CHAPTER 1
INTRODUCTION
Plants have always been common source of medication either in the form of traditional preparations or as pure active principles. In a survey done by WHO it has been estimated that 80% of more than 4000 million inhabitants of the World rely chiefly on traditional medicines for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts of their active principles. In the developed countries too, plants derived drugs are important. In USA, for example, 25% of all prescriptions dispensed from community pharmacies, contain plant extracts or active principles prepared from higher plants.

It is mainly during the last 100 years that some of the active ingredients present in herbal prescriptions have been isolated and introduced into ‘modern’ medicine. Farnsworth et al. pointed out in their review article that there are at least 119 distinct chemical substances derived from plants that can be considered as important drugs currently in use. A few of the drugs are simple synthetic modifications of naturally occurring substances. In some instances, the natural products have now been replaced by commercially available synthetic products. Thus the drugs derived from plants still occupy an important position.

A recent survey of the literature showed that the flavonoid field is still very popular with the chemists and their interest is increasing in isolating new flavonoids and their physiological activity. A large number of naturally occurring new and novel flavonoids are added to the literature every year. Few of the recently isolated flavonoids are listed for ready reference to the studies reported in the thesis.

1. Isoflavone

K.S. Krishnaveni et al., have isolated a new isoflavone from the heartwood of Pterocarpus santalinus. Based on spectral methods, the structure of the new compound was elucidated as 6-hydroxy,7,2',4',5'-tetramethoxyisoflavone(I).
2. **Methylenedioxyisoflavane and prenylatedisoflavanone:**

Gomostsang Bojase et al.\(^1\), have isolated three new flavonoids from the methanolic extract of the stem of *Bolusanthus speciosus*.

The structures of these compounds were determined as 4, 2', 3', 4'-tetrahydroxy-6, 7-methylenedioxyisoflavane (bolusanthol A) (II), 5, 7, 3', 4'-tetrahydroxy-5'-γ,γ-dimethylallylisoflavone (bolusanthol B), (III) and 5, 7, 4'-trihydroxy-6, 3'-di (γ, γ-dimethylallyl)isoflavane (bolusanthol C) (IV) by spectroscopic methods.

![Structure of bolusanthol A (II)]

III : \( R_1 = H; R_2 = H; R_3 = H; R_4 = OH; R_5 = \text{Prenyl} \).

IV : \( R_1 = \text{Prenyl}; R_2 = H; R_3 = H; R_4 = \text{Prenyl}; R_5 = H \).

3. **Isoflavone glycoside:**

F.N. Ngounou et al.\(^1\), have isolated a new isoflavone glycoside, *Pentandrin glucoside* (V), from methanolic extract of the stem barks of *Ceiba Pentandra*.

![Structure of Pentandrin glucoside (V)]
Salwa F. Farag et al.\textsuperscript{20}, have reported two new isoflavonoids, tectorigenin 4'-O-\(\beta\)-D-glucopyranosyl(1→6)-\(\beta\)-D-glucopyranoside (VI), iristectorigenin B 7-O-\(\beta\)-D-glucopyranosyl(1→6)-\(\beta\)-D-glucopyranoside (VII) from rhizomes of \textit{Iris carthaliniae}.

\[
\begin{align*}
\text{VI: } R_1 &= H; R_2 = \text{Glc-Glc}; R_3 = H \\
\text{VII: } R_1 &= \text{Glc-Glc}; R_2 = H; R_3 = \text{OMe}
\end{align*}
\]

4. \textbf{Bi and tetraflavonoids:}

Fernando J. C. Carneiro et al.\textsuperscript{21}, have isolated two bi- and one tetraflavonoid from stems of \textit{Aristolochia ridicula} and identified them as 5, 5", 7", 4"'-tetrahydroxy-7, 4', 3"'-trimethoxy-3,6"'-biflavone (VIII), 7, 7", 5", 4"'-tetrahydroxy-7, 4', 3"'-trimethoxy-3, 6"'-biflavone (IX), (7,5", 4"'-trihydroxy-5, 4', 7"'-trimethoxy-3, 6"'-biflavone)-3"'-O-4"'-\(\text{OMe}\) (V, 5", 7"'-trihydroxy-3', 4', 3"'-trimethoxy-6-O-\(\beta\)-7-\(\alpha\)-flavone-chalcone) (X).

\[
\begin{align*}
\text{VIII: } R_1 &= \text{OMe} \\
&\quad \text{MeO} \\
&\quad \text{OMe} \\
&\quad \text{OMe} \\
&\quad \text{OH}
\end{align*}
\]
5. **Methylaurones:**

Rosa M. Seabra et al.\textsuperscript{22}, have isolated methylaurones from *Cyperus capitatus* and identified them as 4,6,3',4'-tetrahydroxy-5-methylaurone (XI), 4,6,3',4'-tetrahydroxy-7-methylaurone (XII) and 6,3',4'-trihydroxy-4-methoxy-5-methylaurone (XIII), along with previously isolated methylaurone which was revised and shown to be 6,3',4'-trihydroxy-4-methoxy-7-methylaurone (XIV).

![Chemical structures of methylaurones](image)

\[
\begin{align*}
\text{XI: } & R_2 = \text{Me}; R_1 = R_3 = R_4 = \text{H} \\
\text{XII: } & R_1 = \text{Me}; R_2 = R_3 = R_4 = \text{H} \\
\text{XIII: } & R_2 = R_3 = \text{Me}; R_1 = R_4 = \text{H} \\
\text{XIV: } & R_1 = R_3 = \text{Me}, R_2 = R_4 = \text{H}
\end{align*}
\]
6. **Prenylated biaurone:**

Yoshihisa Asada et al.\(^1\), have isolated a new prenylated biaurone (XV) from the hairy root culture of *Glycyrrhiza glabra* named as licoagrone.

![Prenylated biaurone structure](image)

7. **Coumarin:**

Maryam H. Al Yousuf et al.\(^2\), have isolated four coumarins from acetone extract of *Leucas inflata* roots.

![Coumarin structure](image)

- XVI: \( R_1=\text{CH}_3;\ R_2=\text{H};\ R_3=\text{CH}_3 \)
- XVII: \( R_1=\text{CH}_3;\ R_2=\text{OCH}_3;\ R_3=\text{CH}_3 \)
- XVIII: \( R_1=\text{CH}_3;\ R_2=\text{H};\ R_3=\text{H} \)
- XIX: \( R_1=\text{CHO};\ R_2=\text{OCH}_3;\ R_3=\text{CH}_3 \)

8. **Dicoumarin glycoside:**

Nisar Ullah et al.\(^3\), have isolated dicoumarin glycoside from methanolic extract of whole plant of *Daphne oleoides*. Its structure was established as 6, 7-dihydroxy-3-
methoxy-8-[2-oxo-2H-1-benzopyran-7-(O-β-D-glucopyranosyl)-8-yl]-2H-1-benzopyran-2-one (XX).
In the present study, we have tried to carry out systematic chemical investigations of some important medicinal plants with a view to characterise their chemical components preferably flavonoids, which could be the starting point for chemists who are mainly concerned with pharmacological and clinical aspects of the herbal drugs.

Since mainly the spectroscopic techniques, uv, ir, $^1$H-nmr, $^{13}$C-nmr and mass have been used in the identification and structure elucidation of the products isolated from different plants during the course of this work, a short review of each technique has been briefly discussed:

1. **Infra-Red Spectroscopy:**

   The ir spectrum in practice, plays an important role and offers the first clue to the nature of the compound. It provides a valuable information of functional groups in a molecule.

   In case of furanocoumarins (psoralens) (XXI) two or three bands of weak to medium intensity have been observed in the region 3025-3175 cm$^{-1}$. These absorptions are due to the C-H stretching vibrations of the pyrone, benzene and furan rings. The pyrone-carbonyl stretching frequency of coumarin is usually found in the region 1700-1750 cm$^{-1}$.

   Perel'son$^{27}$ noted in the spectra of furanocoumarins that a strong sharp band appears at 1613-1639 cm$^{-1}$ (which was ascribed to the C=C stretching mode of the furan ring) in addition to the aromatic bands at ~1500 cm$^{-1}$ and ~1600 cm$^{-1}$.

   ![XXI](image)

   Psoralene substituted at C-5 gives rise to twin bands in the region 1600-1625 cm$^{-1}$ and can be differentiated from C-8 substituted isomers which exhibit weak absorption between 1620 cm$^{-1}$ and 1625 cm$^{-1}$.\textsuperscript{28,29}

   Two bands found in the region 1088-1109 cm$^{-1}$ and 1253-1274 cm$^{-1}$ in the spectra of furanocoumarins are considered to be characteristic C–O stretching vibrations for the furan group.\textsuperscript{27}
Furanocoumarins have also found to exhibit bands in the 740-760 and 870-885 cm$^{-1}$ regions which ascribed to the in-plane and out-of-plane deformations, respectively, of the furan C-H bonds.

In the case of flavonoids, IR measurements are helpful in providing evidence for the presence of (a) pyrone ring (b) chelated hydroxyl groups and (c) the gem dimethyl groupings. The substitution pattern of the benzene ring can be inferred from bands in the 690-800 cm$^{-1}$ region. Such evidence is helpful in distinguishing between flavonoids and coumarins. IR spectroscopy has mostly been used to adduce corroborative evidences.

The IR spectra of flavones show the carbonyl band at 1660 cm$^{-1}$ owing to the conjugation with olefinic double bond. Introduction of a hydroxyl group at 5-position does not alter the band position appreciably. Luteolin and apigenin show the carbonyl bands at 1655 and 1650 cm$^{-1}$ respectively.

The IR spectrum of unsubstituted flavanone shows the carbonyl absorption at 1680 cm$^{-1}$, the standard value for aromatic ketones. The shift of carbonyl band to 1620 cm$^{-1}$ in 5-OH flavanone is largely due to chelation. Consequently, methylation of the 5-OH produces only a small frequency shift. The existence of chelation is, however, clearly demonstrated by the absence of the hydroxyl bands at the usual position in 5-hydroxy compounds. Apparently it comes to lie in the –OH stretching region and is thus obliterated. A similar high frequency shift of 4'-substituted flavanone is attributed to intermolecular hydrogen bonding. The IR spectra of isoflavones are similar to those of flavones. Another interesting feature of the IR spectra of flavones is that the carbonyl frequency is independent of the substitution pattern in ring-A & -B and is affected only by the introduction of a hydroxyl at 3-position.

The infrared spectra of triterpenes have got much resemblance with the spectra of the steroids. However in C-3 ketones of 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-oxo steroids 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 bonds, ethylenic double bonds and the equatorial or axial nature of the hydroxyl groups, in triterpenic compounds in the IR region.
As a result of infrared spectroscopic studies it might be possible to make a distinction between tertiary equatorial (3613 cm\(^{-1}\)) and tertiary axial (3617 cm\(^{-1}\)) hydroxyl groups, on this basis the band at 3629 cm\(^{-1}\) (CCl\(_4\)) in methyl melaleucate\(^{37}\) (XXII) has been assigned as equatorial secondary, while its 3-\(\alpha\) epimer, obtained by oxidations of the alcohol (XXII) and subsequent reduction gives a band at 3636 cm\(^{-1}\) due to axial secondary nature of OH group.

![XXII](image)

2. **Ultra-Violet Spectroscopy:**

Linear furanocoumarins (Psoralens) show four zones of absorption at 205-225, 240-255, 260-270, and 298-316 nm\(^{38}\). Angular furanocoumarins (angelicins) (XXIII) can readily be distinguished from psoralens since the maxima at 242-245 and 260-270 nm which are characteristic of the linear series are absent.\(^{26}\)

Psoralens mono-oxygenated at C-5 or C-8 gave spectra which are different from those of 5, 8-dioxygenated psoralens. Psoralens mono-oxygenated at C-5 or C-8 show maxima at \(\sim\) 260 nm and minima at \(\sim\) 277 nm, which are absent in spectra of dioxygenated compounds.

Psoralens mono-oxygenated at C-5 or C-8 can also be readily differentiated by their U.V. spectra. The former show maxima at \(\sim\) 268 nm and minima at \(\sim\) 245 nm, which is absent in the later. Moreover, the former show maxima at \(\sim\) 310 nm and the later at \(\sim\) 300.\(^{26}\)

![XXIII](image)
UV spectroscopy has become a major technique for the structure analysis of flavonoids for two main reasons. The first is that only a small quantity of pure material is required. The second reason is that the structural information obtained about flavonoids from UV is considerably enhanced by the use of specific reagents which react with one or more functional groups on the flavonoid nucleus. The addition of these reagents separately to an alcoholic solution of the flavonoid, induces structurally significant shifts in the UV spectrum. The commonly used shifts reagents\(^9\) are sodium methoxide (NaOMe), sodium acetate (NaOAc), sodium acetate / boric acid (NaOAc / H\(_3\)BO\(_3\)), aluminium chloride (AlCl\(_3\)) and aluminium chloride/hydrochloric acid (AlCl\(_3\)/HCl).

The UV spectra of most flavonoids consist of two major absorption maxima, one of which occurs in the range 240-285 nm (band II) associated with ring-A benzoyl system (XXIV) and second at a higher wave length (band I), occurs in the range of 320-380 nm associated with ring-B cinnamoyl absorption (XXV).\(^9\)

![XXIV](image1)

![XXV](image2)

Substitution in ring-B specially at 4' stabilizes the cinnamoyl chromophore resulting in a red shift of band I whereas, substitution in ring-A has a similar effect on the position of band II. The presence of a free hydroxyl group at C-5 and C-3 positions is established by measuring the spectra in the presence of AlCl\(_3\).\(^9\) Compounds having a free 5-hydroxyl group absorb at higher wave length and methylation of this hydroxyl group brings about a blue shift of 1-15 nm of both bands. The hydroxyl groups at C-7 and 4' positions are more acidic than others and their occurrence is established by red shifts of band I & band II on the addition of fused sodium acetate.\(^9\) The presence of hydroxyl group at 4' position is also confirmed by a large red shift in band I without a decrease in intensity on the addition of sodium methoxide.\(^40\) The presence of orthodihydroxy groups in ring-A and ring-B is indicated by a red shift in band I in the presence of AlCl\(_3\)/HCl and sodium acetate/boric acid respectively.
In flavanones and isoflavones, due to the absence of cinnamoyl chromophore the high wave length band is either totally absent or present only as an inflection. Thus it is difficult to distinguish between flavanones and isoflavones with the help of uv spectrum alone. The ultra-violet spectra of biflavonoids are very similar to those of monoflavonoids with the only difference that the molecular extinction coefficient (ε) of the biflavonoids is approximately double as compared to the corresponding monoflavonoids. This demonstrates the presence of two isolated chromophores of flavonoids per molecule of biflavonoids.

3. Nuclear Magnetic Resonance (1H-nmr) Spectroscopy:

For coumarins a pair of doublets centered at δ 6.1-6.4 and δ 7.5-8.3 (J=9.5 Hz) in 1H-nmr spectrum indicates the unsubstituted pyrone ring in coumarins. These characteristic signals arise from H-3 and H-4 protons respectively.

The presence of an unsubstituted furan ring (in the furanocoumarins) is easily recognizable from the pair of doublets, J~2.5 Hz, which arise from H-2' and H-3'. The signals from the former resonate at δ ~7.5-7.7 while the later are found at δ 6.7 in the linear series and at δ ~7.0 in angular furanocoumarins.41-44

Naturally occurring pyranocoumarins (XXVI) are invariably geminally substituted at C-2' with methyl groups which resonate as a six protons singlet at δ 1.45. The two olefinic protons show as a pair of doublet, J=10 Hz, centered at δ 5.3-5.8 (H-3') at 6.3-6.9 (H-4').44

Since flavonoid compounds contain, in general, very few protons, nuclear magnetic resonance spectroscopy is a useful tool in the structural elucidation of this class of compounds. By the use of 1H-nmr studies of silyl derivatives,45 double irradiation techniques46a, solvent induced shift studies46b-48 and lanthanide induced shift studies49 (LIS), one can come to the structure of flavonoids without tedious and time consuming chemical degradation and synthesis.
The valuable contributions in this field have been made by Batterham & Higuet, Mabry, Massicot, Clark-Lewis, Kawano, Pelter and Rahman. ¹H-nmr spectroscopy is highly helpful in determining the substitution pattern of flavonoids. The most commonly occurring hydroxylation pattern in natural flavonoids is 5, 7, 4'-trihydroxy system (XXVII)

¹H-NMR signals in trimethyl silylated flavonoids normally occur between 0 and 9 ppm. The chemical shifts of the protons of ring-A & B prove to be independent of each other but are affected by the nature of ring-C

The signals arising from ring-A protons in most flavonoids occur upfield from those of ring-B protons, and are readily recognized. Different types of substitution in ring-A among the flavonoids and their effects on the proton signals can be discussed as follows:

(i) **H-5, H-6 and H-8 signals in 7-oxygenated flavonoids:**

The additional C-5 proton in these compounds is strongly deshielded by the 4-keto group and its signal appears at a very low field (δ 8.0). It appears as a doublet (J=9 Hz) due to ortho coupling with H-6. The signals for H-6, a d,d (q, J=9 Hz and 2.5 Hz) and for H-8, a doublet (d, J=2.5 Hz) occur at lower field than in the 5, 7-dihydroxy flavonoids and may even reverse their positions relative to one another.

(ii) **H-6 and H-8 signal in 5,7-dioxygenated flavonoids:**

The two ring-A protons, H-6 and H-8 give rise to two doublets (J=2.5 Hz) in the range δ 5.7-6.9 in flavones, flavonols, and isoflavones. The H-8 doublet occurs consistently downfield than the signal for H-6. The doublets for H-8 and H-6 are also clearly distinguished from each other by their widely different paramagnetic induced shifts. Depending upon the nature of the substituents, the chemical shifts may vary.
accordingly. For instance when a sugar is attached to the oxygen at C-7, the signal for both H-6 and H-8 are shifted downfield.

(iii) **H-6/H-8 signal in 5, 7, 8/5,6,7-trisubstituted flavonoids:**

$^1$H-NMR provides the requisite information for differentiating 6 or 8 substituted isomers of 5,7,8 / 5,6,7-trisubstituted flavonoids with a high degree of surety. Horowitz & Gentili$^{38}$ were able to fix up the structure for the two isomers of vitexin, viz. vitexin and isovitexin. The H-6 proton signal appears at about δ 0.2-0.3 upfield than H-8 signal.

All ring-B protons appear around δ 6.7-7.9, a region separate from the usual ring-A protons. The signal for the aromatic protons of an unsubstituted ring-B in a flavone appears as a broad peak centered at about δ 7.45. The presence of ring-C double bond causes a shift of 2', 6'-protons and the spectrum shows two broad peaks, one centered at ≈ δ 8.00 (2', 6') and the other at ≈ δ 7.6 (3',4',5')$^{49}$. The presence of substitution in one or more positions causes a distinct change.

(i) **H-2', 6' & H-3', 5' signals in 4' oxygenated flavonoids:**

With the introduction of 4'-hydroxyl group, the ring-B protons appear as a typical four peaks pattern of two doublets called A$_2$B$_2$ pattern (J=8 Hz, each). The H-3' and H-5' doublet always occurs upfield as compared to the H-2',6' doublet. This is attributed to shielding effect of the oxygen substituent and to the deshielding influence of ring-C functions on H-2' and H-6'. The position of H-2' and H-6' signal also depends to some extent on the oxidation level of ring-C.

(ii) **H-2', H-5' and H-6' signals in 3', 4' -dioxygenated flavonoids:**

The $^1$H-nmr spectrum of 3',4'-dioxygenated flavonoids gives the normal ABX pattern. The H-5' proton in flavones and flavonols in such system appears as a doublet centered between δ 6.7 and 7.1 (J=8 Hz) and the H-2' and H-6' signals which often overlap, usually between δ 7.2 and 7.9.

(iii) **H-2' and H-6'signals in 3', 4', 5'-trioxygenated flavonoids:**

In 3', 4', 5'-trihydroxylated flavonoids H-2' and H-6' are equivalent and appear as a two protons singlet in the range δ 6.5-7.5. Methylation shifts the signal to downfield by about 1 ppm when the compound is analysed in DMSO-d$_6$. 
(iv) **H-2 and H-3 signals in flavanones and flavanonols:**

The spectra of flavanones (saturated heterocyclic ring) contain typical ABX pattern multiplets arising from the C-2 proton and the two C-3 protons. The C-2 proton is split by the C-3 protons into a doublet of doublet ($J_{cis}=5$ Hz, $J_{trans}=11$ Hz) and occurs near $\delta 5.2$, the precise position depending on the substitution of ring-B. The two C-3 protons occur as two quartets ($J_{H-3a, H-3b}=17$ Hz) at $\delta 3.0$. However, they often occur as two doublets, since two signals of each quartet are of low intensity.

The C-2 proton in dihydroflavonols appears near $\delta 4.9$ as a doublet ($J=11$ Hz) coupled to the C-3 proton which appears at about $\delta 4.2$ as doublet.$^{39}$

**Hydroxy protons:**

The position of hydroxyl groups in flavonoids cannot be detected by $^1$H-nmr spectra of their trimethylsilylated derivatives and thus can't be used for their detection. The $^1$H-nmr spectra of parent compound in DMSO-$d_6$, however, can give good information for the detection of phenolic hydroxyl protons. The hydroxyl protons of 3,5,7-trihydroxyflavone give three signals at $\delta 12.40$ (5-OH), $\delta 10.93$ (7-OH) and $\delta 9.70$ (3-OH).$^{39}$

**Sugar protons:**

The sugar protons in the flavone glycosides are denoted as C-1", C-2" protons and so on, while the protons of the terminal sugar in disaccharides are designated as C-1"", C-2"" protons and so on. In the $^1$H-nmr spectra of TMS derivatives of the glycosides, the non-anomeric protons resonate between $\delta 2.9-4.3$, while the anomeric protons resonate between $\delta 4.3-5.8$. The axial anomeric protons are observed between $\delta 4.3-5.0$ and the equatorial anomeric protons between $\delta 4.7-5.8$.

The chemical shift of the C-1" protons of the sugar directly attached to the flavonoids hydroxyl group depends both on the nature of the flavonoid and on the position and stereochemistry of the attachment. For instance, in flavone glycosides with sugar on either C-5, C-7 or C-4', the C-1" proton signal appears near $\delta 5.0$, while in flavonols 3-0-glycosides the C-1" proton signal appears much more downfield i.e. at about $\delta 5.8$. The coupling constant of C-1" proton with C-2" proton in $\beta$-linked glycosides is about 7 Hz$^{39}$, due to diaxial coupling. In the naturally occurring $\alpha$-linked rhamnosides, the diquotorial coupling between H-1" and H-2" gives rise to a coupling
constant of only 2 Hz. The rhamnose C-methyl appears as a doublet (J=6.5 Hz) or a multiplet in the region δ 0.8-1.2.

In flavonoid diglycosides, the C-1" proton of the terminal sugar (H-1"") being relatively remote from the flavonoid nucleus, resonates upfield from H-1". The extent, however, can vary depending upon the position of attachment of terminal sugar. Methylated and acetylated derivatives have also been used for disaccharide linkage determinations.

**Acetoxyl and Methoxyl protons:**

In the $^1$H-nmr spectra of acetylated flavonoids (CDCl₃), the position of methyl signals of acetyl groups can also give useful information about the position of acetyl group by which the position of the hydroxyl groups can be confirmed. The methyl signals of 4' and 7-O-acetyl groups appear in the range of δ 2.30-2.35. While the methyl signal of a 5-O-acetyl group appears at about δ 2.45. The aliphatic acetoxyl signals of sugars generally appear in the range of δ 1.65-2.10. The position of the aliphatic acetoxyl groups of sugars also help in the location of sugar moiety in C-glycoxyl flavonoids. Within the aliphatic acetoxyl group signals, the 2"-O-acetyl signal appears at δ 1.70-1.75 in 8-C-glycosylflavonoids and δ 1.80-1.83 in 6-C-glycosylflavonoids and 6"-O-acetyl signal in 8-C-glycoxyl flavonoids appears at δ 1.90-1.95 while in 6-C-glycosylflavonoids it appears between δ 1.98-2.04. Methoxyl protons signals, with few exceptions appear in the range of δ 3.5-4.1.

(4) **$^{13}$C-NMR Spectroscopy:**

$^{13}$C-NMR spectroscopy has been used in natural product chemistry in variety of ways at various stages of the structure determination. $^{13}$C-NMR spectral data furnish key informations such as the number of carbon atoms and establish if they are primary, secondary, tertiary, aromatic, olefinic or part of functional groups.

The $^{13}$C-nmr spectra of flavonoids and their glycosides are of some interest in the context of compounds isolated during the course of this work. The spectra can be analysed by reference to those of simple compounds such as acetophenones and cinnamic acids which possess structural features characteristic of flavonoids.

It is worthwhile to see how introduction of oxygen at various positions of these, affects the chemical shifts. In hydroxy acetophenone (XXVIII) the nuclear carbon linked directly to oxygen of hydroxyl group gives rise to a singlet at δ 161.5 and the two
Adjacent carbons give two singlets at $\delta 118.0$. The carbon para to the carbonyl is the most deshielded and its singlet appears at $\delta 135.5$. In 2,6-dihydroxy acetophenone (XXIX) the carbon bonded directly to oxygen give rise to singlet at $\delta 161.4$ and the two adjacent carbons produce singlet at $\delta 106.5$. The meta carbon which is para to the acetyl group is deshielded and its singlet appears at $\delta 134.0$.

Thus, chemical shifts correlate to those for protons on these carbons, the protons ortho and para to hydroxyl being shielded more than the one at meta positions and protons para and ortho to carbonyl being the ones most exposed to the deshielding influence of the carbonyl group. In 2, 4, 6-trihydroxy acetophenone (XXX) the oxygenated nuclear carbons show singlet at $165.10$ ppm while in dihydroxy acetophenone (XXIX) it is $\delta 161.4$. This slight deshielding of $\delta 3.70$ can be attributed to the hydroxyl group at meta position. The unsubstituted carbons 3 & 5 are shielded due to enhanced mesomeric effect and their signals appear at $\delta 94.5$. These effects can be assumed to be general and are relied upon in making assignments in flavonoid spectra. The other structural unit of flavonoids is akin to cinnamic acid and the $^{13}$C-nmr chemical shifts of cinnamic acid derivatives are, therefore, of interest. The chemical shifts of the parent cinnamic acid and its mono, di and trisubstituted derivatives are indicated in the structure (XXXI, XXXII, XXXIII, XXXIV).
The 3, 4-type of substitution is the one most commonly encountered in flavones and chemical shifts of carbon 3 and 4 of 3-methoxy 4-hydroxy cinnamic acid (XXXIII) $\delta$ 149.11 and $\delta$ 149.78 respectively are substantially different from those of carbons under oxygen in acetophenone. This makes it possible to distinguish between oxygenated ring-A and B carbons of flavones. The carbons ortho and meta to phenolic hydroxyls are shielded, compared to unsubstituted benzene and appear at $\delta$ 112.28 and $\delta$ 116.89, the cinnamic acid double bond causing a further shift of C-2 resonance. Carbon-1 adjacent to the olefinic double bond of cinnamic acid is almost at the same value as in substituted benzene but different in unsubstituted benzene. The $\alpha$-carbon appears at $\delta$ 116.7 and the $\beta$-carbon at $\delta$ 146.92. In trisubstituted benzene (XXXIV), the carbons attached to oxygen are further shielded and in 3,5-dimethoxy 4-hydroxy cinnamic acid appear at $\delta$ 149.17, $\delta$ 138.79 respectively. The carbon bearing hydroxyl is shielded to a greater extent because of resonance contribution from the flanking methoxyl groups. The same type of resonance effect is responsible for the shielding of 2 and 6 carbons.

The chemical shifts of flavones, substituted flavones and isoflavones are reproduced\textsuperscript{63} in the following table.
Chemical shift (in $\delta$ downfield from T.M.S.)

<table>
<thead>
<tr>
<th>Carbon Number</th>
<th>Flavone</th>
<th>7-methoxy flavone</th>
<th>5-hydroxy flavone</th>
<th>5,7,3',4'-tetrahydroxy Flavones</th>
<th>7-methoxy isoflavone</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>163.2</td>
<td>162.6</td>
<td>164.07</td>
<td>165.07</td>
<td>152.4</td>
</tr>
<tr>
<td>3.</td>
<td>107.6</td>
<td>107.2</td>
<td>105.61</td>
<td>103.94</td>
<td>125.1</td>
</tr>
<tr>
<td>4.</td>
<td>178.4</td>
<td>177.4</td>
<td>182.90</td>
<td>182.63</td>
<td>175.3</td>
</tr>
<tr>
<td>5.</td>
<td>125.2</td>
<td>126.7</td>
<td>155.85</td>
<td>158.24</td>
<td>127.6</td>
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(5) Mass Spectrometry

The introduction of inlet system suitable for volatilisation of high molecular weight ($M^+$, 300-1200) organic materials has greatly increased the utility of mass spectrometry. The presence of furan ring in furanocoumarins does not alter the fundamental fragmentation process observed for structurally simple coumarins (elimination of CO from pyrone ring).\textsuperscript{65-72}

However in methoxyfuranocoumarins, where loss of methyl radical can give rise to a conjugated oxonium ion, this process predominates.\textsuperscript{66, 68, 70}
In pyranocoumarins, the mass spectra of 2',2'-dimethylpyranocoumarin is dominated by the loss of methyl radical and generation of a stable benzopyrylium ion which frequently is the base peak.\textsuperscript{66, 73-79}

Generally the fragmentation is related to the structure of the intact molecule. \textbf{Electron impact mass} spectrometry of both flavonoid aglycones and glycosides serves as a valuable aid in determining their structures, especially when only very small quantities (i.e. less than 1 mg) of the compounds are available. It has been applied successfully to all classes of flavonoid aglycones and also a number of different types of glycosides.\textsuperscript{80-86} The flavonoid aglycones and glycosides have been subjected to \textbf{GC-MS} spectrometry in the form of their permethyl ethers, perdeuteriomethyl ethers\textsuperscript{87-88} and trimethylsilyl ethers\textsuperscript{89-90}.

\textbf{Flavones:}

Most flavonoids yield intense peak for the molecular ion (M\textsuperscript{+}) and indeed this is often the base peak. In addition to the molecular ion, flavonoids usually afford major peaks for [M-1] and [M-15] when methoxylated. Perhaps the most useful fragmentation in terms of flavonoid identification are those which involve the cleavage of intact A and
B-ring fragments. Kingston\textsuperscript{90} had discussed in detail the mass spectra of large number of flavones, flavonols, flavanones and their ether derivatives (Scheme I, II & III). The fragmentation pattern of monoflavones has been summarised as follows:

a) Flavones with fewer than four hydroxyl groups do not readily fragment, a consequence of the stability of their molecular ion.

b) Flavones with fewer than four hydroxyl groups tend to undergo decomposition predominantly by way of the retro-Diels-Alder (RDA) process.\textsuperscript{85, 86} This and other common fragmentation processes are shown in (Scheme-I) using apigenin (XXXV)\textsuperscript{85} as a typical example.

c) An [M-1] ion is often found in the mass spectra of flavones, its origin is however, obscure.

d) The presence of ion m/z 137 (Scheme-II), frequently more intense when a 3-hydroxy group is present, is attributed to the alternative mode of retro-Diels-Alder fragmentation.

e) Doubly charged ions are frequently present.

f) When heavily substituted with hydroxyls and methoxyls, the flavones tend to fragment in a less predictable manner, retro-Diels-Alder process becomes insignificant and the spectrum is dominated by the molecular ion and ions at M-15, M-28 and M-43.\textsuperscript{87,89}

**Flavonoid O-glycosides:**

The position of a sugar residue in a flavonoid aglycone can be easily recognized from the mass spectrum of permethylated glycosides\textsuperscript{89}. The sugar attached to the position 5 and 3 splits more readily than that at position 7 and as a result the molecular ion peak is of very low intensity or totally absent.

On the other hand 7-O-glycosides usually show an intense molecular ion peak amounting to 50\% or higher. The 4'-glycosides represent an intermediate case, having small but distinct molecular ion peak.
(Scheme-II)


34 A R H Cole, *Zeichmester’s Progress in Chemistry of Organic Natural Products* (Wein, spriger Verlag, New York), XIII, **60** (1956)