed by Oppenauer oxidation.

An Example of a Structure Elucidation: Prudomestin

In the earlier years, Seshadri studied floral pigments but later examined other plant parts for their special chemical components. Considerable work was done on the constituents of durable heartwoods of several trees, such as the different species of *Dalbergia*, *Pterocarpus* and *Prunus*. In the following paragraphs, a brief description of the isolation and characterization of two new flavonoid compounds from the heartwood of *Prunus domestica* (the plum tree) is given. An ether extract of the heartwood was fractionated using saturated aqueous sodium bicarbonate, 10% aqueous sodium carbonate and 0.1% aqueous alkali. Neutralisation of the bicarbonate extract yielded one new compound, which was characterized as 3,5,7-trihydroxy-4'-methoxydihydroflavone (dihydrokaemp-feride) (30). More of the same compound was also obtained, along with three other compounds from the carbonate extract after neutralization. Extensive fractionation of the mixture by using benzene and ethyl

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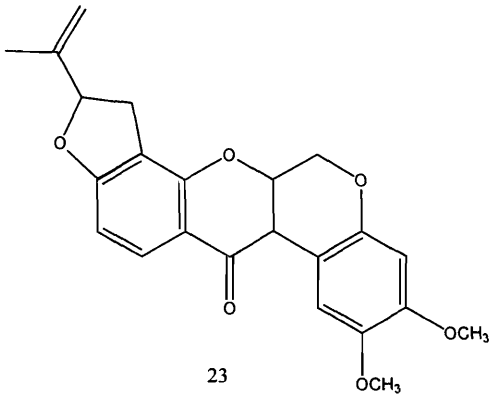
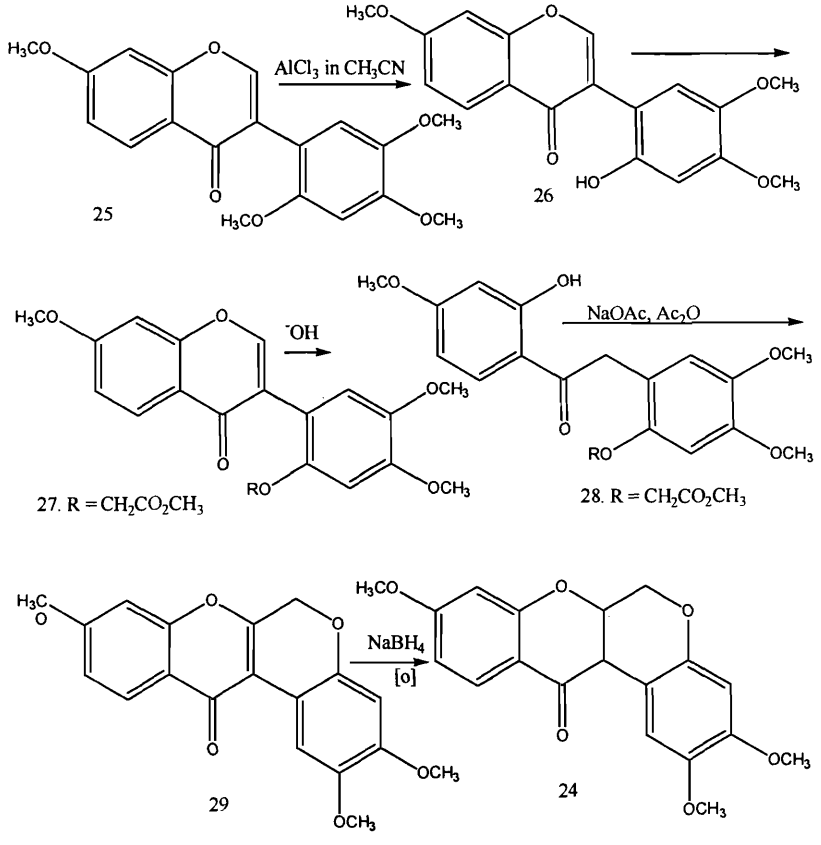


23

Scheme 4.



25 26

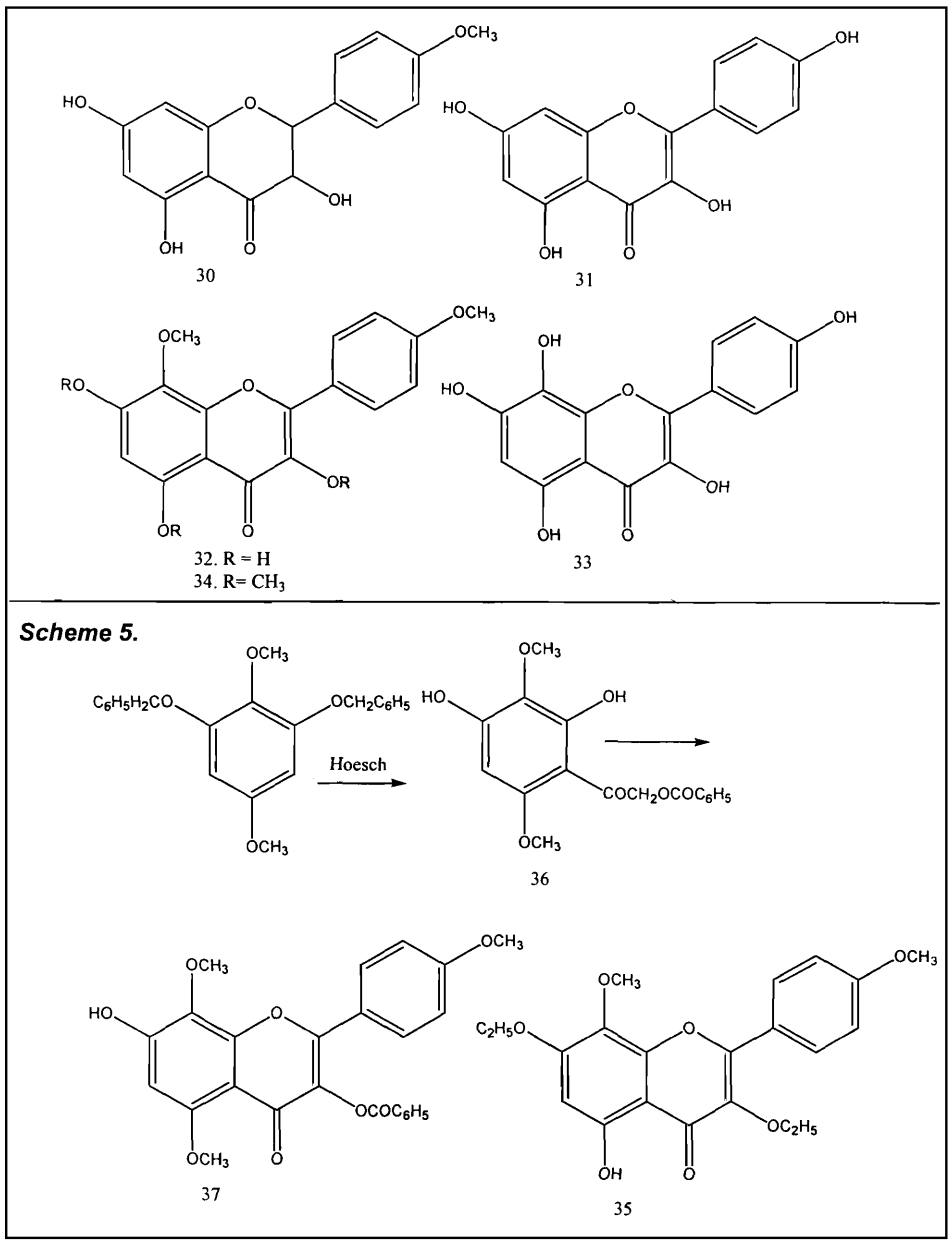
27. R = $CH_2CO_2CH_3$ 28. R = $CH_2CO_2CH_3$

29 24

acetate yielded kaempferol (3,5,7,4'-tetrahydroxyflavone, **31**) and a new compound, named as prudomestin (**32**). The original publication may be referred to for details of the fractionation

An alcoholic solution of prudomestin gave a green colour with alcoholic ferric chloride and a deep pink colour in the Shinoda test, which is a characteristic colour reaction for flavonoids.

procedure, as this is a good example of resolving mixtures of closely related compounds without resorting to chromatography. An alcoholic solution of prudomestin gave a green colour with alcoholic ferric chloride and a deep pink colour in the Shinoda test, which is a characteristic colour reaction for flavonoids. Elemental analysis showed that the compound had the molecular formula $C_{17}H_{14}O_7$. Zeisel estimation revealed the presence of two methoxyl groups. Its ultraviolet spectrum in methanol had absorption maxima at 275, 323 and 375 nm showing close similarity to herbacitin (3,5,7,8',4'-pentahydroxyflavone, **33**). This was confirmed by methylating prudomestin to obtain a flavone pentamethyl ether identical with herbacitin pentamethyl ether (**34**). To locate the position of the two methoxyl groups, prudomestin was ethylated. The product gave a green colour with alcoholic ferric chloride showing the presence of a free 5-hydroxyl group. The product (**35**) was subjected to alkali fusion. One of the products of this reaction was identified as 4-methoxybenzoic acid (anisic acid), thus proving the position of one of the methoxyl groups as 4'. The effect of shift reagents on the UV absorption maxima indicated the presence of free hydroxyls at position 7 (bathochromic shift of the 275 nm band on the additon of sodium acetate) and position 3 (a large bathochromic shift of the 375 nm band on the additon of aluminium chloride). The compound failed to answer the gossypetone colour test, which is characteristic of 5,8-dihydroxyflavones. The presence of a free hydroxyl at position 5 was shown by the long time taken for the formation of the complete methyl ether. Thus, the presence of the second methoxyl at position 8 could be conjectured. The structure thus deduced for prudomestin was confirmed by a synthesis of its diethyl ether **35**. This synthesis, shown in *Scheme* 5, began with a Hoesch condensation of 1,4-dimethoxy-2,6-dibenzyloxybenzene with benzoyloxy acetonitrile. (It is instructive to note that under the Hoesch reaction condition, debenzylation but not debenzoylation occurs). The product **36** was subjected to Allan-Robinson reaction with 4-methoxybenzoic acid anhydride and sodium 4-methoxybenzoate. The resulting flavone **37** was refluxed with aqueous alkali to



liberate the hydroxyl at position 3. Subsequent ethylation followed by selective demethylation using aluminium chloride in acetonitrile yielded **35**. The entire exercise, being a judicious combination of analytical studies, colour reactions, spectral studies, degradation and synthesis, forms a neat package of

The entire exercise, being a judicious combination of analytical studies, colour reactions, spectral studies, degradation and synthesis, forms a neat package of instructional material.

instructional material for anyone on the threshold of a research career.

Conclusion

As mentioned earlier, this article discusses only a small and selective sample of the vast amount of research work carried out by T R Seshadri and his students. References 4-9 may be referred to for a few more examples. The reader can also move on to the remaining one thousand and odd papers!

Suggested Reading

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Address for Correspondence
N R Krishnaswamy
12, 9th Main Road
Banashankari 2nd Stage
Bangalore 560 070, India
Email:
krishnaswamynr@yahoo.com

It is only by introducing the young to great literature, drama and music, and to the excitement of great science that we open to them the possibilities that lie within the human spirit – enable them to see visions and dream dreams.

– Eric Anderson