introduction

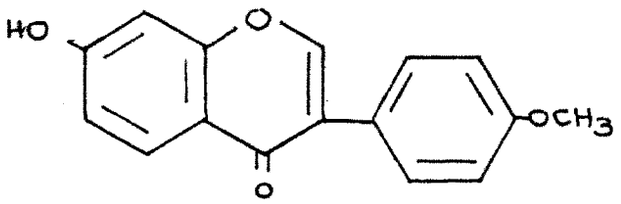
INTRODUCTION

Isoflavones are widely occuring natural products in the form of aglycons as well as glycosides. They are found in the plants with sub-family Lotoideae of Leguminosae.[1,2] They also occur occasionally in the sub-family caesalpionoideae and few other families like Rosaceae, Moraceae, Amaranthaceae, Iridaceae and Podocarpaceae. These groups of products are discovered in the mid 19th Century when glycosides of formononetin (Ononin) and corresponding deoxybenzoin (Onospin) were reported.

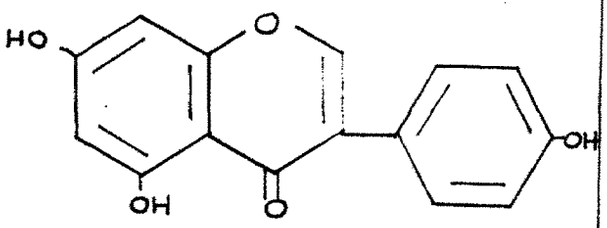
Soyabeans were shown by Walz[3] to contain Diadzein, Genistein and their derived glycosides but in a later study Okano and Beppu [4] claimed that soyabeans contain four other isoflavones, 5,7,2'-trihydroxy isoflavones (Isogenistein), 5,7,2'-trihydroxy-8-methylisoflavone (Methyl Isogenistein), 5,7,4'-trihydroxy-8-methylisoflavone (Methyl Genistein), and 5,4'-dihydroxy-8-methylisoflavone (Tation). This claim was not accepted and it was conclusively proved that these were impure samples of Genistein and Diadzein.[5,6,7]

Extraction and isolation

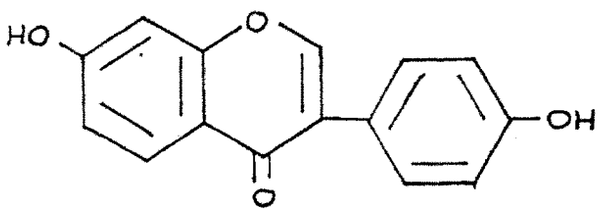
For the isolation of isoflavones, dried plant material is extracted with different solvents iin order as light petroleum ether, benzene, acetone, methanol and ethyl



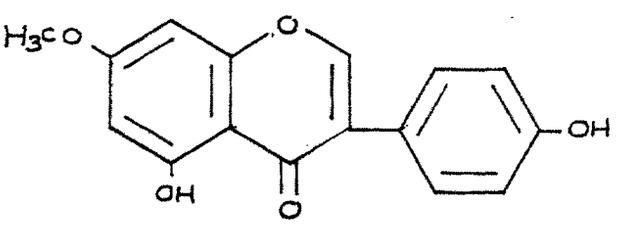
FORMONONETIN



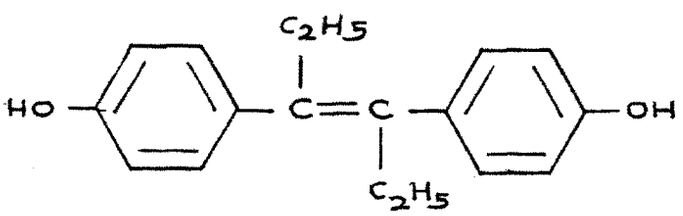
GENISTEIN



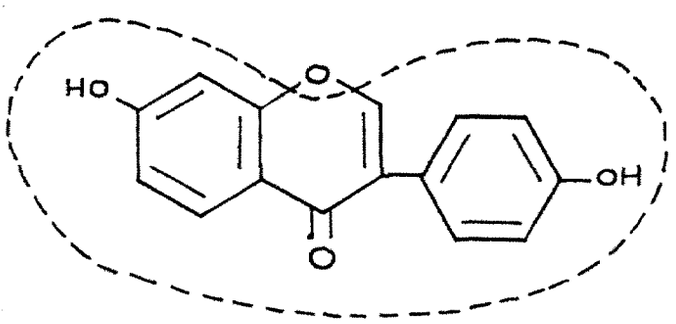
DIADZEIN



PRUNETIN



DIETHYL STILBESTEROL



DIADZEIN

actate. The solvents are evaporated in vacuum and the products are purified by column chromatography.[8,9,10,11]

Pharmacological properties

Isoflavones have estrogenic, insecticidal, pesticidal and antifungal activity. Isoflavones are weak estrogens[12,13] and their presence in forage Legumes such as subterraneum clover (Trifolium sub-terraneum) & red clover (T. Pratense) has been recognised as the cause of infertility problems sometimes occuring in the animals grazing these species.[14,15]

It has been shown that the isoflavone Genistein, Bio-chanin. A, Prunetin, Diadzein and Formononetin have estrogenic activity because of their structure resemblance with diethyl stilbesterol.

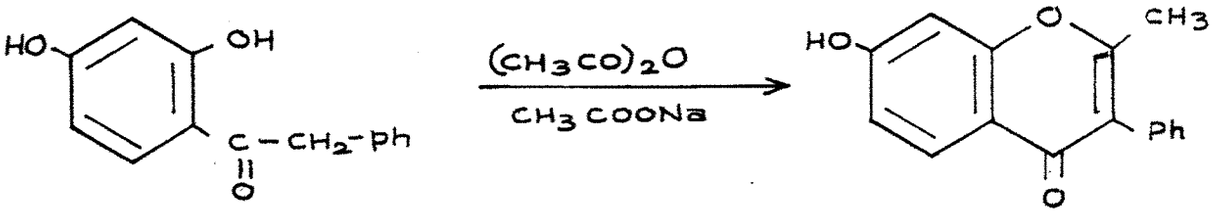
Nilson[16] made a comparative study of the estrogenic activity of Bio-chanin-A and Genistein with diethyl stilbesterol. They[17] tried to establish a structure-activity relationship and reported that a free hydroxyl group at 4'-position of the isoflavone molecule is essential for the estrogenic activity. Braudbury and White[18] found that Genistein and Formononetin possess antifertility activity. They also prepare number of related compounds, some of which have higher potency than Genistein.

Isoflavanoids are showing insecticidal properties in considerable amount. Various rotenoids are used as an effective fish poison. Insecticidal property of isoflavone was observed by Murthy et al.[19] 7-Dimethylallyloxy isoflavone and 7-dimethyl allyloxy-2-methyl isoflavone were tested for their toxicity to fresh water fish by Kukla and Seshadri.[20] The later was found to possess considerable toxicity to fish. Isoflavones with free hydroxyl group are found to be more toxic than its methyl ethers.[19] The toxicity of the compounds having one dihydrofuran ring have been reported. The presence of free hydroxyl group reduce the potency of the compound whereas complete ethers (e.g. 5,7-di-allyloxy compounds) are more powerful.[21] Kukla and Seshadri[20] studied toxicity of various allyl and prenyl derivatives of isoflavone and stated that allyl derivatives are better toxic agents than prenyl derivatives. In the comparative study of flavones and isoflavones it was noted that introduction of methyl group in position 2 and 3 of isoflavones and flavones respectively increased their toxicity. Methoxyl group in 3' and 4' position increased appreciably the activity as in the case of 7-dimethylallyloxy-2-methoxy isoflavone.[22]

Methods of Synthesis

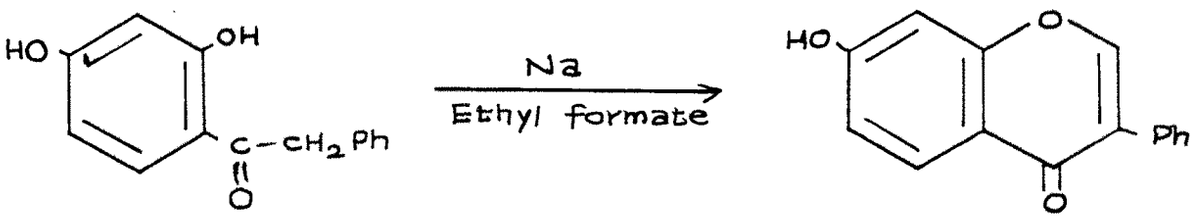
Baker[23,24] synthesized various 2-substituted isofla-

W. Baker et. al.[23,24]



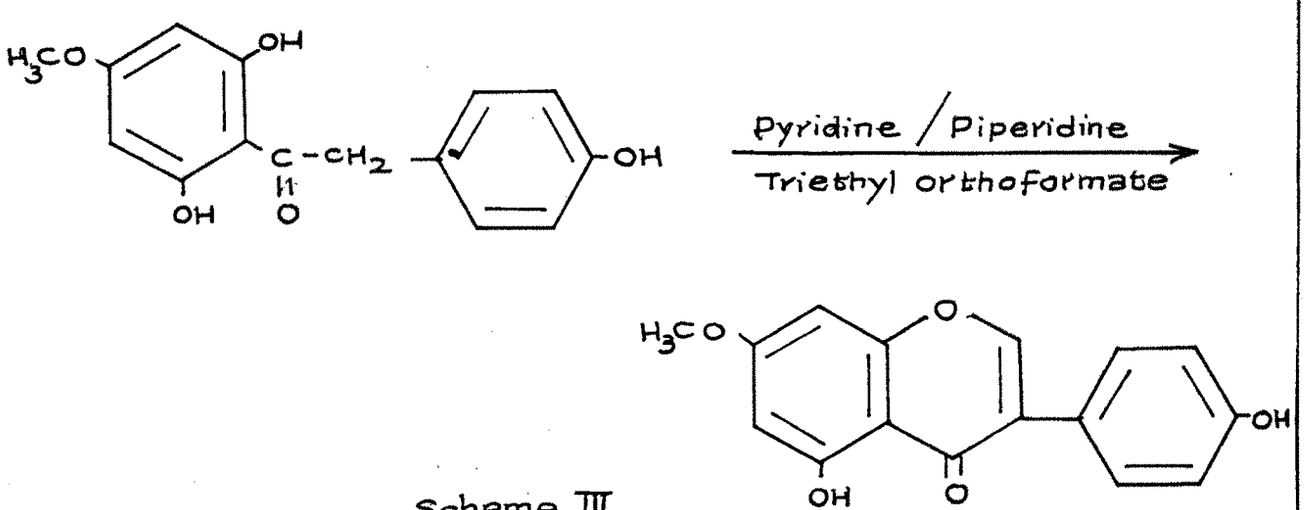
Scheme I

K. Venkataraman et.al[28]



Scheme II

K. Venkataraman et. al.[30]



Scheme III

Prunetin

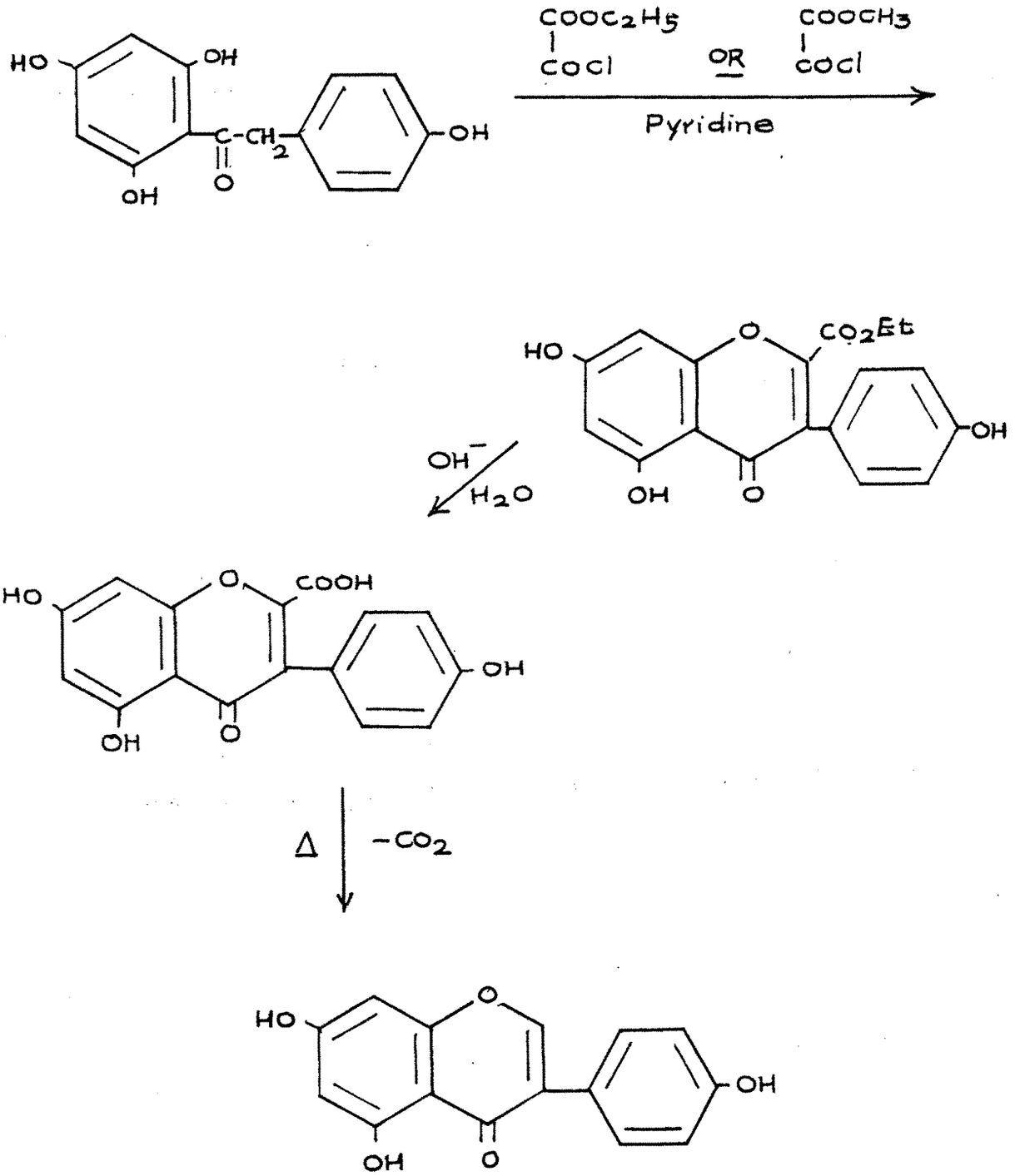
vones obtained by Kostanecki reactions in which the interaction of 2,4-dihydroxy phenylbenzyl ketone or corresponding derivatives are heated with a mixture of anhydride and sodium salt of carboxylic acids at 170-80°C (Scheme-I)

They also synthesized Genistein[25] starting from 2-styryl isoflavone derivatives and 2-methyl Lirigenol[26] The sane group of workers[27] synthesized the natural product Diadzein and other derivatives. Venkataraman et al.[28] synthesized isoflavone by condensing sodium and ethyl formate with deoxybenzoins. They synthesized 7-hvdroxy isoflavone by this method (Scheme-II)

The present, well established method of synthesizing isoflavone through pyridine, pieridine and triethyl orthoformate was first adopted by Venkataraman et al.[29] Prunetin was synthesized by the same method.[30] (Scheme-III)

For the synthesis of polyhydroxy isoflavones Baker et al.[31] condensed polyhydroxy ketone with ethoxalyl chloride in the presence of pyridine. Other group of workers[32] adopted little change in this process in which deethoxy carbonylation was done more effectively by heating it in aniline hydrochloride. Further it was observed that methoxalyl chlorides are more effective as far as yields are concerned.[33] (Scheme-IV)

W. Baker et. al.[31], M.O. Farooq et. al.[33]



Genistein

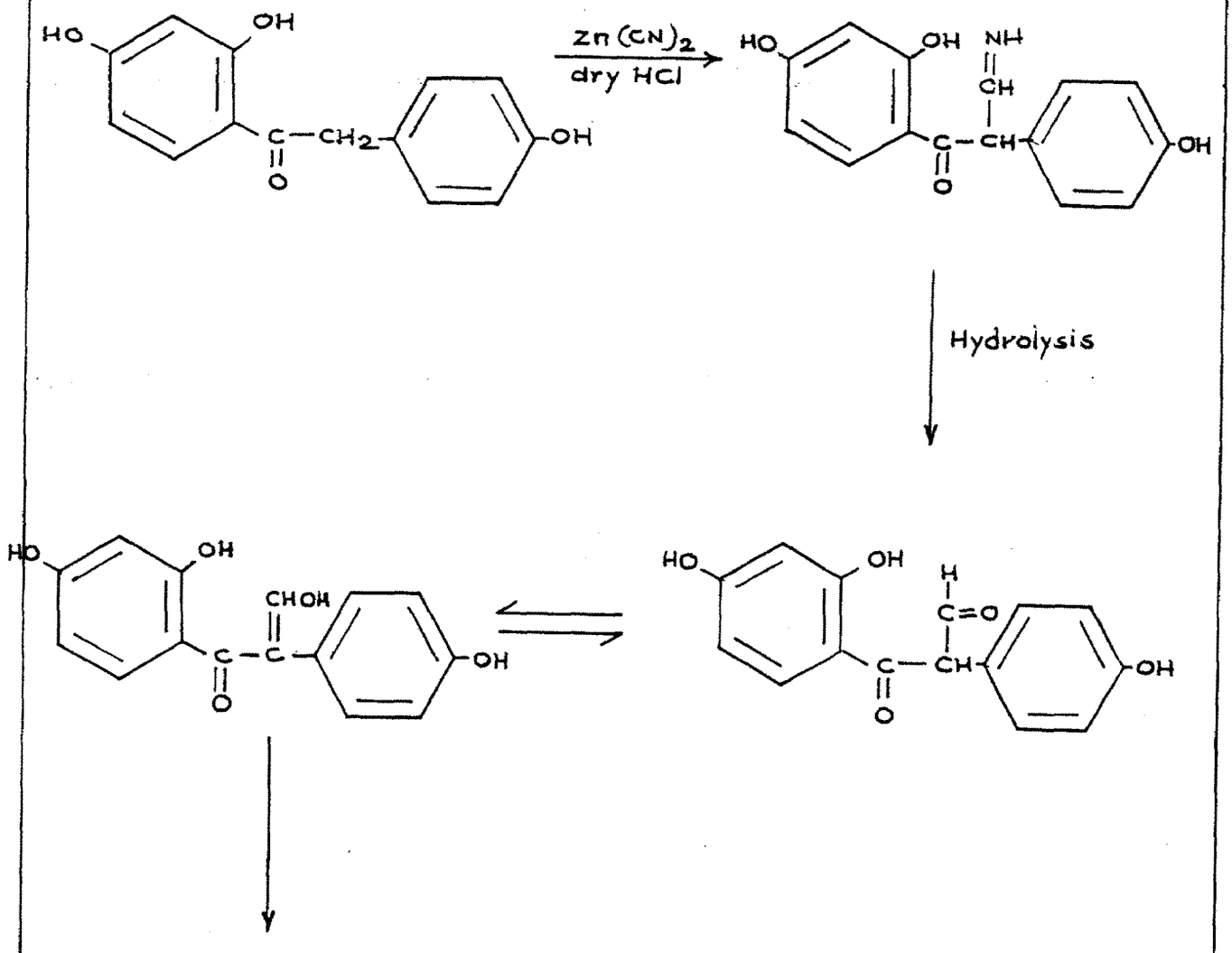
Scheme IV

Farakas et al.[34,35] developed a new method of isoflavone synthesis using Gatterman-Adams reagent $Zn(CN)_2$ and dry HCl gas. They synthesized various isoflavones like Diadzein, Formononetin etc. by this method. (Scheme-V)

Use of hexamethylenetetramine in acetic acid was made in isoflavone synthesis by Fukui et al.[36] On the other hand starting from chalcone epoxide, dihydroflavanols were obtained by treating with BF_3 -etherate which in turn give rise to isoflavones.[37,38] Afrormosin was synthesised using chalcone epoxide method[39] (Scheme-VI). Similarly formononetin and Pseudobaptigenin have been synthesized in good yields subjecting chalcone epoxide to aryl migration using BF_3-etherate reagent.[40]

Catalytic rearrangement for synthesis of isoflavone was carried out by Farakas et al.[41] for which they started with 2'-acetoxy or 2'-hydroxy chalcones. Thallium (III) nitrate in methanol was used as catalyst to obtain oxidative rearrangement product as 1-(2-hydroxy phenyl)-3,3-dimethoxy-2-phenyl-propan-1-ones which was followed by cyclization to furnish various isoflavone derivatives. By this route they have reported synthesis of sorphorol, violanone and few other isoflavone. (Scheme-VII).

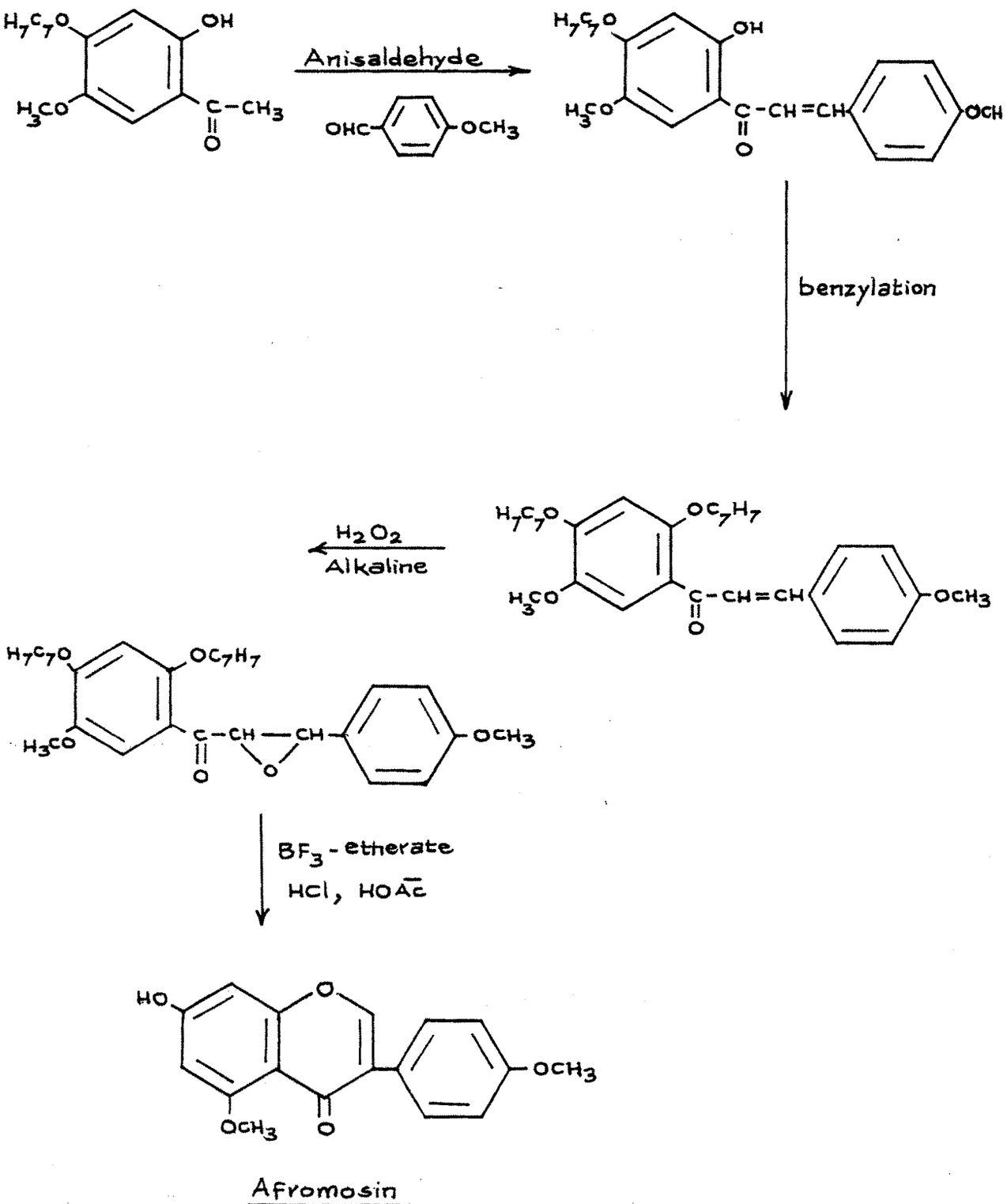
Farkas et al[34,35]



Diadzein

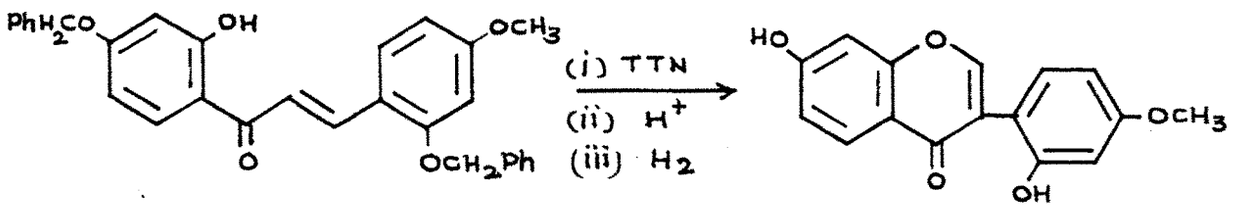
Scheme V

A. C. Jain et al[39]



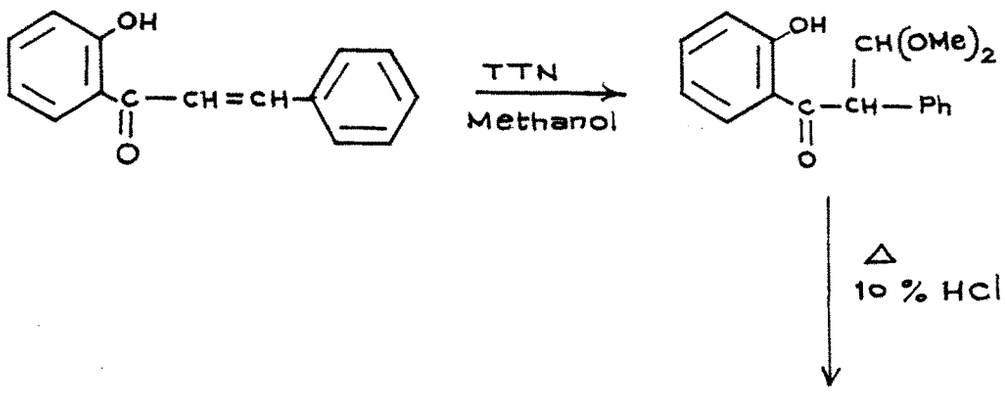
Scheme VI

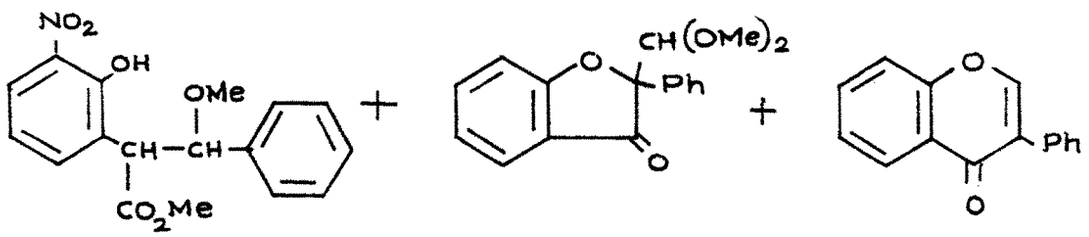
L. Farkas et al [41]



Scheme VII

R. S. Varma [42]





III II I

Scheme VIII

Using this procedure Varma[42] synthesized simple isoflavone (I) alongwith other two side products, 2-dimethoxy-methyl-2-phenyl coumaran-3-one (II) & methyl 2-(2'-hydroxy-3'-nitrophenyl) 3-phenyl-3-methoxypropanoate (III) (Scheme-VIII).

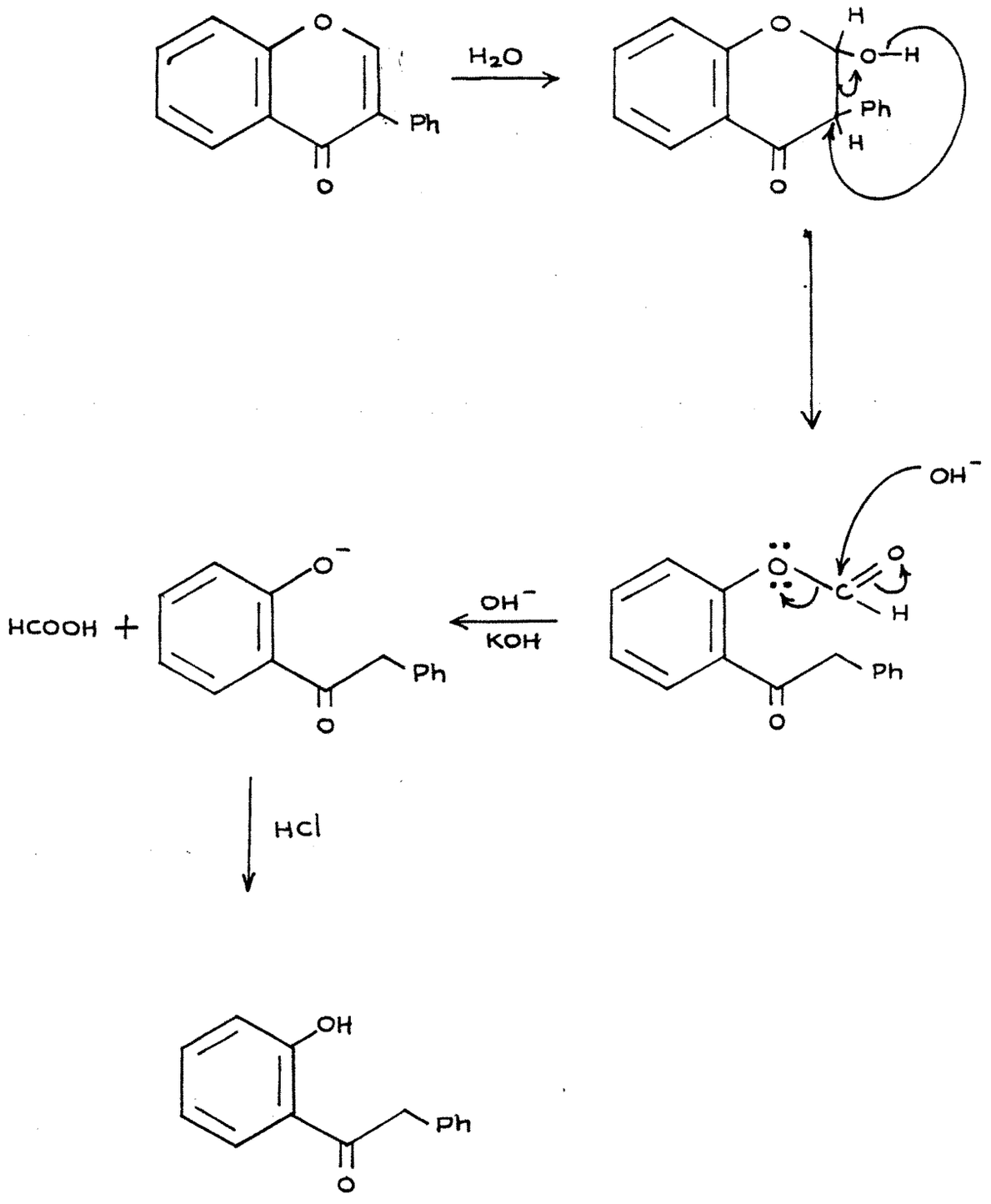
Characterization - Chemical and Spectral Method

Chemical methods for the structure determination of isoflavones are well documented[43,44,45] Isoflavones are much more susceptible to alkali hydrolysis than flavones he production of deoxybenzoins under mild conditions, together with formic acid constitutes an important evidence for an isoflavone structure. (Scheme-IX)

U/V Spectroscopy

Isoflavones are 3-phenylchromones while flavones are 2-phenylchromones. The most distinctive difference betweend isoflavones and flavones is in their ultra violet spectra.[46,47] Isoflavones have maximal absorption in two main regions, band I (270 nm) and band II (290 - 330 nm). In case of flavones these bands are in the region 260 - 280 nm and 340 - 370 nm. Band I in isoflavone is usually more intense than 260 - 280 nm band of flavone.

The values of ultra-violet spectra was determined



Scheme IX

for various structural studies including formonentin,[48] cabreuvin,[49] munetone,[50] jamicine,[51] mundulone[52] and estrogenic isoflavones.[53,54]

H^1-NMR Spectroscopy

Flavanoids with substituents at different positions give signals at different field in nmr spectra.[55] In case of 7-hydroxy flavanoids, C-5 proton in these type of compounds is strongly deshielded by the carbonyl group at C-4 position and appear downfield near δ 8.0 ppm as doublet (d, J=9Hz) due to ortho coupling with proton at C-6. C-6 proton appear at δ 6.7 - 7.1 as double doublet and H-8 appear at δ 6.7 - 7.0 as doublet, which are at lower field compared to signals for 5,7-dihydroxy flavanoids.

The distinguishing feature between the isoflavone and flavone is in the position of the signals due to C_2-H and C_3-H. The former occur at δ 7.8 - 8.0 while the latter shows at δ 6.8. This is because C_2-H proton in isoflavone is β to the carbonyl group and also attached to the carbon atom joined to oxygen. The position of other aromatic protons being almost identical in each case.

C^{13}-NMR Spectroscopy

Isoflavones are also characterized by their C^{13} nmr spectra. Ternai and Markham[56] have reported someof the C^{13} nmr spectra of flavanoids, chalcones, isoflavones and several glycosides. Pelter et al.[57] reported the C^{13}nmr spectra of 7-methoxy isoflavone. It shows characteristic C-2 signal at δ 152.4 while carrbonyl carbon appear at δ 175.3. The other signals appear as follows :

C_3 - 125.1, C_5 - 127.6, C_6 - 114.4, C_7 - 163.8, C_8 - 100,

C_{8a} - 157.7, C_{4a} - 118.3, $C_{1'}$ - 127.9, $C_{2'}$ - 128.2,

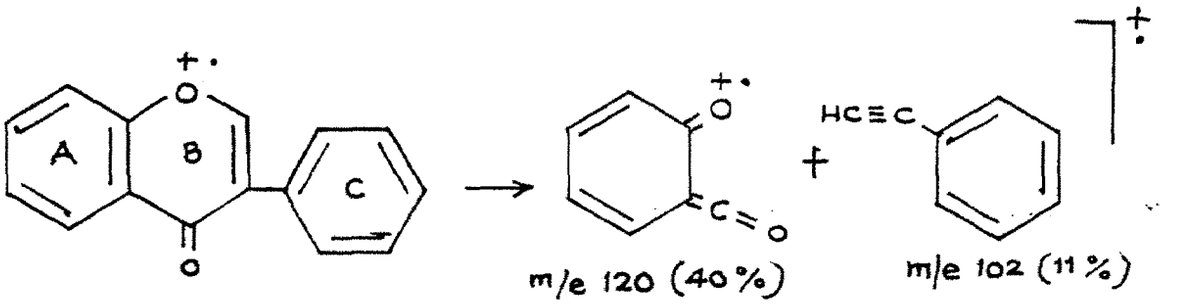
$C_{3'}$ - 128.8, $C_{4'}$ - 131.8, $C_{5'}$ - 128.8, $C_{6'}$ - 128.2.

Mass Spectroscopy

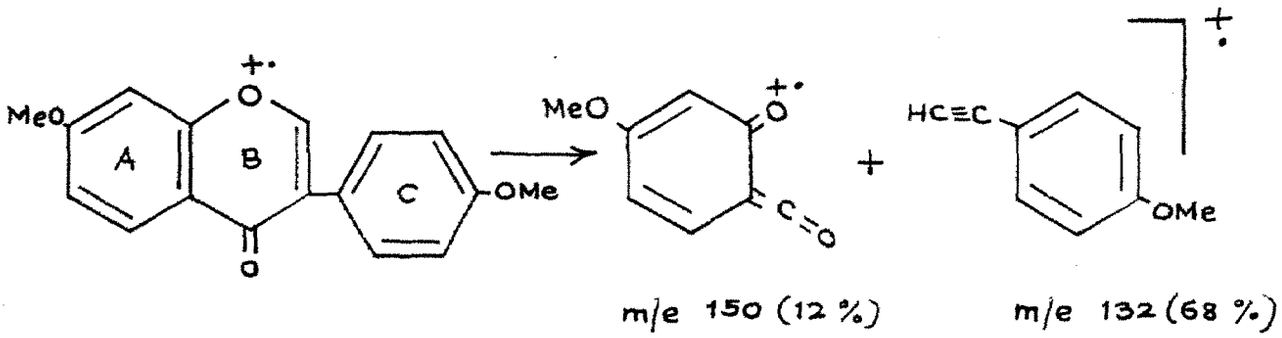
The mass spectra of isoflavones are characterized by intense molecular ion peak indicating stable heterocyclic system.

The mass spectral fragmentation of isoflavone resemble that of the flavone with respect to Retro Diels-Alder fragmentation pattern and charge distribution on the two fragments depends upon the substitution on ring A and C, as in flavones. If the ring C is unsubstituted than the charge resides mainly on ring A, which further fragments by typical loss of CO.[58,59,60] If there is a methoxy group on ring C, then ring C fragmentation is favoured.[61] (Scheme-X)

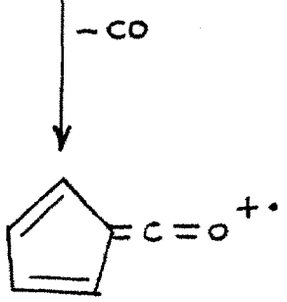
J. H. Beynon [61]



m/e 120 (40 %) m/e 102 (11 %)



m/e 150 (12 %) m/e 132 (68 %)



Scheme X

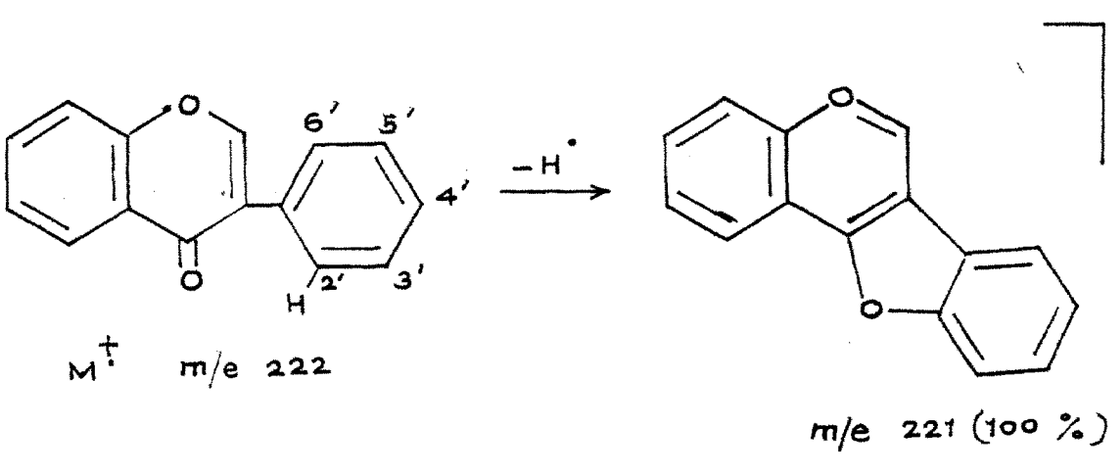
Doubly charged parent ion is prominent in isoflavone. Isoflavones are also characterized by rather intense $(M-1)^+$ ion which is the base peak in the spectra of isoflavone. Loss of hydrogen atom from C-2 position of isoflavone would give resonance stabilized structure, whose stability is responsible for the lack of further fragmentation.[58,62] (Scheme-XI)

Reaction of isoflavone

Seshadri et al.[63,64] introduced an additional hydroxyl group para to existing hydroxyl group viz. Elbs persulfate oxidation of 5,7-dihydroxy isoflavone gave 5,7,8-trihydroxy isoflavone and 5,8-dihydroxy-7-methoxy isoflavone is obtained from 8-hydroxy-7-methoxy isoflavone. (Scheme-XII)

Phenolic hydroxyl group of isoflavone behave normally and can be alkylated and acetylated, provided that they are dnot in the 5th position. Hydroxyl group in the 5th position of isoflavone form hydrogen bond with the carbonyl group at 4 position which makes alkylation and acetylation difficult whereas demethylation of 5-OMe isoflavone derivative is comparatively easier and take place in presence of anhydrous aluminium chloride or BF_3 -etherate at room temperature.

Y. Itagaki et al [58] ; R.V.M. Campbell [62]

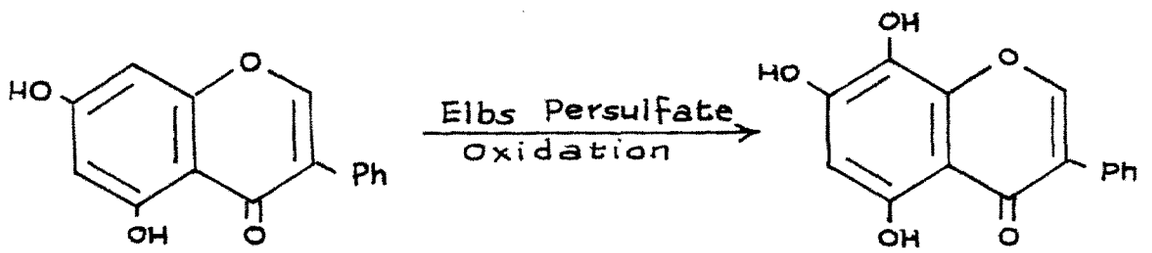


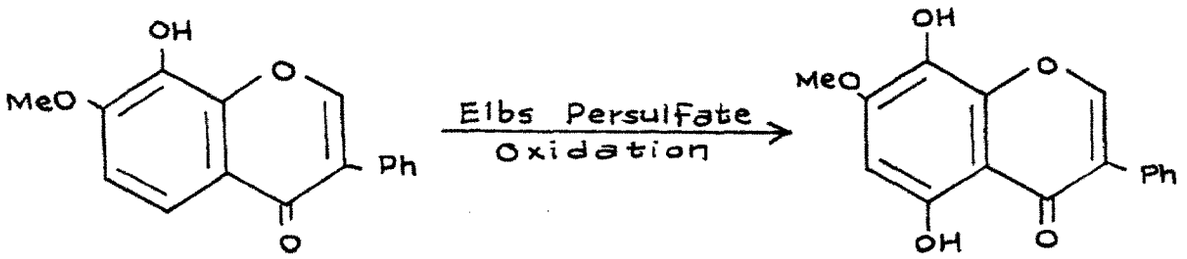
$M^{+\cdot}$ m/e 222

m/e 221 (100 %)

Scheme XI

T. R. Seshadri et al [63,64]





Scheme XII

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