INTRODUCTION
The chemistry of natural products is a rapidly expanding field on which the attention of chemists has been focused since the last several decades. The physiological activity of natural organic compounds and their role in various metabolic activities, the discovery of several drugs (such as corticoids, penicillin and alkaloids)\textsuperscript{1} with dramatic therapeutic effects\textsuperscript{2-16}, offered wealth of material of tremendous interest. Every year a very large number of natural products are screened for their chemical therapeutic, biological and industrial potentials. In addition to their medicinal actions, recently natural products have attracted attention of taxonomists for phytogenetic studies. Flavanoids and terpenoids have been used as taxonomic markers in several cases\textsuperscript{17}. The superiority of chemo-systematic methods over morphological characteristics to plant taxonomy have been advocated in recent years\textsuperscript{18}.

Lack of refined techniques for isolation and characterisation were main barriers for the chemists in the early part of the centuary. The work in natural products chemistry was revolutionised by the introduction in early 1960's of the new physiochemical techniques, nuclear magnetic resonance and mass spectroscopy. NMR affords information on the environment of different protons in the molecule and the mass spectroscopy settled the age old problem of the exact molecular weight of an organic compound, and provides informations of structural value by indicating the preferred cleavage of the molecule. The lite-
ature on the application of both these techniques to natural products such as flavanoids, coumarins, alkaloids and terpenoids has been covered so extensively in various reviews that further comment is hardly needed.

Since mainly the spectroscopic techniques, UV, IR, NMR and Mass have been used in the identification and structure elucidation of the products isolated from different plants, during the course of the present work, a short review of each technique has been given in the theoretical part of the thesis.
Ultra-Violet Spectra

The ultra-violet spectra of different flavanoids are very characteristic and along with colour reactions have been used extensively to distinguish the various groups of this class of compounds. The absorption maxima of flavones have been correlated to the presence of a cinnamoyl (II) and benzoyl (I) groupings, the former giving rise to the high wave length band at 320 to 350 m$\mu$ and the latter to the low wave length band at 240-270 m$\mu$. On the basis of this generalisation, important deductions have been made about the location of substituents in the two rings.

Substitution in the B ring specially at 4' stabilises the cinnamoyl chromophore resulting in a bathochromic shift of band I whereas substitution in the A ring has a similar effect on the position of band II. Compounds having a free 5-hydroxyl absorb at higher wave lengths and methylation of this hydroxyl brings about a hypsochromic shift of 10 to 13 m$\mu$ of both maxima. The presence of a hydroxyl at this position is routinely establi-
shed by measuring the spectrum in presence of AlCl₃. Hydroxyl groups at 7,4' are more acidic than others and a bathochromic shift of band I or II on addition of fused sodium acetate is a good indication of the presence of OH group at these positions, but the results of these measurements have to be interpreted with caution and require further confirmation by degradation. Thus, for example, lucidin, acerosin and scaposin failed to give a bathochromic shift with sodium acetate though they were definitely shown to possess a 7-OH group.

In flavanones absence of cinnamoyl chromophore has the effect at suppressing the high wave length band which is either totally absent or present only as an inflection. The spectra of isoflavones are also marked by the absence of the high wave length band, biochanin A, irigenin and pomiferin absorb only between 261 to 276 m. Thus it is difficult to distinguish between flavanones and isoflavones with the help of UV spectrum alone.

Infrared Spectra

The IR spectrum of flavanone shows the carbonyl absorption at 1680 cm⁻¹, the standard value for aromatic ketones. The shift of the carbonyl band to 1620 cm⁻¹ in 5-OH flavanones is largely due to electron donation by the ortho hydroxyl group, coupled with chelation. Consequently methylation of the 5-OH
produces only a small hypsochromic shift of 10 cm⁻¹. A similar shift towards long wave length of 4' substituted flavanone is however, attributed to inter molecular hydrogen bonding. The IR spectrum of flavone shows the carbonyl band at 1660 cm⁻¹ owing to conjugation with the olefinic double bond. Introduction of a hydroxyl at 5-position does not alter the band position appreciably, luteolin and apigenin shows the carbonyl band at 1655 and 1660 cm⁻¹ respectively. The IR spectra of isoflavones are similar to those of flavones. Chelation of the 5-OH in all cases has the effect of broadening the O-H stretching band to a point where it can no longer be made out. The aromatic region is not of any great usefulness as no reliable prediction about the substitution pattern can be made on the basis of absorption bands in this region. The infrared spectra of alkylated flavanoids give some indication of the presence or absence of gem dimethyl groups and an epoxide linkage but these points can now be better established with the help of NMR spectra.

The infrared spectra of triterpenes have got much resemblance with the spectra of the steroids. However positions in C-3 ketones in the series of steroids, the C-2 and C-4 methylene groups absorb near 1420 cm⁻¹ while in the corresponding (3-oxo) triterpenes, the C-2, methylene group absorbs near 1430 cm⁻¹, a C-11 methylene in 12-oxosteroids absorbs at 1434 cm⁻¹. Whereas the same group in 12-oxo- triterpenes absorbs close to 1420 cm⁻¹. Cole and coworkers have summarised the positions of
carbonyl bands, ethylenic double bonds\textsuperscript{32} and the equitorial or axial nature of the hydroxyl groups in triterpenic compounds\textsuperscript{33} in the IR region.

As a result of infrared spectroscopic studies it might be possible to make a distinction between tertiary equitorial (3613 cm\textsuperscript{-1}) and axial (3617 cm\textsuperscript{-1}) hydroxyl groups. On this basis the band at 3629 cm\textsuperscript{-1} (CCl\textsubscript{4}) in methyl melaleucate\textsuperscript{34} (III) has been assigned as equitorial secondary, while its 3-epimer, obtained by oxidation of the ketone and subsequent reduction, absorbing at 3636 cm\textsuperscript{-1} as axial secondary.

\[
\begin{align*}
\text{HCOOC} & \quad \text{HO} \\
\text{(III)} & 
\end{align*}
\]

\textbf{Nuclear Magnetic Resonance Spectroscopy}

The foregoing discussion of the IR and UV spectra of flavanoids illustrates the usefulness of these two techniques in determining the structure of unknown flavanoids. It is clear that although much useful information regarding the oxygenation pattern
of a flavanoid can be obtained in this way, it falls short of providing complete and unambiguous evidence for or against a presumed structure. Thus the infrared spectrum does not go beyond distinguishing between the \( \gamma \)-pyrone system of flavanoids and the \( \alpha \)-pyrone system of coumarins besides indicating the presence of a chelated 5-hydroxyl. The UV spectrum is much more informative and distinguishes clearly between flavones, isoflavones and coumarins.

The application of NMR spectroscopy has proved to be most powerful tool in the structure determination of flavanoids. By the use of NMR studies of silyl derivatives\(^{35}\) double irradiation techniques\(^{36a}\) solvent induced shift studies\(^{36b-38}\), Lanthanide induced shift\(^{39}\), nuclear overhauser effect\(^{40}\) (NOE) and \( ^{13}C \) NMR\(^{41}\) spectroscopy, one can come to structure of flavanoids occurring even in minor quantities with out tedious and time consuming chemical degradation and synthesis.

The trimethylsilyl derivatives can be conveniently prepared by treatment of the compound with hexamethyl disilazone and trimethylsilyl chloride in pyridine and the spectrum is then measured in carbon tetrachloride with tetramethylsilane as external or internal reference.

Table (I) gives the chemical shifts of different protons of certain representative of flavones and isoflavones. The most detailed and systematic studies of the NMR spectra of...
### Table - I

Chemical shifts (\(\delta\)) for the indicated protons in the NMR spectra of flavones and isoflavones (d, doublet).

<table>
<thead>
<tr>
<th>Location of protons or Substituents</th>
<th>2H</th>
<th>3H</th>
<th>5H</th>
<th>6H</th>
<th>8H</th>
<th>2'H</th>
<th>6'H</th>
<th>5'H</th>
<th>3'H</th>
<th>5-OMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>3.65</td>
<td>3.85(d)</td>
<td>3.5(d)</td>
<td>2.3</td>
<td>3.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteolin</td>
<td>3.7</td>
<td>3.86(d)</td>
<td>3.5(d)</td>
<td>2.65</td>
<td>3.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robinetin</td>
<td></td>
<td>2.05</td>
<td>3.25</td>
<td>3.82</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genistein 5-methyl ether</td>
<td>2.4</td>
<td></td>
<td>3.8</td>
<td>2.7</td>
<td>3.25</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orobol</td>
<td>2.3</td>
<td>3.82(d)</td>
<td>3.61(d)</td>
<td></td>
<td>3.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**APIGENIN** - \(R_1:O\)H; \(R_2, R_3, R_4, = H\)

**LUTEOLIN** - \(R_1, R_2, = O\)H; \(R_3, R_4, = H\)

**ROBINE TIN** - \(R_1 = H\); \(R_2, R_3, R_4, = O\)H

**GENISTEIN 5-METHYL ETHER**

\(R' = CH_3\); \(R = H\)

**OROBOL** - \(R' = H\); \(R = O\)H
flavanoids are due to Mabry\textsuperscript{42,43}, Batterham and Hight\textsuperscript{44}, Clark-Lewis\textsuperscript{45}, Massicot\textsuperscript{46}, Kawano\textsuperscript{39a,b} and Pelter and Rahman\textsuperscript{47-49}.

These studies have simplified the task of determining the substitution pattern of flavanoids with the help of NMR spectroscopy. An obvious advantage of this technique in its application to flavanones is that they can be readily distinguished not only from flavones and isoflavones but also from the isomeric chalkones which in view of the extreme ease of the isomerisation process, is often not possible with other methods.

The most commonly occurring hydroxylation pattern in natural flavanoids is 4',5,7-trihydroxy (IV) system. The chemical shifts of the protons of ring A and B prove to be independent of each other but are affected by the nature of ring (C).

![Chemical structure of IV](image)

The two A ring protons of flavanoids with 5,7-hydroxylation pattern give rise to two doublets (J=2.5 Hz) between 3.34-4.0 from tetramethylsilane. There are however small but
predictable variation in the chemical shifts of the C-6 and C-8 proton signals depending on the 5- and 7-substituents. In flavonones the 6,8-protons give a signal peak near \( \tau \approx 4.05 \), with the addition of a 3-hydroxy group (flavanols) the chemical shift of these protons are slightly altered and the pattern changes to very strongly coupled pair of doublets. The presence of double bond in ring C of flavones and flavanols causes a marked downfield shift of these peaks again producing the two doublet pattern out of 6- and 8-protons, the latter appears downfields.

**Ring B**

All B ring protons appear around \( \tau 2.3-3.3 \) a region separate from the usual A-ring protons. The signals from the aromatic protons of a substituted B-ring in a flavone appear as a broad peak centred at about \( \tau 2.55 \). The presence of C-ring double bond causes a shift of 2',6'-protons and the spectrum shows two broad peaks one centred at \( \tau 2.00 \) (2',6') and other at \( \tau 2.4 \) (3',4',5')

With the introduction of 4'-hydroxyl group the B-ring protons appear effectively as a four peak pattern, this is called \( A_2B_2 \) pattern. Introduction of one more substituent to ring B gives the normal ABC pattern, the hydroxyl group increases the shielding on the adjacent 3',5'-protons and their peak moves substantially upfield. The 2',6'-protons of flavanones give signals centred at about \( \tau 2.65 \).
Ring C

Considerable variations are generally found for the chemical shifts of C-ring protons among the several flavanoid classes. For example, the C-3 proton in flavones gives a sharp singlet near \( \tau 3.7 \), the C-2 proton of isoflavones is normally observed at about \( \tau 2.3 \), while the C-2 proton in flavanones is split by C-3 protons into a doublet of doublet (\( J_{\text{cis}} = 5 \text{ Hz}, J_{\text{trans}} = 11 \text{ Hz} \)) and occur near \( \tau 4.8 \). The two C-3 protons occur as two quartets (\( J_{\text{3a-3b}} = 17 \text{ Hz} \)) near \( \tau 2.3 \). However, they often appear as two doublets since two signals of each quartet are of low intensity. The C-2 proton in dihydroflavanols appears near \( \tau 5.1 \) as a doublet (\( J = 11 \text{ Hz} \)) coupled to the C-3 proton which comes at about \( \tau 5.8 \) as doublets.\(^{50}\)

In the structure elucidation of biflavonoids certain useful information can be obtained by comparison of their NMR spectra with those of their corresponding monomers. Such a choice, however, is compelling but by no means infallible. Comparison of the NMR spectra of methyl and acyl derivatives of a biflavonoid with those of biflavonoids of the same series as well as with those of biflavonoids of other series in which at least one monoflavonoid unit is similarly constituted is very helpful in assigning each and individual proton and the position of methoxy groups. The problem of interflavanoidal linkage has been successfully solved by solvent induced shift studies of
methoxy resonances\textsuperscript{48,49a,51} and lanthanide induced shift\textsuperscript{52-55} studies.

In biphenyl type biflavones such as amentoflavone, cupressuflavone, agathisflavone etc., the peaks, of ring protons involved in interflavanoid linkage, appear at somewhat lower field (\textasciitilde 0.5 ppm) as compared with the peaks of the same protons in monomer due to extended conjugation.

It has been observed\textsuperscript{49b} both in biphenyl as well as in biphenyl ether type biflavanoids that the 5-0-Me group of a 8-linked monoflavonoid unit in a biflavanoid shows up below \textasciitilde 6.00 in deuterochloroform in all the cases examined so far (Table II). This observation may be explained on the basis of extended conjugation. 5-Methoxyl group of a 8-linked monoflavonoid unit in biflavonoids of BGH-series\textsuperscript{56-62}, WGH-series\textsuperscript{56,60} and GB-series\textsuperscript{63-66} does not show up below \textasciitilde 6.00 as the linkage is through heterocyclic ring.

\textsuperscript{13}C Nuclear Magnetic Resonance Spectroscopy

A nucleus can have a net magnetic moment only when the concerned nucleus has non-zero spin quantum number. The abundant \textsuperscript{12}C isotope of carbon, with both atomic and mass numbers even, has zero spin quantum number and hence can not give rise to nuclear magnetic resonance. The \textsuperscript{13}C isotope of carbon has a very low natural abundance (1.108\%) and because of its lower gyromag-
### Table - II

Methoxy protons (τ values) of fully methylated biflavanoids.

<table>
<thead>
<tr>
<th>Biflavanoid</th>
<th>I-5-OMe</th>
<th>II-5-OMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupressuflavone</td>
<td>5.85</td>
<td>5.85</td>
</tr>
<tr>
<td>[I-8,II-8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amentoflavone</td>
<td>6.13</td>
<td>5.94</td>
</tr>
<tr>
<td>[I-3',II-8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agathisflavone</td>
<td>6.14</td>
<td>5.95</td>
</tr>
<tr>
<td>[I-6,II-8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Hinokiflavone</td>
<td>6.00</td>
<td>5.92</td>
</tr>
<tr>
<td>[I-4'-O-II-8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3-Dihydroamentoflavone</td>
<td></td>
<td>5.95</td>
</tr>
<tr>
<td>[I-3',II-8]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Synthetic.
netic ratio compared to that of proton gives rise to week NMR signals. The technique was therefore applied only in work of biological importance employing enrichment of $^{13}$C isotope. The lack of sensitivity leads to high signal to noise ratio but accumulation of spectra in digital computer involving averaging of noise, computer averaged transients (CAT) produce interpretable spectra. But the most economical and efficient method of sensitivity enhancement in $^{13}$C NMR is the Pulse Fourier Transform (PFT) technique which in combination with decoupling methods such as proton broad band and off resonance decoupling becomes a very powerful tool of structure analysis.

In conventional NMR spectroscopy the radio frequency power is kept low to avoid saturation and the spectrum is obtained by sweeping the rf field in the range of Larmor frequencies of the observed nuclei. This method is applicable to nuclei which have sensitivity of the order of protons and is described as the static absorption or continuous wave (CW) NMR spectroscopy. With less sensitive nuclei the signal to noise ratio is high and useful spectra were only obtained when a new technique "The pulse Fourier Transform" was developed. The complete understanding of this new technique requires considerable command over the principles of quantum mechanical energy exchange but the underlying principle can be discussed in general terms.

In PFT technique all nuclei of the sample are elevated to excited state through irradiation by short intense rf pulses.
During the free induction decay of the high energy state the transverse magnetisation vector has a phase shift of $\pi/2$ relative to rf field $H_1$. Owing to appropriate experimental arrangement induction current stemming from the decay of this magnetisation vector is built up in receiver coil following the rf pulse. Now if the Larmor frequency of the nuclei differs from the rf, the magnetisation vector periodically rephases and dephases with applied magnetic field resulting in a pulse interferogram which is then analysed through Fourier transform. When one compares the CW and FT spectra of the same sample at low concentration, the difference is striking.

In $^{13}$C NMR spectroscopy coupling of the protons to $^{13}$C nuclei gives rise to complicated spectra which are simplified through double resonance which involves irradiation of the sample not only by rf frequency $H_1$, at resonance with nuclei to be observed, but additionally with a second alternating field $H_2$, at resonance with the nuclei to be decoupled. If $H_2$ is such that it covers the whole range of protons, the spectrum is described as proton broad band or noise decoupled. In it the resonances of individual carbons appear as singlets the position of which i.e. their chemical shift, depend on their environment. No information is, however, available from such spectra on whether the carbon atom is primary, secondary or tertiary. In order to know this single frequency off-resonance decoupling is applied. In it the perturbing field $H_2$ is not at resonance with the nuclei $X$ of
an A–X system but is so adjusted that the coupling constant $J_{AX}$ is larger than zero but smaller than $J_{AX}$. By proper adjustment of $H_2$ the multiplets are so narrowed that no, or only slight overlap occurs. Such spectra are normally obtained along with broad band spectra to facilitate assignments.

**Chemical Shifts**

The factors which influence $^{13}$C chemical shifts are not always the same as for protons though a broad correspondence does exist. In this context one might usefully compare the effect of ring current, electron withdrawing and electron donating substituents on neighbouring protons and carbons. It is well known that ring current, as in aromatic compounds, and circulating π electrons, such as in acetylene and carbonyl compounds, generate magnetic fields which oppose or enhance the applied field in different regions of the molecule. Due to this interaction acetylenic hydrogens are shielded whereas the aldehydic and benzenoid hydrogens are deshielded.

Ring current is not of much importance in determining $^{13}$C chemical shifts and benzylic methyl is only slightly deshielded as evident from comparison of methyl cyclohexene (V) with toluene (VI). Similarly the methylene carbon facing the ring in ansa compounds is shifted upfield by only 0.7 ppm. The acetylenic carbons appear, however, upfield (63–88 ppm) from
olefinic carbons and the methyl carbons of ketones are also at about the same value as the olefinic methyl.

\[ \text{[V]} \]

\[ \text{[VI]} \]

It is thus apparent that $^{13}$C spectra cannot unequivocally differentiate between allylic and ordinary carbons but electron withdrawal as expected leads to deshielding of \( \alpha \)-carbons. This is borne out by measurement of shifts of halogen substituted alkanes. It is surprising, however, that the effect is not transmitted to \( \beta \), \( \gamma \) and \( \delta \) positions with decreasing intensity, as one would expect, instead the \( \gamma \)-carbon is shielded. Probably factors other than inductive effect are involved.

Crowding by alkyl substituents also causes deshielding. The methyl carbons of ethane resonating at 5.7, the methylene carbons of isopropane at 15.4, the methine of neopentane at 24.3 and the totally substituted carbon of tetra-methyl methane at 31.4. The carbons of the carbonium ions are the most deshielded, trimethyl carbonium ion ([VII]) resonating at 330 ppm and the resonance stabilised carbocations ([VIIa]) and ([VIIb]) at 248 and
256 ppm respectively. Much use of this has been made in work on controversial non-classical carbonium ion.

Unshared electrons, as in carbon monoxide (VIII) and nitrile ion (IX), bring out deshielding as shown by comparisons of carbon dioxide (X) with carbon monoxide and of methylcyanide (XI) with HCN. Mesomeric effects operate in the same way as in the case of protons and the effect of introducing electron donating and electron withdrawing substituents on various sites of the benzene molecule is given below:
Consideration of the chemical shifts given above shows that benzenoid carbon carrying substituents is the most affected and that this cannot be attributed to inductive effect alone, is evident from the chemical shift of C-1 of toluene (VI). The ortho and para effects are not sharp as they are in the case of protons and the importance of electric fields in the vicinity of the carbon atom is shown by shielding as against the expected deshielding of C-2 of nitrobenzene (XII). Most important from structural viewpoint is the extreme deshielding of carbonyl carbon. In simple ketones (XIII) it appears at 201.5, introduction of α,β-unsaturation leads to shielding by about 10 ppm (XIV) and hydrogen bonding brings a further shift of about 5-8 ppm (XV, XVI).
It is upsetting to find the carbonyl carbon at 180.1 ppm in carboxylic acids (XVII) as one would have expected the predominant inductive effect of the hydroxy oxygen to lead to further deshielding. The olefinic carbons are deshielded through conjugation but $\alpha$ and $\beta$ positions remain undifferentiated whereas in pmr spectra the $\alpha$ proton suffers much greater deshielding.

**Coupling Constants**

Because of the low natural abundance of $^{13}$C carbon–carbon coupling is not detectable in the spectra i.e. it is obliterated by noise. Carbon–hydrogen couplings of the A–X type depend on bond hybridisation. For Sp$^3$ hybridised carbon it is 125 Hz, for Sp$^2$ 156 Hz and for Sp 249 Hz. In short the greater
the 'S character of carbon, the larger the coupling constant. Coupling constants are also effected by the nature of substituents and the degree of substitution. Thus C-H coupling in CH$_3$X is about 140 Hz and in CHX$_3$ it is 180 Hz, for X = OCH$_3$. The effect is more pronounced in fluorides i.e. CH$_3$X, it is 150 Hz and CHX$_3$ (240 Hz) for X = F.

The above coupling constants are not to be confused with the coupling constants in off resonance spectra which depend on the rf employed to bring about decoupling. This rf is so adjusted as to provide the best resolution and the residual coupling is of value only in determining the number of hydrogen atoms.

**Mass Spectroscopy**

Recently mass spectrometry has been successfully employed for the structure elucidation of mono and biflavonoids$^{61,68-72}$ and a conclusive structure fragmentation pattern relationship established. Since most of the naturally occurring flavanoids and biflavonoids possess at least 5,7,4'-hydroxylatation pattern. The present discussion is mainly centred at such compounds.
Flavones

The principal modes of fragmentation in flavones involve (i) fission of the heterocyclic ring via reverse Diels-Alder reaction (ii) loss of carbon monoxide from molecular or fragment ion. This is illustrated in scheme (I) for an unsubstituted flavone \(^{75}\) (XVIII). Apigenin \(^{71}\) (XIX) has the parent molecule ion as base peak which loses a molecule of carbon monoxide to give a major fragment ion m/e 242 (XX). Fragment ions in much less abundance correspond to RDA fission in the heterocyclic ring (Scheme II).

By comparison of the mass spectrum of an unsubstituted flavone with a highly oxygenated flavone (apigenin), it is observed that fragmentation via RDA reaction is less favoured in the latter. This is due to stabilisation of the initially produced ion radical by mesomerism over a number of oxygen atoms. These minor break downs may still prove to be of diagnostic value as they frequently represent the only even numbered peaks in their particular region and hence are readily distinguished.

In case of apigenin trimethyl ether (XXI)\(^{73-77}\) the molecular ion appears as the base peak. Further fragmentation of the molecular ion via RDA process yields the ketone m/e 180 (XXII) and the acetylene m/e 132 (XXIII) (Route I) and the carbonyl ion, m/e 135 (XXIV) (Route II) Scheme III.
SCHEME - (I)
SCHEME-(II)
SCHEME - (III)
Flavanones

In the case of flavanone (XXV), fragmentation by path A (RDA fission of the heterocyclic ring) and path B are of great importance as they lead to clean out, characteristic spectra. Another method of breakdown that helps to characterise the flavanone is the loss of either a hydrogen atom (XXVI) or an aryl radical at C-2 (XXVII) from the molecular ion to give even electron fragments.
SCHEME-(IV)
These fragmentation processes are illustrated in the case of 4'-methoxy flavanone (XXVIII) (Scheme-IV).

The fragment with methoxyl group takes nearly all the charge. A further peak is at m/e 108 (XXIX) arising from a hydrogen transfer reaction.

\[
\begin{align*}
&\text{(XXVIII)} \\
\rightarrow &\text{m/e} 108^{(10)} \\
&\text{(XXIX)}
\end{align*}
\]

The presence of a hydroxyl (XXX) or methoxyl group at C-4 position of ring B facilitates, by enhanced resonance stabilisation of the resulting fragment ion, the formation of $\beta$-hydroxy benzyl (XXXI) or $\beta$-methoxy benzyl ion respectively (or their equivalent tropolium ions). These ions appear as peaks of significant intensity in the mass spectrum of naringenin/its trimethyl ether\(^{69,71}\).

\[
\begin{align*}
&\text{(XXX)} \\
\rightarrow &\text{m/e} 107 \\
&\text{(XXXI)}
\end{align*}
\]
SCHEME (V)
The mass spectrum of 3,5,7-trihydroxy-4′-methoxy flavanone (XXXII) is of particular interest, as the base peak is neither the molecular ion nor a fragment arising from breakdown via path A (Scheme-V).

Biflavones

In biphenyl ether type biflavones, molecular ion is usually the base peak72. Apart from the fragmentation processes mentioned for apigenin trimethyl ether, these compounds also undergo (i) fission of the C–C or C–O–C linkage between the aromatic residues (ii) elimination of CO (28) or CHO (29) from the biphenyl ethers and (iii) rearrangements involving condensation between the phenyl rings. Steric factors seem to play an important role in influencing the breakdown mode and internal condensations. Formation of doubly charged ions is frequently observed.

The mass spectra of amentoflavone hexamethyl ether and cupressuflavone hexamethyl ether are similar, molecular ion being the base peak in each case. Difference lies in the intensities of the corresponding peaks due to variation in substitution and steric factors. The main peaks in the mass spectrum of amentoflavone hexamethyl ether (XXXIII) are given below (Scheme-VI).
SCHEME (VI)
Amentoflavone hexamethyl ether (XXXIII)

622 (100), 621 (13), 607 (33), 592 (8), 576 (10), 312 (2), 311 (5), 245 (5), 181 (2), 180 (3), 155 (16) and 132 (3).

The mass spectral studies of the biflavonoids isolated from natural sources reveal that their fragmentation patterns depend not only on constituent monomeric flavanoid units but also on the nature and the position of interflavanoid linkage.73