THEORETICAL
The term glycosides embraces a large and remarkably varied group of organic compounds having the properties in common of furnishing saccharides or their oxidation products, the glycones and one or more other substances (not infrequently aromatic in nature) the aglycones, when hydrolysed by acid or a specific hydrolytic enzyme. In combination with sugars, representatives of nearly all classes of compounds occur in plants, chiefly in fruits, in flowers and barks. These compounds correspond in structure to the simple synthetic methyl glucoside having hemiacetal linkage and accordingly a glycoside can be represented by a general formula (I), where R stands for the non-sugar portion.

Glycosides are thus derivatives of sugars in which the reducing or the potential aldehydic group of the sugars is substituted by condensation with an alcohol or a phenol to form a hemiacetal. The oligo- and polysaccharides are also the
glycosidic condensation products of monosaccharides, one of the component sugars behaving as a reducing sugar and the other one acting as an alcohol. Customarily they are neither included nor dealt with glycosides for they fail to furnish a non-sugar part - the aglycone on hydrolysis.

Laurent7 (1852) was the first to collect together all substances which gave sugar on hydrolysis into a special group which he called glucosamid and Berthelot1 termed them later as saccharides. It is not known who originated the term glucoside. However, in the past, glucoside was the general name given to a class of organic substances which on hydrolysis gave sugar or a mixture of sugars. The term glucoside was based on the meagre knowledge of the group as no member was known which did not contain glucose as one of the products of hydrolysis.

The term glycoside is now officially used as a general name for the group, irrespective of the sugar present, glucoside is the specific name used for those glycosides, the sugar constituent of which is glucose. In the past the glycosides were named ending in-in, based on the plant in which they occur. It has now been proposed in France to substitute the suffix-oside, to indicate the glycosidic nature. Thus asperulin becomes asperuloside. The non-sugar part of the glycoside is named as aglycone, a term originated by Japanese chemists2.
The classification of glycosides is based upon the nature of aglycone. The aglycones include representatives of many of the numerous groups of hydroxyl compounds occurring in plants, ranging from small molecules such as ethyl alcohol, acetone cyanhydrin to large ones such as the triterpenes, steroids (cardiac glycosides, saponins etc.), hydroxyanthraquinones, anthocyanins and anthoxanthins. The following is the brief outline of the classification representing one member of each group:

(1) **Natural Glycosides of Alcohols and Phenols.**

(a) **Alcoholic Glycosides**: Very few glycosides of aliphatic alcohols have been isolated from plant tissue. Gaultherioside (II) m.p. 185\(^0\), a glycoside extracted from fresh Gaultheria procumbens yields on hydrolysis ethyl alcohol, glucose and xylose.

![Gaultherioside](attachment:glycoside.png)

A glucoside of the aromatic alcohol, benzyl alcohol, is believed to occur in maize and tolu balsam.
(1b) Phenolic Glycosides: In these glycosides the sugar residue is attached to the aglycone through the phenolic hydroxyl group. Gein, occurs in Gymnurus urbicann (roots) and is hydrolysed by the enzyme geinase to eugenol and viclanose and has the following constitution.

\[
\begin{align*}
\text{Eugenol} & \quad \text{D-Glucose (III)} & \quad \text{L-Arabinoose} \\
\text{Gein} & \quad \text{Vicanose}
\end{align*}
\]

(2) Cranogenic Glycosides:

Vicianin (IV) m.p. 147-48°C, occurs in the seeds of the wild vetch (Vicia angustifolia). It is hydrolysed by Vicianase yielding D-mandelonitrile and vicianose, a disaccharide of D-glucose and L-arabinose.

(3) Thioglycosides:

Sinainin (V) m.p. 85°C (m.p. 130-140°C anhyd.), a glycoside found in white mustard seed (Sinapis alba), is hydrolysed by myrosin present in the seeds yielding glucose, p-hydroxybenzylisothiocyanate and sinapin sulphate.
(4) Anthocyanins:

The red and blue pigments in flowers, fruits and beans are mostly anthocyanins. Hydrolysis of the anthocyanin by dilute hydrochloric acid yields anthocyanidin chloride and a sugar.

(5) Coumarin Glycosides:

Many naturally occurring fish poisons and insecticides are derivatives of coumarins. The structure of the glycosides is based upon coumarin, iso-coumarin or their derivatives.
Aesculin (IX) m.p. 160° (205° anhyd.) is the glycoside of the bark of horse chestnut (Aesculus hippocastanum). It is also found in the roots of the wild jasmin (Gelsemium sempervirens). Aesculin (IX) is hydrolysed by dilute mineral acids or by emulsin to glucose and aesculetin.

Hydrangenol (X) m.p. 181°, an isocoumarin derivative occurs as glucoside in the flowers of the garden hydrangea (Hydrangea opuloides). The constitution of aglycone has been established by alkali fission.

(5) The Saponins, Phytosterols and Solanum Alkaloids:

(a) The Saponins: The saponins are widely distributed in nature. They all exhibit the characteristic property of foaming strongly when shaken in aqueous solution. They form emulsions with oil. The saponin when taken orally are non-toxic or only slightly toxic, but when injected intravenously they exert a powerful haemolytic effect, dissolving the red corpuscles even at extreme dilution.

Hydrolysis of saponins yields sugars or their oxidation products and sapogenins (aglycones) which may be triterpenic or steroidal in nature. Tschesche divided the saponins into two groups according to the nature of their dehydrogenation products:

(i) The Triterpenic Saponins: The dehydrogenation of triterpenic sapogenins furnishes the following main products.
The Steroid Saponins: On dehydrogenation with selenium they yield Dietz's hydrocarbon (3-methyl cyclopentano-phenanthrene) which indicates the presence of a steroid nucleus.
Diehl's hydrocarbon

Sarsasapogenin

(b) Phytosterols (sterol glycosides):

In addition to the steroidal saponins a few naturally occurring sterol glycosides have been reported. They possess a common stigmasterol skeleton.

Stigmasterol

(c) Solanum Alkaloids:

A group of nitrogen containing glycosides with physiological function similar to that of the saponins has been isolated from various species of Solanum. The aglycones of these glycosides are alkaloids. Solmargine (XXI) has been isolated from the fruits of Solanum marginatum. This appears to be a rhamno-glycoside of the following constitution.20
The cardiac glycosides are steroidal glycosides with a heart stimulating action. The best known source of these glycosides is Digitalis (fox-glove) of the order Scrophulariaceae, the orders Apocynaceae, Asclepiadaceae, Moraceae, Ranunculaceae and Liliaceae yield other members of the group.

Hydrolysis with acid or enzymes yields one to four molecules of sugar and the aglycone or genin. In addition to the common sugars glucose and rhamnose the unusual deoxysugars antiarose, cymarose, diginose, digitalose, digitoxose, oleandrose and sarmentose have been identified in the sugar moiety of the cardiac glycosides.
(8) Anthraquinone Glycosides:

The aglycones of a small group of naturally occurring glycosides are anthraquinone derivatives. Ruberythric acid m.p. 253° is the chief glucoside of madder root (Rubia tinctorum). Hydrolysis gives xylose, glucose and alizarin (1,2-dihydroxyanthraquinone). It has been established that ruberythric acid is 2-β-primeveroside-alizarin.

\[
\text{(XXIII)}
\]

(9) The Nucleosides:

The naturally occurring nucleosides are N-glycosides of ribose or 2-deoxyribose as carbohydrate moiety and purine or pyrimidine base as aglycone. They occur both in plants and animals. The carbohydrate molecule is attached through imino group which is equivalent to the hydroxyl of other aglycones.

Adenosine is the N-ribosyl of the purine, adenine, which may be prepared from yeast nucleic acid.

\[
\text{Adenosine}
\]

\[
\text{(XXIV)}
\]
(10) **Phenyl Benzopyrone Glycosides:**

**Anthoxanthin glycosides:** (Flavones, Isoflavones, Flavonols, Flavanones and Chalkones). The flavones are water soluble pigments which occur in the cell sap and in flower petals. They may be free or combined as glycosides with glucose, galactose, rhamnose, pentose, or a methyl pentose. All pigments of the flavone type may be regarded as derivatives of benzopyrone (chromone).

\[ \text{L-Pyrone} \]
\[ \text{V-Pyrone} \]
\[ \text{Bi-benzo-γ-pyronc} \]

The sugar residue may be a monosaccharide, a disaccharide or even a tri-saccharide. Thus xanthorhamn yields two L-rhamnose and one D-galactose molecules.

\[ \text{2-Phenyl benzopyrone} \]
\[ \text{3-Phenyl benzopyrone} \]
\[ \text{Benzopyrone} \]

These sap soluble pigments having the C\textsubscript{15} skeleton belong to a large number of categories. They can be tabulated in the following order on the basis of oxidation levels of the oxygen ring or of the carbon atoms bridging the two benzene rings:

(a) Flavonols (Highest); (b) Flavones, flavanones, iso-flavones and anthocyanidines; (c) Flavanones and chalkones; and (d) Catechin and phloretin.
### Table I

**FLAVONES.**

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>Position of the hydroxyl groups</th>
<th>Position of the methoxyl groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavone</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Pratol</td>
<td>7</td>
<td>6'</td>
</tr>
<tr>
<td>5-hydroxy flavone</td>
<td>5</td>
<td>7'</td>
</tr>
<tr>
<td>(Primuletin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysine</td>
<td>5, 7</td>
<td></td>
</tr>
<tr>
<td>Apigenin</td>
<td>5, 7, 4'</td>
<td></td>
</tr>
<tr>
<td>Apigenin di-methyl ether</td>
<td>5</td>
<td>7', 4'</td>
</tr>
<tr>
<td>Acacetin</td>
<td>5, 7</td>
<td>4'</td>
</tr>
<tr>
<td>Genkwanin</td>
<td>5, 4'</td>
<td>7</td>
</tr>
<tr>
<td>Luteolin</td>
<td>5, 7, 3', 4'</td>
<td></td>
</tr>
<tr>
<td>Chryscericol</td>
<td>5, 7, 4'</td>
<td>3'</td>
</tr>
<tr>
<td>Diosmetin</td>
<td>5, 7, 3'</td>
<td>4'</td>
</tr>
<tr>
<td>Tricin</td>
<td>5, 7, 4'</td>
<td>3', 5'</td>
</tr>
<tr>
<td>Primetin</td>
<td>5, 8</td>
<td></td>
</tr>
<tr>
<td>Wogonin</td>
<td>5, 7</td>
<td>8</td>
</tr>
<tr>
<td>Baicalien</td>
<td>5, 6, 7</td>
<td></td>
</tr>
<tr>
<td>Oroxylin-Á</td>
<td>5, 7</td>
<td>6</td>
</tr>
<tr>
<td>Scutellarin</td>
<td>5, 6, 7, 4'</td>
<td></td>
</tr>
<tr>
<td>Pectolinarigenin</td>
<td>5, 7</td>
<td>6, 4'</td>
</tr>
<tr>
<td>Nobiletin</td>
<td></td>
<td>5, 6, 7, 3', 3', 4'</td>
</tr>
<tr>
<td>Techtchrysin</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Alpinatin</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Lotoflavin</td>
<td>5, 7, 2', 4'</td>
<td></td>
</tr>
<tr>
<td>Flavonols</td>
<td>Position of the hydroxyl groups</td>
<td>Position of the methoxyl groups</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Fisetin</td>
<td>$3,7,3',4'^{\text{a}}$</td>
<td></td>
</tr>
<tr>
<td>Robinetin</td>
<td>$3,7,3',4'^{\text{a}},5'^{\text{c}}$</td>
<td></td>
</tr>
<tr>
<td>Kamynin</td>
<td>$3,7,3'^{\text{a}},4'^{\text{a}},5'^{\text{c}}$</td>
<td>$3,7,5'(3',4'^{\text{a}}$ methylene dioxy)</td>
</tr>
<tr>
<td>Galangin</td>
<td>$3,5,7$</td>
<td></td>
</tr>
<tr>
<td>Galangin-3-methyl ether</td>
<td>$8,7$</td>
<td>$3$</td>
</tr>
<tr>
<td>Izalpinin</td>
<td>$3,5$</td>
<td>$7$</td>
</tr>
<tr>
<td>Knespyronol</td>
<td>$3,5,7',4'^{\text{a}}$</td>
<td></td>
</tr>
<tr>
<td>Knespyronol</td>
<td>$3,5,7$</td>
<td>$4'^{\text{a}}$</td>
</tr>
<tr>
<td>Quercetin</td>
<td>$3,5,7,3'^{\text{a}},4'^{\text{a}}$</td>
<td>$7$</td>
</tr>
<tr>
<td>Rhamnetin</td>
<td>$3,5,3'^{\text{a}},4'^{\text{a}}$</td>
<td>$7$</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>$3,5,7,4'^{\text{a}}$</td>
<td>$3'^{\text{a}}$</td>
</tr>
<tr>
<td>Rhamnazin</td>
<td>$3,5,4'^{\text{a}}$</td>
<td>$7,3'^{\text{a}}$</td>
</tr>
<tr>
<td>Myricetin</td>
<td>$3,5,7,3'^{\text{a}},4'^{\text{a}},5'^{\text{a}}$</td>
<td>$7$</td>
</tr>
<tr>
<td>Datiscatin</td>
<td>$3,5,7,2'^{\text{a}}$</td>
<td></td>
</tr>
<tr>
<td>Morin</td>
<td>$3,5,7,2'^{\text{a}},4'^{\text{a}}$</td>
<td></td>
</tr>
<tr>
<td>Hesperetin</td>
<td>$3,5,7,8,4'^{\text{a}}$</td>
<td>$8$</td>
</tr>
<tr>
<td>Tambuletin</td>
<td>$3,5,7,4'^{\text{a}}$</td>
<td>$8$</td>
</tr>
<tr>
<td>Tambulin</td>
<td>$3,5$</td>
<td>$7,8,4'^{\text{a}}$</td>
</tr>
<tr>
<td>Gossypetin</td>
<td>$3,5,7,8,3'^{\text{a}},4'^{\text{a}}$</td>
<td>$3,5,7,8(3',4'^{\text{a}}$ methylene dioxy)</td>
</tr>
<tr>
<td>Melitenin</td>
<td>$3,5,7,8,3'^{\text{a}},4'^{\text{a}},5'^{\text{a}}$</td>
<td>$3,5,7,8(3',4'^{\text{a}}$ methylene dioxy)</td>
</tr>
<tr>
<td>Aglycone</td>
<td>Position of the hydroxyl groups</td>
<td>Position of the methoxyl groups</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Tangeretin</td>
<td></td>
<td>3,5,6,7,4</td>
</tr>
<tr>
<td>Quercetagetin</td>
<td>3,5,6,7,3',4'</td>
<td></td>
</tr>
<tr>
<td>Matuletin</td>
<td>3,5,7,3',4'</td>
<td>6</td>
</tr>
<tr>
<td>Gardenin</td>
<td>5</td>
<td>3,6,8,3',4',5'</td>
</tr>
<tr>
<td>Auranetin</td>
<td></td>
<td>3,6,7,8,4'</td>
</tr>
<tr>
<td>Calycopterin</td>
<td>5,4'</td>
<td>3,6,7,8</td>
</tr>
<tr>
<td>Nesomorol</td>
<td>7,2',4',3</td>
<td></td>
</tr>
<tr>
<td>Rhamnocitrus</td>
<td>5,4',3</td>
<td>7</td>
</tr>
<tr>
<td>Iso-Kaempferide</td>
<td>4',5',7</td>
<td>3</td>
</tr>
<tr>
<td>Trianthin</td>
<td>5,7</td>
<td>3,6,8,3',4'</td>
</tr>
</tbody>
</table>

![Flavonoloid Diagram](image)

**Table III**

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>Position of the hydroxyl groups</th>
<th>Position of the methoxyl groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fustin</td>
<td>3,7,3',4'</td>
<td></td>
</tr>
<tr>
<td>alpinone</td>
<td>3,5,(2-CH₃)</td>
<td>7</td>
</tr>
<tr>
<td>3-Hydroxy naringenin</td>
<td>3,5,7,4'</td>
<td></td>
</tr>
<tr>
<td>3-Hydroxy eriodictyol</td>
<td>3,5,7,3',4'</td>
<td></td>
</tr>
<tr>
<td>Ampeloptin</td>
<td>3,5,7,3',4',5'</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE IV

#### ISOFLAVONES

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>Position of the hydroxyl groups</th>
<th>Position of the methoxyl groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>$7,4'$</td>
<td></td>
</tr>
<tr>
<td>Formononetin</td>
<td>$7$</td>
<td>$4'$ (3', 4'-methylenedioxy)</td>
</tr>
<tr>
<td>Paardo-baptigenin</td>
<td>$7$</td>
<td></td>
</tr>
<tr>
<td>Tattein</td>
<td>$5,4'(8-CH_3)$</td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td>$5,7,4'$</td>
<td></td>
</tr>
<tr>
<td>ß-Methyl genistein</td>
<td>$5,7,4'(8-CH_3)$</td>
<td></td>
</tr>
<tr>
<td>Prunetin</td>
<td>$5,4'$</td>
<td>$7$</td>
</tr>
<tr>
<td>Biochanin</td>
<td>$5,7$</td>
<td>$4'$</td>
</tr>
<tr>
<td>Iso-genistein</td>
<td>$5,7,2'$</td>
<td></td>
</tr>
<tr>
<td>ß-methyl isogenistein</td>
<td>$5,7,2'(8-CH_3)$</td>
<td></td>
</tr>
<tr>
<td>Orobol</td>
<td>$5,7,3',4'$</td>
<td></td>
</tr>
<tr>
<td>Santal</td>
<td>$5,3',4'$</td>
<td>$7$</td>
</tr>
<tr>
<td>Toctorigenin</td>
<td>$5,7,4'$</td>
<td>$6$</td>
</tr>
<tr>
<td>Irigenin I</td>
<td>$5,7,3'$</td>
<td>$6,4',5'$</td>
</tr>
<tr>
<td>Trunusetin</td>
<td>$4',7$</td>
<td>$5$</td>
</tr>
</tbody>
</table>
### Table V

**Anthocyanidins**

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>Position of the hydroxyl groups</th>
<th>Position of the methoxyl groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelargonidin</td>
<td>3, 5, 7, 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cyanidin</td>
<td>3, 5, 7, 3&lt;sup&gt;b&lt;/sup&gt;, 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Delphinidin</td>
<td>3, 5, 7, 3&lt;sup&gt;b&lt;/sup&gt;, 4&lt;sup&gt;c&lt;/sup&gt;, 5&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Peonidin</td>
<td>3, 5, 7, 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Petunidin</td>
<td>3, 5, 7, 4&lt;sup&gt;a&lt;/sup&gt;, 5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Malvidin</td>
<td>3, 5, 7, 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;, 5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hirsutidin</td>
<td>3, 5, 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7, 3&lt;sup&gt;a&lt;/sup&gt;, 5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gesneridin (Apigenidin)</td>
<td>5, 7, 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Carajuridin</td>
<td>6, 7</td>
<td>5, 4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Betanidin</td>
<td>Nitrogenous</td>
<td></td>
</tr>
<tr>
<td>Methoxy Butol</td>
<td>7, 3&lt;sup&gt;a&lt;/sup&gt;, 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pankanetin</td>
<td>5, 6, 7, 8, 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

### Table VI

**Flavanones**

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>Position of the hydroxyl groups</th>
<th>Position of the methoxyl groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquiritigenin</td>
<td>7, 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Butin</td>
<td>7, 3&lt;sup&gt;a&lt;/sup&gt;, 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Aglycone</td>
<td>Position of the hydroxyl groups</td>
<td>Position of the methoxyl groups</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Deoxymethoxy-matteucinol</td>
<td>5,7(6,3-dimethyl)</td>
<td></td>
</tr>
<tr>
<td>Maringenin</td>
<td>5,7,4</td>
<td></td>
</tr>
<tr>
<td>Sakuranetin</td>
<td>5,4</td>
<td>7</td>
</tr>
<tr>
<td>Cetrifoliol(iso-Sakuranetin)</td>
<td>5,7</td>
<td>4'</td>
</tr>
<tr>
<td>Matteucinol</td>
<td>5,7(6,8-dimethyl)</td>
<td>4'</td>
</tr>
<tr>
<td>Citronetin</td>
<td>5,7</td>
<td>2'</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>5,7,3',4'</td>
<td></td>
</tr>
<tr>
<td>Homoeridictyol</td>
<td>5,7,4'</td>
<td>3'</td>
</tr>
<tr>
<td>Nesperetin</td>
<td>5,7,3'</td>
<td>4'</td>
</tr>
<tr>
<td>Carthamidin</td>
<td>5,7,8,4'</td>
<td></td>
</tr>
<tr>
<td>Iso-carthamidin</td>
<td>5,6,7,4'</td>
<td></td>
</tr>
<tr>
<td>Iso-pedicin</td>
<td>6</td>
<td>5,7,8</td>
</tr>
</tbody>
</table>

**TABLE VII**

CHALKONES

```latex
\begin{array}{|c|c|c|}
\hline
\text{Aglycone} & \text{Position of the hydroxyl groups} & \text{Position of the methoxyl groups} \\
\hline
\text{Butein} & 2,4,3',4' & - \\
\text{Pedicin} & 2,5 & 3,4,6 \\
\text{Pedicellin} & - & 2,3,4,5,6 \\
\text{Pedicinin} & 2,5(3,6=quino-) & 4 \\
\hline
\end{array}
```
The estimation of glycosides is done by two types of methods, the determination of sugar released by hydrolysis and the measurement of some specific property of the aglycone. Many glycosides are coloured or fluorescent and others are chromogenic so that simple methods for their estimation may easily be devised along colorimetric or fluorimetric lines. All such methods are liable to suffer from the interfering effects of hydrolytic enzymes and other substances present in the plant. This necessitates the inactivation of enzymes by treatment with acids, heat etc. and the removal of interfering substances before proceeding.
for the analysis. This is all the more essential when powerful reagents are to be used, say in the development of colour. Under such conditions the glycosides being very reactive in nature may give various products by side reactions with other compounds present in the extract.

No accurate general methods are available for the determination of $\beta$-glycosides in plant extracts, but two techniques of wide application are worth attention.

1. The Biochemical Method of Bourquelot
2. The Charcoal Adsorption Method

No general methods are available for the isolation of glycosides. A method which works well in one case may be a complete failure in the other. The occurrence of contaminants so similar in properties to the compounds to be isolated renders the process of separation very difficult. There are, nevertheless, certain general principles of the isolation of glycosides which should be kept in view.

The isolation of the glycosides involves three main stages:

1. The simultaneous extraction of the glycosides from the plant material and the inactivation of any glycosidase which may be present.

The inactivation of enzymes is accomplished by dropping fresh or dried parts of plants into boiling water or boiling ethanol. The glycosidases are also inactivated with acid, which
has the advantage of avoiding heat. The hydrogen ion concentration need only be taken to pH 1-2 and if the temperature is kept low by the addition of ice even some of the most acid-labile glycosides can be extracted with only small losses. Inactivation of enzymes may also be effected by working at very low temperature.

(ii) The separation of the glycoside from substances which impede crystallization.

The following general treatments may be employed to separate glycosides from unwanted substances which occur quite generally in plants and which interfere with crystallization:

(a) The removal of waxy and fatty substances from dried, powdered plant material by extraction with petroleum ether.

(b) The preferential extraction of some glycosides from dried powdered plant material with an organic solvent.

(c) Filtration of extracts.

(d) The removal of sugars from extracts by fermentation with Baker's yeast and the subsequent removal of the acids so produced by precipitation with bases such as MgO, Ba(OH)$_2$ and Sr(OH)$_2$ or with CaCO$_3$.

(e) The removal of tannins, pectins, mucilages, resins and organic acids by the addition of Ba(OH)$_2$, Sr(OH)$_2$ or MgO, followed by alcohol. These contaminants form compounds with bases and the sugars are also degraded to some extent yielding products precipitable by alcohol. The use of this method is limited because some glycosides are degraded under alkaline conditions and others are precipitated.
(f) The same components are removed from aqueous extracts by heavy metals, usually lead, under acid condition without the use of alcohol. Certain glycosides are also precipitated by the same treatment and this may be employed as a stage in their isolation.

(g) The precipitation of glycosides by treatment with basic lead acetate or lead acetate in presence of ammonia.

(h) Chromatography: In recent years paper chromatography has been extensively used by various workers for the isolation and identification of flavonoids and their glycosides. References are also found for the use of column and ion-exchange chromatography in this field. A brief review of some of the important works done in this connection is being given.

Natural and some synthetic chlorides of anthocyanins and flavones were identified and separated by partition chromatograph on paper by making use of their colour in visible or ultraviolet light with and without treatment with NH₃ vapours, their reaction with ammonical AgNO₃, and their Rf values in Butanol: Acetic Acid: Water (40:10:50). The Rf increases on hydrolysis of the pigments with acid.

The detection of Rf values for eleven flavonoid pigments in chloroform, ethyl acetate, phenol and butanol, acetic acid; the separation of mixtures containing four to six of these pigments; and the use of colour developing sprays to locate and identify the pigment zones are given. The characteristic colours in ordinary light and an intense fluorescence in U.V. light have been
observed by treating the chromatogram with basic and normal lead acetate, alcoholic AlCl₃, Na₂CO₃, and boric-citric acid reagent.

Two-dimensional paper chromatography has been used to separate and identify nine phenolic constituents of pine heartwood extracts. The solvent consists of a water saturated mixture of equal volumes of benzene and ligroin containing traces of methyl alcohol. Best results are obtained by using the above solvent mixture in one direction and chloroform:methanol:ligroin (2:1:7 + 3H₂O) in the other (at right angles to the previous direction). Tetrazotized benzidine is used as a spray reagent.

The acetone extract of the heartwood of Pinus clausa on a paper chromatogram revealed six spots which have been identified. In addition a paper partition chromatographic investigation of 48 Pinus species has also been done. Results are summarized of experiments with 47 flavone derivatives by using Butanol-acetic acid mixture; ethyl acetate-cresol and phenol-water as solvents.

The behaviour of 38 flavonoids in 11 solvents has been studied. Colour development has been given by 8 chromogenic sprays (alcoholic AlCl₃, ThCl₄, FeCl₃, each 1%; aqueous basic lead acetate, lead acetate, Na₂CO₃, each 1%; Ammonical AgNO₃ and Benedict's solution) or spots have been located under U.V. light before spraying. The results are given in the following table (Table IX).

Simpson and Garden have discussed the application of paper chromatography to the study of chelate system. It has been shown that the pyrone carbonyl group of flavones forms stronger hydrogen bonds with the 5-than with the 3-hydroxyl group. The ability of the
Flavonol
Rhoapyrt
Sapifer
Serins...
Morange
Patuletr?
Inerece
Percecti
Rhamneti
Robinet?

Flavone
Accetin
Apigenin
Aurzeti
Chrysin
Cicerwani
Asowagon
Norwagon
Broxylin
Wegonin

Flavanon
Homoerio
3, 3', 4',
Hesperdi
Harlingin.
Nachesper

Flavene e
C-Catech i
l-Spicate

Miscellan
Resperidi
ch
Phloretin
2', 3', 4- Tr
chal
Esculetin
Pemiferin.
carbonyl group to form bonds with both hydroxyl groups simultaneously has been observed. A group of 30 flavones, selected to provide most of the possible combinations of hydroxyl and methoxyl substitution in the 3-, 5-, 7- and 4'-positions and a few simple phenols have been run on paper chromatograms. Rₜ values have been determined with these solvents, under substantially the same conditions as those recommended by Bate-Smith and Westall. Comparison of the Rₜ values of a number of 4'-, and 3'-hydroxy flavones shows that a 4'-hydroxyl group stabilises the carbonyl-3-hydroxyl more than the carbonyl-5-hydroxyl chelate system.

Ice and Mender have reported the details of the isolation in pure form of quercetin and five of its glycosides from the leaves of Vaccinium myrtillus. The concentrate of mixed flavonoids was separated by adsorption chromatography on a column of Magnesol.

Although the complex mixtures of phenolic glycosides present in plant extract may readily be separated by paper chromatography, the individual components can not usually be identified by Rₜ values and colour reactions alone because there may be present a large number of glycosides related to a single aglycone (cf. Ice and Mender). Even direct measurement of U.V. absorption spectra on paper chromatograms does not permit differentiation between glycosides related to one aglycone. Attempts were made therefore to separate the mixture before examination of individual components. Nordstrom and Swain found, however, contrary to Gage and Mender and Bradfield and Ficca, that provided the solvent is removed
from developed paper chromatogram at room temperature, individual bands can be quantitatively eluted in 24-48 hours. The extracted bands may be then further purified if necessary by use of a second solvent. Similar techniques for eluting flavonoid compounds have been described \(^{44,45}\), although the elution time (2 hours) used by the later authors was possibly too short for complete extraction. Nordstrom and Swain\(^ {42}\) describes the method for the separation and identification of flavones and their glycosides of Dahlia tithymalbus "Mandy". They used Whatman's filter paper No. 3 and methanol:acetic acid:water (6:1:2) as the solvent mixture. The chromatogram was allowed to run overnight. After careful drying, the portions of the bands were marked under an ultra-violet lamp. The separated components were eluted in a chromatographic tank with either cold aqueous ethanol (40-70\%) or cold aqueous dilute hydrochloric acid for the anthocyanins. The results of the primary separation are described\(^ {42}\). The \(R_p\) values of the separated components in several solvents and colour reactions of the glycosides were determined in Whatman's filter paper No.1. The results are given in Table X.
### Table X

<table>
<thead>
<tr>
<th></th>
<th>Spina</th>
<th>Spine</th>
<th>acetyl spino</th>
<th>O.4%</th>
<th>O.4%</th>
<th>U.V. light</th>
<th>U.V. light +</th>
<th>NH3 vapour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>plain</td>
<td>paper</td>
<td>phosphat.</td>
<td>plain</td>
<td>paper</td>
<td>paper</td>
<td>paper</td>
<td></td>
</tr>
<tr>
<td>Compound</td>
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<td></td>
<td></td>
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</tr>
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<td>Apigenin 7-glucoside</td>
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<td>0.44</td>
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<tr>
<td>Luteolin 7-glucoside</td>
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<td></td>
<td>0.35</td>
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<td></td>
<td></td>
<td>0.42</td>
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<tr>
<td>Dihydro-apigenin</td>
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<td>0.32</td>
<td>0.43</td>
<td>0.64</td>
<td>0.35</td>
<td>0.39</td>
<td>0.37</td>
<td>0.39</td>
</tr>
<tr>
<td>Luteolin</td>
<td>0.62</td>
<td>0.73</td>
<td>0.62</td>
<td>0.66</td>
<td>0.66</td>
<td>0.30</td>
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</tr>
<tr>
<td>Baringenin</td>
<td>0.93</td>
<td>0.63</td>
<td>0.87</td>
<td>0.93</td>
<td>0.91</td>
<td>0.94</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

*Prepared from partially hydrolysed "Crude apin".*
C O N S T I T U T I O N

The problem of the elucidation of the structure of glycosides involves the following important steps:

(i) Hydrolysis giving rise to glycone and aglycone components.
(ii) Characterisation of glycone or carbohydrate moiety.
   (a) Paper chromatography of sugars.
   (b) Column chromatography of sugars.
   (c) Quantitative estimation of saccharides.
   (d) Study of the stereochemical nature of the glycosidic union.
   and (e) location of the carbon atoms involved in the glycosidic union.
(iii) Characterisation of the aglycons:
   (a) Colour reactions.
   (b) Comparison of the aglycone and its derivatives with synthetic standards by mixed melts and chromatographic methods.
   (c) Spectrophotometric methods, ultraviolet and infra-red absorption spectra.
   (d) Degradation
   and (e) Synthesis and isomerisation.

(i) Hydrolysis. All glycosides are hydrolysed by treating with dilute mineral acids, sulphuric and hydrochloric, with the production of sugar and aglycone. They are hydrolysed at different
rates; some glycosides, e.g. gynocardin, being extremely resistant to acid hydrolysis. The same reaction may be affected by the agency of enzymes whose action is, however, specific; the $\beta$-glycosides being hydrolysed by $\beta$-glycosidase (emulsin), while $\alpha$-glycosides are hydrolysed by $\alpha$-glucosidase (maltase). It is important to note that both di- or tri-saccharides present in glycosides as carbohydrate moiety are converted into monosaccharides during hydrolysis.

The large group of glycosides containing only a single monosaccharide residue in the glycone are in most common cases derivatives of $\beta$-D-glucose, although other monosaccharide glyccones, e.g. the hexose, D- and L-galactose, D-mannose and D-fructose have also been isolated. The methyl pentose (6-deoxy hexose) L-rhamnose is of fairly frequent occurrence and D-glucose has been reported as a component of Jalanin and terpethin.

Disaccharide glycosides are quite common and the following have been identified: gentibiose (4-$\beta$-D-glicosido-D-glucose) in amygdalin and crocin, vicenose (4-$\beta$-L-arabinosido-D-glucose) in vicenalin, violitin and gein; primrose (6-$\beta$-D-xilosido-D-glucose) in primaverin, prismavarin, and mono-triptin; rutinoside (6-$\beta$-L-rhamnosido-D-glucose) in rubin and datisin. Higher saccharides are unusual except among cardiac glycosides and the saponins.

The following pentoses are known in glycosides: D- and L-arabinose, D-xylose and D-ribose. The occurrence of D-arabinose is rare in nature. Barbaloxin, from Barbados albes, sapindus saponin and albizia saponin appear to be some of the only
authenticated sources of D-arabinose among the plant glycosides. 
Apiin, a glycoside from parsley seeds contains the unusual pentose 
apiose, which has a branched chain.

The cardiac glycosides, on hydrolysis, yield in addition 
to the common sugars, D-glucose and L-rhamnose, certain deoxy 
sugars which are found nowhere else in nature. Thus digitalis 
glycosides contain digitalose (3-methyl-6-deoxy-D-galactose or 
3-methyl-D-fucose) and digitoxose (2:6-dideoxy-D-allose or altrose; 
cymarin and periplocymin contain cymarose (3-methyl digitozose) 
oleandrin and adynerin contain oleandrose (3-methyl-2-deoxy-1- 
quinobose or 3-methyl-2:6-dideoxy-L-glucose). Diginose (3-methyl- 
2:6-dideoxy-D-galactose or 3-methyl-2-deoxy-3-fucose) and sarmentose 
(a methyl ether of 2:6-dideoxy hexose isomeric with cymarose) are 
found in diginin and sarmentocyminar respectively.

Uronic acid is a rare constituent of glycosides. Baicalin 
and scutellarin, favone glycosides, contain D-glucoronic acid. It 
is also present in the saponin aescin from horse chestnut seeds, 
in the saponin from the bark of quillaia saponaria, from mistletoe 
from sugar beet and randia dumaturum etc.

The cyclic form of the sugar is generally pyranose with the 
exception of ribofuranose and deoxyribofuranose in the nucleosides.

Neutralisation of the hydrolysate: At the conclusion of the hydro-
lysis it is necessary to neutralise the acid before proceeding to 
concentration of the solution. Concentration should always be done 
below 40°. When sulphuric acid is used as hydrolytic agent it is 
customary to use barium carbonate for neutralisation. Barium
carbonate should be pure and freshly precipitated and to avoid certain transformations it should be used in cold, when this is done it is sometimes difficult effectively to remove the barium sulphate formed. This can be done however, by shaking with an intimate mixture of both acid and base-binding resins. If resins are used care should be taken lest the acid-binding resin should be sufficiently alkaline to isomerise the sugars. A control experiment should always be done. Another disadvantage of using barium carbonate is that it probably absorbs partially uronic acids as well as glucoronic acid lactones which appear to be formed in course of hydrolysis of glycosides containing glucoronic acid. Columns of suitable resins such as Amberlites IR 100 and 4B or "Dowex 2" etc. are used with advantage for acid removal from hydrolysates.

Hydrochloric acid when used as hydrolytic agent is usually neutralised by pure silver carbonate. Here it is necessary subsequently to remove dissolved ("colloidal") silver by means of hydrogen sulphide. Hydrochloric acid may also be removed under vacuum over KOH solid.

(ii) Characterisation of glucose or carbohydrate moiety:

In the last several years the use of chromatography for the separation of different compounds has sprung as an uncontestable technique and is widely used.
(a) PAPER CHROMATOGRAPHY

The use of paper chromatography as a means of gaining rapid and specific information regarding the saccharide composition is well established. In addition, the paper chromatogram provides a means of carrying out quantitative analysis with a confidence and accuracy previously unknown. But it must be borne in mind that the characterisation of a saccharide solely by its chromatographic behaviour is not unequivocal. In general, the isolation and identification of crystalline compounds and the preparation of appropriate crystalline derivatives having characteristic physical and analytical properties should always be the analyst's ultimate aim.

Qualitative Paper Chromatography: The separation of monosaccharides was first described by Partridge53,54,55. The chromatograms were run with a number of mixtures of solvents. Partridge has published a fairly complete Rp value table which is given below (Table XI)54.

Ascending as well as descending techniques by the mobile fluid phase are used. The circular technique developed55,57,58,59 has also been utilized and the spraying reagents for revealing the sugar spots are to be numbered by the scores viz analine phthalate, analine phosphate, para-anisidine hydrochloride, ammonical silver nitrate, benzidine, p-anisidine phosphate etc59.

Spots of authentic sugars should always be included in runs along with the unknown sugars. The inclusion of such control 'spots'
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Phenol</th>
<th>t-Butanol</th>
<th>l-Butanol</th>
<th>n-Butanol</th>
<th>1-Acetone</th>
<th>n-Butanol</th>
<th>Isobutyric acid</th>
<th>Methyl ethyl ketone</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H$_3$O$^+$</td>
<td>lone</td>
<td>H$_3$O$^+$</td>
<td>lone</td>
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<td>H$_3$O$^+$</td>
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<tr>
<td>d-Glucose</td>
<td>0.49</td>
<td>0.36</td>
<td>0.18</td>
<td>0.16</td>
<td>0.16</td>
<td>0.070</td>
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<td>0.100</td>
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<tr>
<td>d-Sorbitol</td>
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<td>0.47</td>
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<td>0.19</td>
<td>0.19</td>
<td>0.120</td>
<td>0.15</td>
<td>0.050</td>
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<td>0.15</td>
<td>0.095</td>
<td>0.12</td>
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<td>0.110</td>
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<tr>
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<td>0.21</td>
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<td>0.110</td>
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<td>0.075</td>
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<td>0.18</td>
<td>0.120</td>
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<td>0.075</td>
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<td>0.120</td>
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<td>0.19</td>
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<tr>
<td>R-Glucose</td>
<td>0.50</td>
<td>0.51</td>
<td>0.20</td>
<td>0.18</td>
<td>0.18</td>
<td>0.120</td>
<td>0.15</td>
<td>0.075</td>
</tr>
<tr>
<td>R-Mannose</td>
<td>0.73</td>
<td>0.80</td>
<td>0.21</td>
<td>0.19</td>
<td>0.19</td>
<td>0.110</td>
<td>0.15</td>
<td>0.075</td>
</tr>
</tbody>
</table>

Values in brackets due to lacunae.

Values in brackets due to free base (most connects the two spots).
greatly minimises the chances of erroneous deductions arising from unforeseen temperature changes etc. The selection of suitable spraying reagents also aids the certainty of identification.

Rough et al. have done considerable amount of work on this aspect of sugar chemistry and sufficiently detailed reviews on the subject have appeared.

(b) COLUMN CHROMATOGRAPHY

An important limitation of paper chromatography lies in the small amount of material that can be satisfactorily handled. Recently a number of thick papers are available to overcome partially this difficulty and are being used more and more, but still for the isolation of substances in the crystalline form and appreciable quantity a recourse is taken to the technique of column chromatography. In addition considerable differences in the relative proportions of sugars of neighbouring Rf values frequently cause interference with their separation. Column chromatography, on the other hand can be used to separate amounts of material sufficient for ordinary chemical and physical identification.

The first application of partition chromatography to sugars was published by Bell who separated 2,3,4,6-tetramethyl glucose from 2,3,5-trimethyl and 2,3-dimethyl glucose on columns of silica gel.

Rough, Jones and Wadman used powdered cellulose columns for the separation of monosaccharides. Hydrocellulose columns are reported to have a higher resolving power, for methylated sugars than cellulose columns, as well as greater capacity.
Besides cellulose columns which are used abundantly, other columns such as charcoal columns are also used. An entirely new type of chromatographic procedure is the separation of polyhydroxy compounds using strongly basic ion-exchange resins and borate buffers of various pH-values. The use of ultraviolet and infrared absorption spectra in structural detection of sugars has also been reported.

(c) Quantitative Determination of Saccharides

A large number of quantitative methods for the determination of total sugars are known, such as Bartrand’s method and Somogyi’s method. Nowadays micro-methods are being used more and more. The number of sugar groups attached to each aglycone in the purified glycoside can be tentatively reduced from the $R_g$ value ($R_g = \log (1/R_p) - 1$), but it can be determined more accurately after careful hydrolysis of each component (ca. 0.2 mg) by estimation of the resulting aglycone spectrophotometrically and the sugars either by one of the several micro-methods (given later) such as Somogyi’s copper micro-method or by the anthrone reagent, hydrolysis being unnecessary in the latter case. The anthrone method is specially useful for glycosides containing rhamnose since low concentration of the sugar give erratic results with the Somogyi’s reagent.

A host of methods are available for the quantitative determination of reducing sugars but a few are known for the determination of non-reducing sugars. There are three requisites for successful
quantitative analysis based on paper chromatography (a) sufficient material must be applied to the paper to yield an accurate determination (b) the position on the paper of the area to be analysed must be found and (c) the satisfactory elution of the material from the paper. A list of some of the colorimetric and titrimetric methods is given below.

**Elution and Determination**

**Colorimetric Methods:**

(i) The Nelson Colorimetric method for reducing sugars (copper sulphate solution and arsenomolybdate solution)\(^7\) and Eastwood\(^7\) and Laidlaw and Reid\(^8\) have successfully applied the method of Nelson\(^9\) to sugars eluted from paper.

(ii) Determination of reducing sugars and furanoses non-reducing sugar by benzidine and acetic acid\(^10\).

(iii) Determination of reducing and non-reducing sugars (Phenol-sulphuric acid method)\(^11\).

(iv) Anthronesulphuric acid method\(^12,13\).

(v) Anilino phthalate method\(^14\).

(vi) A direct colorimetric method for reducing sugars (alkaline triphenyl tetrazolium halide as spray reagent). The method is unique as the coloured spot is eluted from the paper\(^15\).

**Titrimetric Methods:**

(i) Determination of saccharides by hot periodate oxidation\(^16\).

(ii) Somogyi's copper method\(^17\).

Spot intensity measurements:

The method differs from other methods in that it does not require elution of the spot. (Direct measurement of spot intensity after reaction with silver nitrate, using standard photovolt electron-transmission densitometer)\(^3\).

(d) **STUDY OF THE STEREOCHEMICAL NATURE OF THE GLYCOSIDIC LINK**

The naturally occurring glycosides have almost exclusively the \( \beta \)-configuration for the glucosidic carbon and as a result are laevorotatory; e.g. extrorotating phillyrin from Forsythia suspensa and Uloa fragrans is believed to be an \( \alpha \)-glycoside\(^9\). The stereochemical problem of the glycosidic linkage is readily revealed by the study of the behaviour of glycosides towards a particular enzyme. The action of enzyme is entirely specific. The enzyme emulsin attacks the \( \beta \)-glycosides only while the \( \alpha \)-isomer is hydrolysed by maltase. Studies of the optical rotation of the glycoside can also be used to determine the stereochemical nature. Before coming to the problem of the location of the carbon atoms involved in glycosidic linkage the following points should be kept in view:

In the flavone glycosides, the sugars occupy \( \beta \)-position generally (apiin, acaciin, diosmin, lotusin, baicalin etc.). Chrysin is an exception as the glucose molecule is attached to \( \alpha \)-position.

In the flavonol glycosides, the sugars are attached mostly in \( \beta \)-position, thus robinin is 3-rhamnogalactoside, multiflorin is 3-rhamnoglucoside, kaempferitin is 7-dirhamnoside, xanthorhamnin is 2-trirhamnoside, etc. In the quercimeritrin the glucose is in
7-position and also in gossypitrin, in quercetagitrin and also in herbacitrin. It may be pointed out that in the glycoside of Crocus St. John Bright, one glucose molecule is in 3-position while the other in 4'-position.

In the falconosides it seems that the position 7' is the most usually occupied by the sugars (naringin, hesperidin), while the glucose of Sakuramin is in 5', that of liquiritin is in 4', and the butrin has two sugar molecules in 3' and 7-positions in the two nuclei like the glucoside of crocus.

In the group of isoflavones only the 7-position is occupied by the sugars (daidzin, genistin, prunifrutis, iridin etc.).

Location of the carbon atoms involved in the glycosidic union.

Here one has to study the carbon atom of the glycosidic linkage between carbohydrate molecule and aglycone and also between two or three molecules of the sugar if the carbohydrate moiety happens to be a di- or tri-saccharide respectively.

The location of the carbon atoms involved in glycosidic linkage is determined by methylation followed by hydrolysis. During hydrolysis of methylated glycosides fresh hydroxyl groups are introduced in each of the sugar units as well as in aglycone at the carbon atoms which were involved in the glycosidic linkages. Thus the location of the hydroxyl groups in the partially methylated hydrolytic products decides the positions of the glycosidic linkage. The partially methylated sugars are characterised by the methods described earlier and the partially methylated glycones are identi-
characterization of aglycones". In this connection spectrum of
the compounds in 0.002 M sodium ethoxide has been of great diag-

tic value.

The order of mono-saccharides in the case of biosides
containing two different sugars is determined by separation and
examination of the intermediate monoside resulting from partial
hydrolysis.

(iii) IDENTIFICATION OF AGLYCONES

The aglycones are identified with certainty by comparing
their spectra in ethyl alcohol and 0.002 M sodium ethoxide. By
values in various solvents or on borate impregnated filter
paper and their colour reactions with synthetic standards.

(a) Colour Reactions

A number of colour reactions are used for detecting certain
structural features among the hydroxy flavones. These tests are not
infallible, however, and have been criticized.

One common test which detects the presence of flavones,
flavonols. Flavanones is based upon reduction. Reduction with
magnesium and hydrochloric acid, as well as sodium amalgam followed
by acidification gives the same salmon-pink colour. Ashina and

Inamase first found that flavones may be reduced to anthocyanidin
only in alkaline solution and flavonols only in acid solution but
flavanones in both acid and alkaline solutions. This conclusion
was later modified by the discovery that quercitin pentamethyl
ether and rutin (quercitin-3-rhamno-glucoside) could be reduced
in alkaline solution. By the extension of the reactions, Briggs and Locker found that flavonols with a methoxyl group at C (3) in contrast with those with a free hydroxyl group at C (3) are reduced by sodium amalgam. Products isolated from this reduction and partially responsible for the colour are flavylum salts, salt from 4-hydroxy flavones100,101 and bimolecular products102.

Wilson's Boric acid Test103 is specific for 5-hydroxy and 5-methoxy flavones and flavonols and o-hydroxy and methoxy chalcones. With fisetin, naringenin and hesperitin, the reaction is however, negative. The curcumin gives in these conditions a pink colouration Flavanones do not respond to this test. The boric acid reaction has been extended to the quantitative colorimetric estimation of flavones by Weatherby and Cheng104. Taubock105 observed that flavonols evaporated to dryness with various dibasic organic acids in acetone give a yellow dye with a yellow-green fluorescence at high dilution and is specific for flavonols.

Fluorescence Tests: Fluorescence in flavone, isoflavone and chromone series is brought out by certain solvents and ions. The phenomenon are almost general in concentrated sulphuric acid but absent in alkali. This fluorescence is not dependent on the hydroxyl groups in the ring since flavone itself fluoresces. In fact the presence of a large number of groups seem to have an adverse effect106. Further it should be noted that fluorescence is a characteristic of 3-hydroxy flavones but not of 5-hydroxy flavones107,108. Acetic anhydride as a solvent brings out a fluorescence with 5-methoxy flavones but not with 5-hydroxy flavones109.
A further colour test\textsuperscript{110} reported as specific for flavanones depends on the formation of a blue fluorescent spot (UV light) when the compound, on paper, is sprayed with magnesium acetate.

**Gossypetone Reaction**\textsuperscript{111}: Gossypetin, the aglycone of the flavone glycoside gossypin, in alcoholic solution gives a red precipitate with p-benzoquinone and is believed to be specific for flavonols which are hydroxylated at positions 5 and 8.

**Bargellini Test**\textsuperscript{112}: 5,6,7-trihydroxy flavone when treated with sodium amalgam in absolute ethanol formed green flocculent precipitate and is not general since herbacetin (5,7,3':4'-penta-hydroxy flavone) shows a similar behaviour\textsuperscript{113}.

**Ferric chloride colours**: The flavones and related compounds present various colourations in the presence of ferric chloride but they are not very characteristic as to serve their complete identification. However, they are very helpful in determining the constitution of the molecule, especially the location of the hydroxyl groups. Briggs and Locker\textsuperscript{114} have given a resume of these reactions.

Both 5- and 3-hydroxy flavones give intense ferric chloride colours but the colours given by the 5-hydroxy compounds are also invariably green\textsuperscript{115} and occasionally brown\textsuperscript{115} or purple\textsuperscript{117} while the colour given by 3-hydroxy flavones is invariably brown\textsuperscript{118}. 3-hydroxy compounds also furnish either brown or green colouration with ferric chloride\textsuperscript{113}.

It was thus pointed out\textsuperscript{114} that the production of a colour with ferric chloride solution (carried out by the addition of
aqueous ferric chloride to an alcoholic solution of the flavone) is a property of 3-, 5, or 6-hydroxy compounds but not of 5-, 7-, or 4-hydroxy derivatives (cf.) also for 5-hydroxy flavones\textsuperscript{120}, for 7-hydroxy flavones\textsuperscript{121} and for 4-hydroxy flavones\textsuperscript{122}).

Here are the various colours obtained by the few derivatives of flavones, flavonols and flavanones in alcoholic solution\textsuperscript{123}.

Table XII

<table>
<thead>
<tr>
<th>Substance</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flavones</strong></td>
<td></td>
</tr>
<tr>
<td>luteolin</td>
<td>Green</td>
</tr>
<tr>
<td>baicalein</td>
<td>brownish yellow</td>
</tr>
<tr>
<td>wogonin</td>
<td>violet brown</td>
</tr>
<tr>
<td>tricin</td>
<td>brown red</td>
</tr>
<tr>
<td>genkwanin</td>
<td>brown</td>
</tr>
<tr>
<td>primetin</td>
<td>green + NaOH, brown red</td>
</tr>
<tr>
<td><strong>Flavonols</strong></td>
<td></td>
</tr>
<tr>
<td>izalpinin</td>
<td>dirty green; + Na acetate, red</td>
</tr>
<tr>
<td>galangin</td>
<td>blackish green</td>
</tr>
<tr>
<td>fisetin</td>
<td>violet</td>
</tr>
<tr>
<td>robinetin</td>
<td>violet</td>
</tr>
<tr>
<td>kaempferide</td>
<td>olive green</td>
</tr>
<tr>
<td>quercetin</td>
<td>deep green</td>
</tr>
<tr>
<td>rhamnetin</td>
<td>precipitation</td>
</tr>
<tr>
<td>iso-rhamnetin</td>
<td>blackish green</td>
</tr>
<tr>
<td>morin</td>
<td>deep olive green</td>
</tr>
<tr>
<td>myricetin</td>
<td>blackish brown</td>
</tr>
<tr>
<td>gossypetin</td>
<td>olive green</td>
</tr>
<tr>
<td>quercetagetin</td>
<td></td>
</tr>
<tr>
<td><strong>Flavanones</strong></td>
<td></td>
</tr>
<tr>
<td>butin</td>
<td>deep green</td>
</tr>
<tr>
<td>naringenin</td>
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</tr>
<tr>
<td>homoeriodictyol</td>
<td>brown red</td>
</tr>
<tr>
<td>hesperitin</td>
<td>no modification</td>
</tr>
<tr>
<td>liquiritigenin</td>
<td>violet brown</td>
</tr>
<tr>
<td>mattucinol</td>
<td></td>
</tr>
</tbody>
</table>
Miscellaneous colour reactions:

The literature concerning colour reactions of flavones is notoriously confused and many conclusions about the specificity of certain reactions have been reached on insufficient evidence. One aspect of this field has been clarified by an investigatic of the colours given by 57 flavone derivatives with two reagents (magnesium-hydrochloric acid and zinc-hydrochloric acid). Where-as the first reagent appears to give stable anthocyanin-like colours with all flavone derivatives, the second gives black colours only with flavonols substituted in the -position, faical colours are produced with flavones and 3-hydroxy flavones.

Chalcones and the flavone pigments can be distinguished by means of colour reaction. When a solution of antimony pentachloride in methylene carbon tetrachloride is added to a solution of a chalcone in the same solvent an intense red or violet red precipitate is formed. Flavones, flavanones and flavonols give yellow orange precipitates. Basic lead acetate gives a red salt with flavonoids and a yellow salt with flavones.

The change in colour observed in buffers of various pH is dependent upon the benzopyrone portion and is valuable in structural determination if the colour change can be compared with a compound of known structure. Flavones containing 7, 3, 6, 8-hydroxyl group, for example, show very little colour change in alkal solution. 3-Hydroxy flavones give addition compounds with stannic chloride. Flavonols are reported to be oxidized in cold alkali by air more easily than flavones. This test is not completely reliable.
The ultra-violet spectra came into use long long ago for the elucidation of the structure. It was followed by Raman spectra since its discovery in 1928. The infra-red spectra came into use recently and nowadays it is utilised more and more usually for the qualitative and quantitative analysis of the unknown substances.

**Ultra-violet Spectra:** It is mostly the Japanese school with Shibata and Kimotsuki, Tasaki, Kattori and Hayashi who have engaged themselves to precise the spectral aspects of these pigments and have connected their particulars to their chemical constitution. In this connection the researches of Iajos and Gerendas, Grinshteyn and Marchlewski and above all the memoirs of Skarzynski are worthy of note. These authors, by the aid of most modern technique have studied systematically a large number of these pigments and related compounds. Aranoff has made a critical study of the results of all these authors already cited and is well reviewed by Sannie and Sauvain.

Skarzynski has found the presence of two characteristic bands in the molecule of benzopyrone nucleus or chromone at approximately 3000 Å and between 2000-2500 Å. It has also been noted that the presence of a phenyl group at position 2= has got no effect on the fundamental spectra. Also the spectra of hydroxy and methoxy chromone donot differ from the spectra of corresponding flavones.

The flavone in itself presents well characteristic two independent bands, one at 2875 Å (Band I) and the other at 2500 Å (Band II). It is probable that in the rest there exists
a third band near 2000 Å but it is not present in the usual spectra without a special technique. Meanwhile it is to be remarked that the attribution of these bands as specific of the benzopyrone nucleus is not absolutely certain. On the one hand the bands are present at 2000 and 2500 Å in the spectra of benzene and γ-pyron.

On the other hand spectra of 2-hydroxy hydroxychalcone is comparable to that of flavanone with the maximum at 2500 and 3200 Å. Therefore it is not the structure of benzopyrone nucleus responsible for the characteristic spectra of flavones but the presence of an analogous nuclear system which is present or is formed by chelation. Thus it is actually the form of resonance o- or p- responsible for it.

Skarzynski has given a table showing the maxima of each band with the value of log ε.

Table XIII

<table>
<thead>
<tr>
<th>Substance</th>
<th>Band I</th>
<th></th>
<th>Band II</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Maximum A</td>
<td>Log ε</td>
<td>Maximum A</td>
<td>Log ε</td>
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<tr>
<td>Flavone</td>
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<td></td>
<td></td>
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<td>6-hydroxy flavone</td>
<td>2975</td>
<td>4.20</td>
<td>2500</td>
<td>4.07</td>
</tr>
<tr>
<td>7-methoxy flavone</td>
<td>2860</td>
<td>4.49</td>
<td>2475</td>
<td>4.12</td>
</tr>
<tr>
<td>3′-oxy flavone</td>
<td>2975</td>
<td>4.21</td>
<td>2415</td>
<td>4.15</td>
</tr>
<tr>
<td>4′-oxy flavone</td>
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<td>4.37</td>
<td>2550</td>
<td>3.92</td>
</tr>
<tr>
<td>5,7-dioxy flavone</td>
<td>3300</td>
<td>5.90</td>
<td>2700</td>
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<td>5,2′-dioxy flavone</td>
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<td>4.16</td>
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<td>4.28</td>
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<td>5,7,3′-trioxy flavone</td>
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<td>Substance</td>
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<td>--------</td>
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<td>---</td>
</tr>
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<td>Maximum</td>
<td>Log ε</td>
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<tr>
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<tr>
<td>5,7,5',4'-tetracetoxy flavonol</td>
<td>3800</td>
<td>4.42</td>
<td>2575</td>
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</tr>
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<td>7,8,5',4'-tetracetoxy flavonol</td>
<td>3700</td>
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<td>2675</td>
<td>4.30</td>
</tr>
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<td>2'-methoxy flavonol</td>
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<td>4.15</td>
<td>2450</td>
<td>4.25</td>
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<tr>
<td>4'-methoxy flavonol</td>
<td>3600</td>
<td>4.24</td>
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<td>7,3',4'-trimethoxy flavonol</td>
<td>3600</td>
<td>4.25</td>
<td>2600</td>
<td>4.10</td>
</tr>
<tr>
<td>7,8,2'-trimethoxy flavonol</td>
<td>3550</td>
<td>3.92</td>
<td>2550</td>
<td>4.28</td>
</tr>
<tr>
<td>7,8,3',4'-tetracetoxy flavonol</td>
<td>3600</td>
<td>4.33</td>
<td>2550</td>
<td>4.30</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>3550</td>
<td>4.12</td>
<td>2630</td>
<td>4.22</td>
</tr>
</tbody>
</table>
Briggs and Locker have studied the ultra-violet spectra of some of the natural and synthetic flavonoids. They have found that there exists in most cases two pronounced peaks ca. 250 μ (band I) and ca. 350 μ (band III) but a less pronounced peak or point of inflexion at ca. 270 μ (band II). The results of their investigation have been given in the following table.

### Table XIV

<table>
<thead>
<tr>
<th>Substance</th>
<th>λ max, Log λ</th>
<th>λ max, Log λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>255 4.32</td>
<td>275 4.04</td>
</tr>
<tr>
<td>Quercitrin (β-rhamnoside)</td>
<td>250 4.35</td>
<td>262 4.34</td>
</tr>
<tr>
<td>Quercetin (7,5,4-trihydroxy-8,3-dimethoxy flavone)</td>
<td>255 4.37</td>
<td>252 3.27</td>
</tr>
<tr>
<td>5,7-dihydroxy-3-methoxy-4,6-methylene dihydro flavone</td>
<td>256 4.29 259 4.23 253 4.25</td>
<td></td>
</tr>
<tr>
<td>5-hydroxy-5,7-dimethoxy-3,4,6-methylene dihydro flavone</td>
<td>255 4.32 259 4.29 254 4.29</td>
<td></td>
</tr>
<tr>
<td>5-hydroxy-5,7,3,4-tetramethoxy flavone (quercetin tetramethyl ether)</td>
<td>254 4.37 259 4.29 252 4.34</td>
<td></td>
</tr>
</tbody>
</table>
Substance | λmax, Log ε | λmax, Log ε | λmax, Log ε | λmax, Log ε
--- | --- | --- | --- | ---
3,5,7-trimethoxy-4',4'-dimethylene dihydroxy flavone | 250 | 4.35 | 257 | 340 | 4.32
5,7,4',2'-dihydroxy-5,5'-dimethoxy flavone | 265 | 4.31 | 256 | 360 | 4.31
5,4',2'-dihydroxy-3',3'-trimethoxy flavone | 257 | 4.32 | 255 | 360 | 4.33
4'-hydroxy-3,5,7,3'-tetra-methoxy flavone | 251 | 4.32 | 250 | 345 | 4.34
Melisimplexin (natural) | 235 | 4.27 | 235 | 355 | 4.23
" (synthetic) | 235 | 4.30 | 235 | 355 | 4.29
Melitarnatin | 240 | 4.37 | 235 | 355 | 4.40
Quercetagetin hexamethyl ether | 240 | 4.37 | 235 | 355 | 4.40
6-hydroxy-3,5,7-trimethoxy-5',4'-methylene dihydroxy flavone | 243 | 4.24 | 237 | 330 | 4.33
7-ethoxy-6-hydroxy-3,5,3'-dimethoxy 3',4'-methylene dihydroxy flavone | 244 | 4.25 | 237 | 337 | 4.32
Quercetagetin | 250 | 4.25 | 249 | 337 | 4.34
" (synthetic) | 253 | 4.36 | 249 | 337 | 4.29
Gossypetin hexamethyl ether | 238 | 4.34 | 237 | 337 | 4.33
Terratin | 238 | 4.37 | 237 | 337 | 4.38

A low intensity inflexion at ca. 300 nm is a common feature of quercetin derivatives not shared by quercetagetin or gossypetin derivatives.

The usual technique involving elution of appropriate portions of the paper chromatogram was impossible in some cases as the substance was eluted with boiling water or boiling alcohol. A similar difficulty has been reported by Gage and Fenner. Elution may be avoided and time saved by direct measurement of the absorption spectrum on the paper. Bradfield and Flood measured the ultra-violet spectra of some the compounds on paper chromatogram. For the flavonol quercetin they have found that the methylation of 4'-hydroxyl group for introduction of an additional hydroxyl...
group in the 5-position has little effect on the position or relative intensity of the bands (cf. isorhamnetin and myricetin). The 3-rhamnoside of quercetin, quercitrin gives the same spectrum as quercetin. Removal of the 3'-hydroxyl group from quercitrin, to give Kaempferitrin, results in coalescing of the K and the K2 bands to give one at 345 m, i.e. less displaced to longer wavelength on the other hand the removal of 5'-hydroxyl group from quercetin to give fisitin leaves the positions of the bands practically unchanged. The 3-hydroxyl group, whether bound as a glycoside or not, has little effect (cf. the spectra of butein and apigenin with those of quercetin and kaempferitrin respectively). The spectrum of the glycoside apigenin is similar to that of its aglycone apigenin. It may be noted, however, that in the spectra of apigenin and apinin a very weak additional band appears at 240-250 m,.

It is well known that the ultra-violet absorption spectra of the ions of many polar-substituted compounds are markedly different from the spectra of the compounds themselves, usually the main band having shifted 20 m, or more towards the red and increase in intensity, in the case of flavones which contain a number of similar polar groups, each group will contribute to the total bathochromic shift in ionisation, and if or more is blocked (for example by methylation or glycosidation) the resulting shift should be substantially different. Since it has been shown that except for flavones and their 3-glycosides, the normal ultra-violet spectra of most flavones and flavonol glycosides are similar to those of the parent aglycone, it was felt that the spectra of
the ions might afford an elegant method of differentiating such compound which had been obtained in too small a quantity (for example, separation by paper chromatography \(^{40,95}\)), for the normal chemical methods to be applied. It is evident that the position of attachment of sugar in flavone glycosides can be determined from the spectra of the ions of either the compounds themselves or the partial methylation of the corresponding aglycone obtained from them on a micro-scale \(^95\) by methylation and subsequent hydrolysis. This has been illustrated beautifully by the spectrophotometric study \(^21\) of apigenin and its 5,7,8,6- and 7,4'-dimethyl ethers first in absolute ethanol and then in 0.002 M sodium ethoxide in absolute ethanol. The spectra of two apigenin moroglucosides (7- and 4'-glucosides) have also been compared.

The absorption and fluorescence spectra of substituted flavones \(^{143}\) flavonols \(^{144}\), benzoyl derivatives \(^{145}\) and the absorption spectra of dihydroflavonols \(^{146}\) have been studied and discussed.

**Infra-red spectra.**

Infra-red spectroscopy has proved to be an especially useful tool for the identification of flavones and related compounds. Gargant and \(^5\) have measured the infra-red frequencies of a series of flavonoids and related compounds in Hugol or perfluoro hexafluoro in 146.

The carbonyl and hydroxyl frequencies of this series of compounds are presented in the following table \(^{147}\).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Frequency cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flavonone</strong></td>
<td></td>
</tr>
<tr>
<td>3',4'-Dihydroxy-</td>
<td>1660</td>
</tr>
<tr>
<td>3',4'-Diacetoxy-</td>
<td>1665</td>
</tr>
<tr>
<td>3',4',5,7-Tetrahydroxy-</td>
<td>1620</td>
</tr>
<tr>
<td>3',4',5,7-Tetraacetoxo-</td>
<td>1650</td>
</tr>
<tr>
<td>5-Hydroxy-3',4',7-trimethoxy-</td>
<td>1610</td>
</tr>
<tr>
<td>3',3',4',5,7-Pentahydroxy-</td>
<td>1542</td>
</tr>
<tr>
<td>3',3',4',5,7-Pentacetoxo-</td>
<td>1764, 1703</td>
</tr>
<tr>
<td>3',5-Dihydroxy-3',7-trimethoxy-</td>
<td>1606</td>
</tr>
<tr>
<td><strong>Chalcones (benzylacetophenones)</strong></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxy-</td>
<td>1635</td>
</tr>
<tr>
<td>2'-Acetoxy-</td>
<td>1732, 1678</td>
</tr>
<tr>
<td>2'-Benzoxy-</td>
<td>1736, 1634</td>
</tr>
<tr>
<td>2-Methoxy-</td>
<td>1649</td>
</tr>
<tr>
<td>4-Hydroxy-</td>
<td>1628</td>
</tr>
<tr>
<td>4-Acetoxy-</td>
<td>1763, 1668</td>
</tr>
<tr>
<td>4-Methoxy-</td>
<td>1657</td>
</tr>
<tr>
<td>4-Methoxy-2-hydroxy-</td>
<td>1618</td>
</tr>
<tr>
<td>2',4-Dihydroxy-</td>
<td>1620</td>
</tr>
<tr>
<td>2',4-Dimethoxy-</td>
<td>1649</td>
</tr>
<tr>
<td>2',4-Diacetoxy-</td>
<td>1764, 1666</td>
</tr>
<tr>
<td><strong>Flavone</strong></td>
<td></td>
</tr>
<tr>
<td>3,3',4',5,7-Pentacetoxo-</td>
<td>1733, 1640</td>
</tr>
<tr>
<td>3,3',4',5,7-Pentahydroxy-</td>
<td>1654, 1627</td>
</tr>
<tr>
<td>3,3',4',5,7-Pentamethoxy-</td>
<td>1637</td>
</tr>
<tr>
<td>3,4',5,7-Tetrahydroxy-flavone</td>
<td>1655</td>
</tr>
<tr>
<td>Rutinones</td>
<td></td>
</tr>
<tr>
<td>3,3',4',5,7-Pentahydroxy-</td>
<td>1655</td>
</tr>
<tr>
<td>3,3',4',5,7-Pentamethoxy-</td>
<td>1764, 1648</td>
</tr>
</tbody>
</table>

*Broad band, not sharply defined.*

*Exact position in doubt because of interference by phenyl band at 1605-1590 cm⁻¹.*

*liquid.*
It is a well known observation in infra-red spectroscopy that conjugation of ethylenic double bond, or a carbonyl group and double bonds causes shift from the normal position to a longer wave-length. Thus a conjugated carbonyl group in acetone shows a band at 1716 cm\(^{-1}\) while conjugation with one phenyl group as in acetoxyacetophenone lowers the frequency to 1687 cm\(^{-1}\) and conjugation with 3-6 phenyl groups as in naphthoacetophenone lowers the frequency to 1655 cm\(^{-1}\).

**Flatulones:** Unsubstituted flavanones shows a band at 1680 cm\(^{-1}\). Introduction of hydroxyl groups in the 3' and 4'-positions cause the carbonyl frequency to shift to 1665 cm\(^{-1}\). Acetylation of these groups causes a shift back to 1680 cm\(^{-1}\), a frequency identical with that of unsubstituted flavanones.

Incorporation of hydroxyl groups into the 5' and 6'-positions shifts the carbonyl frequency 7-20 cm\(^{-1}\). It is concluded that the following are important resonant structures having hydrogen bonding between the 3'-hydroxyl and the 6'-group.

\[
\text{\[H2\]}\quad \text{\[H2\]}
\]

7',4',1,7-tetrahydroxy-5-hydroxy derivative has a carbonyl frequency of 1618 cm\(^{-1}\) and, shows no band attributable to a hydroxy group.

Acetylation of the 3,3',4',5,7-penta hydroxy flavone shifts the carbonyl band from 1642 to 1703 cm\(^{-1}\). Since the acetoxy derivative...
derivatives has a higher carbonyl frequency than unsubstituted flavanone and 3',4',5',7-tetraacetoxy flavone, it appears likely that the 5-substituent is responsible for this effect.

**Chalcone:** Unsubstituted chalcone shows a carbonyl band at 1689 cm\(^{-1}\) which is due to conjugation with a phenyl group and an aliphatic double bond. Introduction of a hydroxyl group in the conjugated chelated 2'-position lowers the carbonyl frequency to about 1620 cm\(^{-1}\). Acetylation causes a return to the original unsubstituted position. The same considerations governing the carbonyl frequencies in acetophenone and flavanone derivatives apparently are operative in chalcones with the exception that enhancement due to the conjugated 2- and 4-position is increased.

**Flavone:** The flavone derivatives examined did not show marked lowering of carbonyl frequency when a hydroxyl group was present in the 5-position. Acetylation of the hydroxyl group decreased, rather than increased the carbonyl frequency. The 5-hydroxyl group is involved in chelation as apparent since the OH-band is absent in 5-hydroxy-3',4',7-tetramethoxy flavone. Introduction of a methoxyl group in the 5- position causes a shift to 1627 cm\(^{-1}\) for 1,3',4',6,7-pentamethoxy flavone. This value is 23 cm\(^{-1}\) lower than that of the corresponding flavone derivative. The lowering is due, at least partially, to increased conjugation, which is not possible in the chalcones.

As the infrared spectra in Nujol or perfluorocarbon melts have significant changes in the absorption spectra due to the interaction in the solid phase, Shaw and Simpson have measured,
the infra-red absorption spectra, in carbontetrachloride solution of a number of flavanones and flavones, in the carbonyl stretching frequencies, are recorded in the Table XVI.

Table XVI

<table>
<thead>
<tr>
<th>Compounds</th>
<th>3°C Frequency cm⁻¹</th>
<th>1°C Frequency cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flavanones:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Unsubstituted)</td>
<td>1605</td>
<td>1647</td>
</tr>
<tr>
<td>7-methoxy</td>
<td>1635</td>
<td>1652</td>
</tr>
<tr>
<td>7,4’-dimethoxy</td>
<td>1636</td>
<td>1637</td>
</tr>
<tr>
<td>5-hydroxy</td>
<td>1646</td>
<td>1649</td>
</tr>
<tr>
<td><strong>Flavones:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Unsubstituted)</td>
<td>1649</td>
<td>1659</td>
</tr>
<tr>
<td>7-methoxy</td>
<td>1645</td>
<td>1645</td>
</tr>
<tr>
<td>3’-methoxy</td>
<td>1625</td>
<td>1649</td>
</tr>
<tr>
<td>4’-methoxy</td>
<td>1657</td>
<td>1657</td>
</tr>
<tr>
<td>7,3’-dimethoxy</td>
<td>1638</td>
<td>1657</td>
</tr>
<tr>
<td>7,4’-dimethoxy</td>
<td>1649</td>
<td></td>
</tr>
</tbody>
</table>

* These compounds were not sufficiently soluble to give carbonyl absorption of greater than 20°. The reported figures may therefore be inaccurate.

**Flavanones:** It is seen from Table XVI that introduction of a methoxy group in the 7-position of the Flavanone nucleus causes a frequency shift of +10 cm⁻¹.

**Flavones:** Table XVI lists the differences between the carbonyl stretching frequency of substituted flavones and the corresponding flavanones. The unchelated compounds show the expected frequency decrease resulting from the increased conjugation of the carbonyl group. The difference between the carbonyl frequencies of 5-hydroxy flavone and 5-hydroxy flavanone is due to the unusual properties.
of the former chelated system (see below).

**Table XVII**

<table>
<thead>
<tr>
<th>Flavone substituent</th>
<th>None</th>
<th>7-MeO</th>
<th>7,3'-diMeO</th>
<th>5-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency difference (cm⁻¹) flavone-flavonone</td>
<td>-38</td>
<td>-45</td>
<td>-40</td>
<td>-4</td>
</tr>
</tbody>
</table>

All the 3-hydroxy flavones examined show only feeble absorption bands at 3360 cm⁻¹ suggesting partial suppression of hydroxyl character by internal hydrogen bonding. The shifts in carbonyl stretching frequency caused by the introduction of a hydroxyl group at the 3-position of the flavone nucleus are listed in Table XVIII.

**Table XVIII**

<table>
<thead>
<tr>
<th>Flavone substituents</th>
<th>Frequency shift(cm⁻¹) on addition of 3-OH group</th>
<th>Flavone substituents</th>
<th>Frequency shift on addition of 3-OH group</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>7,3'-diMeO</td>
<td>30</td>
</tr>
<tr>
<td>3'-OMe</td>
<td>-38</td>
<td>7-MeO</td>
<td>-19</td>
</tr>
<tr>
<td>3,3'-di-OMe</td>
<td>-52</td>
<td>7,3'-diMeO</td>
<td>-22</td>
</tr>
</tbody>
</table>

These again indicate chelation between the carbonyl group and 3-hydroxy groups and are interpreted by Shaw and Simson by the stabilisation of the ionic structure (and similar form) by hydrogen bonding.

Chemical evidence, chromatographic data and the small depression of melting point on admixture with water clearly establish 5-hydroxy flavones as chelated compounds. Moreover these compounds show no absorption in the region 3200-3800 cm⁻¹. It is
but however (Table XIX) that introduction of a 5-hydroxyl group into flavone increases or at most only slightly decreases the carbonyl stretching frequency $^{147}$. In this respect 5-hydroxy flavones differ from other peri-hydroxy carbonyl compounds.

<table>
<thead>
<tr>
<th>Flavone sub-</th>
<th>Frequency shift</th>
<th>Flavone sub-</th>
<th>Frequency shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-OMe group</td>
<td>(cm$^{-1}$) on addition</td>
<td>C-OMe group</td>
<td>(cm$^{-1}$) on addition</td>
</tr>
<tr>
<td>None</td>
<td>$\delta$</td>
<td>4'-OMe</td>
<td>$\delta$</td>
</tr>
<tr>
<td>7-OMe</td>
<td>$\delta$</td>
<td>7, 3'-OMe</td>
<td>$\delta$</td>
</tr>
<tr>
<td>3', 4'-OMe</td>
<td>$\delta$</td>
<td></td>
<td>$\delta$</td>
</tr>
</tbody>
</table>

Introduction of a 3', 4'-OMe group into 5-hydroxy flavones causes the absorption of the C=O stretching to be intensified and shifted to $165$ cm$^{-1}$, indicating a decrease in overall hydrogen bonding. These observations are in agreement with the conclusion from chromatographic evidence, that the two chelate systems are opposed. Table XIX shows the change in carbonyl stretching frequencies caused by the introduction of a 7-methoxyl group into unchelated flavones.

<table>
<thead>
<tr>
<th>Flavone sub-</th>
<th>Frequency shift</th>
<th>Flavone sub-</th>
<th>Frequency shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-OMe group</td>
<td>(cm$^{-1}$) on addition</td>
<td>C-OMe group</td>
<td>(cm$^{-1}$) on addition</td>
</tr>
<tr>
<td>None</td>
<td>$\delta$</td>
<td>11-OMe</td>
<td>$\delta$</td>
</tr>
<tr>
<td>7-OMe</td>
<td>$\delta$</td>
<td>3', 4'-OMe</td>
<td>$\delta$</td>
</tr>
</tbody>
</table>

As with flavanones these decreases are ascribed to conjugation of methoxyl and carbonyl group. Addition of the less conjugated 4'-methoxyl or the unconjugated 3'-methoxyl groups causes the
expected small changes in carbonyl frequency, but these are of the same order as the expected errors in measurement.

In the interpretation of infra-red spectra the analyst should take into consideration all the empirical constants established by the numerous workers throughout the world. It is absolutely necessary to know all the published data about infra-red spectroscopy and great care must be taken to ensure that they are up to date. But unfortunately they are so much widely distributed in different journals some of which are very difficult to obtain. Any way we find in the literature few tables resuming these informations which are undoubtedly of great value but the details are always missing.

The infra-red spectroscopy is a technique which has a great future and permits to elucidate the structure of the natural or synthetic molecule and differentiate between the isomers which are difficult to study by other methods.

(d) DISTRIBUTION OF THE AGLYCON.

The tool in the hands of a chemist for establishing the structure of any compound is to degrade it in to its simpler fragments and to compare them with the original substances. Finally the structure is confirmed by synthesising the compound from simple known substances and the product so obtained is compared with the original one. The same technique has been applied in case of aglycone by Kostanecki, Herzig and Perkin.
(i) **Alkaline fusion:** By fusion with alkali the pyrone nucleus is disintegrated into a phenol and an aromatic acid. Thus with flavone we have phenol and benzoic acid.

![Chemical Structures](image)

(XXXIII) → Phenol (XXXIV) + Benzoic Acid (XXXV)

(ii) **Boiling hydrolysis:** By boiling the flavone with a concentrated solution of alkali (NaOH) 6 hours, the degradation of the molecule takes place in stages and adopts two courses.

![Chemical Structures](image)

- (XXXVII) → (XXXVIII) + (XXXIX)
- (XXXIX) → (XL) + U-vinylc acid dimethyl methane (XLII) + (XLIII)
It is worthy of note in this connection that if glycoside itself is boiled with alkali solution (25°, 5 hours), the molecule undergoes similar degradation without any change of the carbohydrate position into p-hydroxy acetophenone and a phloroglucinol glycoside which Vongerichten named as apiose-glucose chloroglucin.

Methods introduced by Paul Karrer for the purpose of establishing the precise nature of the phenyl residue in position 2 and the point of linkage of the sugar residue have proved fruitful and reliable. Prior to Karrer's work the position of the methoxyl residue into anthocyanidin groups, peonidin, malvidin and himidalin was not known, since the concentrated alkali employed to degrade the pigment also removed the methoxyl groups. Karrer's degradation of the sugar-free pigment, with dilute barium or sodium hydroxide (10") in an atmosphere of hydrogen, which yielded the phenolic acid with the methoxyl group intact was therefore a significant advance. The results obtained by this method were confirmed through a second method wherein a degradative oxidation with hydrogen peroxide was first employed to open the ring.
of the anthocyanidin between carbon atoms 2 and 3 without removing either the sugar residue or the methoxyl group. The resulting intermediate could subsequently be hydrolysed by dilute alkali solution to the corresponding methylated phenolic acid and the phloroglucinol derivative which would contain sugar residue and an acid side chain. Unfortunately the phloroglucinol derivative has not so far been isolated in crystalline form.

The course of these degradations can be illustrated with the di-glucoside of malvin chloride, (III). Starting with malvidin chloride (II) degradation with dilute alkali gives on the one hand phloroglucinol (I) and syringic acid (3,5-dimethoxy gallic acid) (II).

The oxidative degradation with hydrogen peroxide transformed the di-glucoside (malvin chloride) (III) into an intermediate malvin, whose exact constitution is not known but which can be represented either by structure III or IV. The different products obtained by the degradation of malvin with dilute sodium hydroxide have been shown schematically blow.
c) SYNTHESIS OF FLAVONES

Kostanecki and his students (1898-1907) have shown that flavones are obtained by treating the dibromo-derivatives of chalcone of type LVIII, that is to say o-hydroxy phenyl-styryl ketones by the alcoholic potash.

\[
\text{LVIII} \quad + \text{Br}_2 \quad \rightarrow \quad \text{LIX} \quad + \text{KOH Alc.}
\]

\[
\text{LX} \quad \leftarrow \quad \text{LX} \quad \text{(isolated)}
\]
Kostancki’s first method for the synthesis of flavones which involves the action of cot alcoholic alkali on dibromide of o-hydroxy or o-acetoxyl phenyl ketones is limited in its application, as instead of flavones the isomeric 2-benzylidene-coumaranones are frequently obtained.

Several attempts have been made to explain the dual product ion of flavones and the isomeric benzylidene coumaranones. Hutchings and Wheeler, however, observed that o-hydroxy chalcone dibromides in general, give flavones when they are heated above their melting points under reduced pressure, or are heated with alcoholic potassium cyanide.

O-hydroxy acetophenones (LXV) condense with substituted benzaldehyde in the presence of basic or acidic condensing agent, to give either a chalcone (LXVI), flavanone (LXVII), a 3-arylidene flavanone (LXVIII), or a mixture of these.
Dry hydrogen chloride in dry ethyl acetate converts benzoyl derivatives of o-hydroxy acetophenones and benzaldehyde into benzoyl chalcones. These compounds can be hydrolysed to the free chalcones with alkali under nitrogen. Resacetophenone gives the flavanone in place of chalcone, with dry HCl in absolute alcohol the flavanone or e-aryliden flavanone, or a mixture of these is formed.

The o-hydroxy chalcones prepared by these various processes may be cyclised to flavanones by dilute base or by acids. Bases which accomplish cyclisation are 1.5% sodium hydroxide and sodium acetate. The latter is of special value in the preparation of glucoside derivatives, acids that have been used are dilute hydrochloric acid, sulphuric 0.2% sulphuric; d-camphorsulphonic and phosphoric acids.

Chalcones of the type o-hydroxy-α-methoxy (LXXX) when cyclised with HCl give 3-hydroxy flavanone (LXXI). Similar products can be obtained by mild treatment of chalcone (LXXXI) in alkali with hydrogen peroxide.

The cyclisation of arylxy or alkoxy-dibenzoyl-methane under the action of sulphuric acid, hydrochloric acid, hydroiodic acid alone or in presence of acetic anhydride or by hydrobromic acid in acetic acid or sodium acetate in acetic acid leads in all cases to the formation of flavones. These dibenzoyl-
methanes (LXXIV) may be obtained by the condensation of (LXXII) c-alkoxy acetophenone with the ester of the acrylic acid (LXXIII) in presence of sodium, c-alcoyl esters (LXXV) with hydroxy acetophenone (LXXVI) in presence of sodium and by the rearrangement of c-benzoic ester of c-hydroxy acetophenone (LXXVII) in toluene, benzene or ether with potassium or sodium carbonate,178, sodium175, sodamide174, sodium ethoxide or sodium hydroxide175 (Baker-Venkataraman rearrangement).

\[
\text{(a) } \begin{array}{c}
\text{CH}_3 \text{O} \\
\text{C} \equiv \text{N} \\
\text{OCH}_3 \\
\text{OCH}_3 \\
\text{OCH}_3
\end{array}
\quad + \quad \begin{array}{c}
\text{C}_2\text{H}_5\text{O} \quad \text{CO} \quad \text{C}_6\text{H}_5
\end{array}
\quad \rightarrow \quad \begin{array}{c}
\text{CH}_3 \text{O} \\
\text{CH}_3 \text{O} \\
\text{OCH}_3
\end{array}
\text{(LXXII)}
\]

\[
\text{(b) } \begin{array}{c}
\text{CH}_3 \text{O} \\
\text{C} \equiv \text{N} \\
\text{OCH}_3 \\
\text{OCH}_3 \\
\text{COOC}_2\text{H}_5
\end{array}
\quad + \quad \begin{array}{c}
\text{C}_2\text{H}_5\text{O} \quad \text{CO} \quad \text{CH}_3
\end{array}
\quad \rightarrow \quad \begin{array}{c}
\text{C}_2\text{H}_5\text{O} \\
\text{CH}_3 \text{O} \\
\text{OCH}_3
\end{array}
\text{(LXXV)}
\]

\[
\text{(c) } \begin{array}{c}
\text{CH}_3 \text{O} \\
\text{C} \equiv \text{N} \\
\text{OCH}_3 \\
\text{OCH}_3 \\
\text{COOC}_2\text{H}_5
\end{array}
\quad + \quad \begin{array}{c}
\text{Na} \\
\text{C}_2\text{H}_5\text{CNa}
\end{array}
\quad \rightarrow \quad \begin{array}{c}
\text{C}_2\text{H}_5\text{O} \\
\text{CH}_3 \text{O} \\
\text{OCH}_3
\end{array}
\text{(LXXVII)}
\]

\[
\text{(LXXVIII) } \begin{array}{c}
\text{OH} \\
\text{OH} \\
\text{OH}
\end{array}
\quad + \quad \begin{array}{c}
\text{H}_3\text{HBr}, \text{HCl} \quad \text{etc.}
\end{array}
\quad \rightarrow \quad \begin{array}{c}
\text{H}_3\text{COONa} \quad + \quad \text{CH}_3\text{COOH}
\end{array}
\]

\[
\text{(LXXX) mono-methyl ether of primetine}
\]

174. Venkataraman rearrangement.
These syntheses have been utilised by the numerous authors authors174,175,186.

The use of ω-2-dibenzoyl acetophenone (LXXI) with potassium acetate and alcohol brings about the formation of flavonol167,185 (LXXXII).

\[
\text{COO-C}_6\text{H}_5 + \text{CH}_3\text{COOK} + \text{Alcohol} \rightarrow \text{(LXXXII)}
\]

The method put forward by Baker and by Chavan and Robinson has got the advantage that it does not require very strong conditions of operation. Chavan and Robinson167 obtained galangin in boiling alcohol (mild condition) by this method. Baker188 has shown that the transformation of the dibenzoyl methanes to flavones goes on equally well at the ordinary temperature. This is of great importance for the synthesis of glycosides of the flavones corresponding to natural flavonoids.

The above method suffers from the disadvantage of using alcoyld derivatives of the acetophenone from which are obtained alcoxy flavones and flavonols. The protected groups are then liberated to free hydroxyl group which is not always possible.

On the contrary the synthesis proposed by Alina–Robinson permits directly to obtain flavones or flavonols with free hydroxyl nomenclature. It consists in heating the acetophenone with anhydride and the sodium salt of the aromatic acid at 180–185° for 3 hours. If a ω-methoxyacetophenone is employed the corresponding
flavonols are obtained. The acetophenones employed are most usually resacetophenone, phloroacetophenone and their methoxy derivatives.

\[
\text{(LXXXIII)} \quad \begin{array}{c}
\text{HO} \\
\text{CH} \\
\text{COCH₃}
\end{array} + \begin{array}{c}
\text{C₆H₅COONa} \\
\text{(C₆H₅CO)₂O}
\end{array} \rightarrow \begin{array}{c}
\text{HO} \\
\text{CH} \\
\text{CO-CH₂-CO=C₆H₅}
\end{array}
\]

(LXXXIV)

(LXXXV)

The intermediate β-diketone formed is cyclised by heating for 30–45 minutes with an aqueous alcoholic potassium hydroxide solution. The employment of the methylated starting material with partial or total demethylation with hydroiodic acid or aluminium chloride in nitrobenzene results in the formation of the whole of the series of flavones and flavonols. This reaction has been abundantly utilised by Robinson and his students in particular and more recently by the Indian school.

The method of Allan and Robinson is certainly one of the most practically and most frequently utilised for the synthesis of flavonols and flavones for on the one hand it is very easy to synthesise the starting material, hydroxy acetophenone and acids and on the other hand due to its great usefulness and generality.

Shinoda and Sato have applied the synthesis of Kostanecki to the compounds containing free OH groups in employing the reaction shown by Behn in condensing the chloride of cinnamic acid or hydrocinnamic acid with polyhydroxy phenols by AlCl₃ in nitrobenzene and thus obtained the chalcone or hydrochalcone which then leads to the flavanones.
With resorcinol the principal product is the chalcone while
with phloroglucinol it is a flavanone. The authors think that the
presence of hydroxyl group in 6-position makes the hydroxyl group
in 2-position so reactive that the intermediate chalcone formed
cyclizes immediately to flavanone. The optimum conditions of the
synthesis have been precised by Huzise and Tatsuji.122

The findings of Narasimhachari and Seshadri133 explain these
results easily. These authors in affect well placed the evidence
of the role played by hydroxyl group in 6-position in the molecule
of the flavanone (therefore in the 6-position in the corresponding
chalcone) on the stability of these products.

The flavanones having a hydroxyl group in 5-position like
that of the derivatives of phloroglucinol do not transform in chalcone
by heating with $\text{H}_2\text{SO}_4$, while those which do not have
them, like the derivatives of resorcinol transform easily. All the
same, the former dissolved easily in $\text{NaOH}$, in cold and are
precipitated intact by acidification, while the latter dissolved in
$\text{NaOH}$ on heating only, and the acidification of the solution gives
the chalcones. It gives by chelation one hydrogen bonding between
hydroxyl groups in 5-position and C-G group in 4 which stabilizes
the molecule and hinders its easy transformation to chalcone.

_From Chalcones, Flavanones and Benzovillium Salts; Chalcones
and Flavanones can be converted into Flavones and Flavonols in
several ways. Oyamada has shown that the hydroxy chalcones trans-
form to flavonols when treated with \( \text{H}_2\text{O}_2 \) in presence of dilute
alkali. Algar and Flyn have proposed the mechanism for explaining
the reaction. Evidence indicates that the reaction proceeds through
the chalcone (LXXXIX) to give an intermediate (XC) which give a
3-hydroxy flavanone (XI). This compound is then oxidised to
flavonol (XCI). The 3-hydroxy flavanone (XI) also may be dehydro-
genated to the flavonol (XCI) by means of palladium with cinnamic
acid or maleic anhydride as hydrogen acceptor.

This method was used in the synthesis of 7,8- and 6,7,8-hydroxy
flavonoids. Recently Geismann and Fukushina reported the forma-
tion of brazili-coumaranones by the oxidation of 2-hydroxy chalcones
with a 3-hydroxy group in the 6-position. A similar observation
was made by Seshadri and co-workers who showed that even a 6-methyl
group produces the same effect. Venkataraman et al observed that
when a chalcone with a free hydroxy in the 4'-position was treated with alkaline hydrogen peroxide, a flavonol was produced even when a methoxyl was present in the 6-position. Recently Seshadri and co-workers have shown that the presence of a methoxyl group in the 6-position of the chalcone eliminates the formation of benzal coumaranones and gives only flavonols even when the 6-position is substituted.

For the preparation of a flavonol, Kostanekci and Lampe first converted the chalcone into the flavanone by boiling with alcoholic sulphuric acid. On treatment with amyl nitrite and concentrated hydrochloric acid, the flavanone yielded an iso-nitroso derivative which was hydrolysed subsequently by boiling with dilute mineral acids to the flavonol. An improvement was made by Seshadri et al. who combined the last two stages in one operation.

The first method of synthesis of flavones employed chalcones as the starting materials. But the chief defect in this method of Kostanekci using bromine as the reagent is the possibility of nuclear bromination and of the predominant tendency to form benzal coumaranones (XCVII) instead of flavones (XCVIII). A recent improvement is that of Zemplen and Boglar who have submitted the acetate of hydroxy flavonones to bromination in the presence of
ultra-violet light whereby the bromine atom enters the 3-position alone (XCVIII). This is subsequently eliminated as hydrogen bromide by means of alcoholic potash.

An important advance is the direct dehydrogenation of chalcones or flavanones by means of selenium dioxide. But it has been found to be successful only in a few simple cases and seems to fail when the chalcone or flavanone contains a number of free hydroxyl groups. Phosphorous pentachloride may also be used for the dehydrogenation of flavanone methyl ethers, but the use of iodine in the presence of sodium acetate seems to be more satisfactory. In alcoholic solution and in the presence of sodium acetate, iodine brings about the oxidation of hydroxy flavanones rapidly and very good yields of the corresponding flavones are obtained. The method is suitable also for the oxidation of hydroxy flavanone glycoside.

Methods of interconversion have interest not only in structural studies but also in connection with biogenesis. The most attractive was the conversion of a flavonol, quercetin, into an anthocyanidin, cyanidin by reduction. The reverse process of...
oxidising a pyyllium salt of a flavone has not attracted much
attention but has been used by Robinson and Schwarzenbach\textsuperscript{218}
for an ingenious synthesis of Scutellarcin.

Particularly interesting are the researches of Karrer on the
oxidation of benzopyryllium salts to flavonols. The gentle oxidation
of anthocyanidin by monoperphthalic acid gives flavonol. Also the
chloride of 2-phenyl-5-methoxy benzopyryllium treated with perphthalic
acid gives 2:3-dimethoxy flavanone, which is transformed to flavonol
by heating at reflux with a mixture of 20 cc methanol, 2 cc of
water and 80 cc HCl acid for 3 hours. The chloride of 7:7:4-trimethoxy-
2-phenyl-benzopyryllium by treatment with perphthalic acid at the
ordinary temperature for a few hours, then by the treatment of
sodium bicarbonate solution gives 2:3-dimethoxy flavanol.

Very recently Miss Grove and collaborators\textsuperscript{225} have proposed
an elegant synthesis of flavones and flavonols by simple thermal
cyclisation of the ester of o-hydroxy aceto-phenones. The heating
at \( 350^\circ \) for 30 minutes in the medium of anhydrous glycerol transforms
the ester of o-hydroxyaceto-phenone to flavones or flavonols with the
yield several times comparable to that of method of Baker and
Vankatarasan. It is possible that it forms a dibenzo1 methane by
a thermal mechanism but the analogy has been observed with the
rearrangement of Baker and Vankatarasan. In presence of an acid
anhydrons flavonoid-pyrones are obtained.
Iyer and Venkataratnam²¹⁴ have put forward a curious reaction by the help of which 3-hydroxy flavones are transformed to 5,6-dihydroxy flavones by coupling with benzene diazonium chloride $\text{C}_6\text{H}_5N_3\text{H} = \text{HCl}$. The coupling takes place in 5-position giving 5-phenylazo-6-hydroxy flavones which by reduction with $\text{Zn} + \text{CH}_3\text{COOH}$ is transformed to 5-amino derivative. The 5-amino derivative by boiling with $\text{H}_2\text{SO}_4$ decomposes and gives 5,6-dihydroxy flavone.

The introduction of a hydroxyl group in ortho to a free CH₂ group can still be realised by the reaction given for the chromones and flavones by Rangaswami and Seshadri²¹⁵. It consists in condensing the phenol with hexamethylene tetramine in acetic acid at 100° for six hours, then heating with half or one-third HCl. The corresponding o-aldehydes are obtained which by oxidation with $\text{P}_2\text{O}_5$ in alkaline solution are transformed to o-diphenols.

Method based on Nuclear Oxidation: Seshadri and collaborators²¹⁶ have shown that the partial oxidation of benzopyrone nucleus can be realised in the flavones and flavonols by treating their alkaline
solution with a solution of potassium persulphate (4 g in 60 cc of water) at the cold temperature (15-20°) by shaking 3 hours. It is left overnight, neutralised with hydrochloric acid and the untransformed flavone is precipitated. It is extracted with ether. The product of oxidation after hydrolysis is isolated by crystallisation.

The application of this method to 5-hydroxy flavones or 5-hydroxy flavonols leads to 5,3-dihydroxy derivatives. The oxidation of acetophenone itself can also be accomplished. With the 7-hydroxy flavones and flavonols, 7,3-dihydroxy compounds can be obtained but with a very poor yield. Later on Seshadri et al. observed that higher members of 6-hydroxy flavones are more facile to persulphate oxidation than those of 5-hydroxy flavones. The reason for the difficulty in the oxidation of higher members may be that in these compounds the 5-hydroxy group is not activating adequately the 3-position. It may be mentioned that these substances are sparingly soluble in aqueous alkali because of the chelate bond between the 5-hydroxyl and the carboxylic group.

Method based upon nickel reduction. In the course of work on Raney nickel reduction Ramanaidu and Venkataraimu observed that the application of the method of Kenner and Murray for the cleavage of sulphonate esters with Raney nickel leads to a convenient method for the preparation of \( \gamma \)-resorcylic acid by the hydrogenolysis of the mono-o-p-toluene sulphonyl derivative of phloroglucinol carboxylic acid. Extending this procedure to the flavone series, the authors have converted chrysin (5,7-dihydroxy flavone) to 5-hydroxy flavone and galangin 3-methyl ether.
A general method has thus become available for the synthesis of 5-hydroxy flavones, which are of interest because of their possible occurrence in nature and also as intermediate for the synthesis of naturally occurring 5,2-dihydroxy flavones by persulphate oxidation.

The process involves two steps (i) tosylation (p-toluenesulphonyl chloride, excess of anhydrous potassium carbonate in boiling acetone and (ii) hydrogenolysis of the tosyl derivatives under controlled and mild conditions with Raney nickel.

Jain and Seshadri\textsuperscript{222} in continuation of their previous publication\textsuperscript{221} carried out the reduction of hydroxyl groups in 5-position partial methyl ethers of chrysin, apigenin, galangin and quercetin were subjected to nuclear reduction through their tosylxy compounds. The products were 7-methoxy flavones, p-tetol methyl ether, 8,7-dimethoxy flavone and fisatin tetramethyl ether respectively. Nuclear reduction was also carried out successfully by the same authors\textsuperscript{222} in the side phenyl nucleus. Thus o-tetramethyl quercetin with OH in 3'-position and trimethyl ether of kaempferol with a free
OH group in 4'-position, yielded maempherol tetramethyl ether and galangin trimethyl ether. Ramathan and Venkataraman prepared 3-hydroxy-3',4'-trimethoxy flavone from 5,7-dihydroxy-3',4'-trimethoxy flavone by nuclear reduction.

Kostanecki and his collaborators showed that Brull and Frielander had obtained 2-arylidene coumaran-3-ones (CXIII) and tot flavones (CXII) by the reaction of ω-halogeno-o-hydroxy acetophenone (CXI) with aromatic aldehydes in presence of alkali. It has now been found that if the condensation is carried out in the cold in presence of excess of alkali, the corresponding flavonol (CXVI) is generally obtained in a yield up to 55%. Twelve flavonols have been prepared in this way. With increase in temperature or a deficiency in alkali 2-arylidene coumaran-3-ones (CXIII), hitherto the only known products are obtained.
Isomerization of Flavones and Related Compounds.

In the synthesis of most flavones, demethylation is an essential step and hydroiodic acid is the reagent most commonly used. During hydroiodic acid demethylation rearrangement of a 5,7,3'- to a 5,6,7-trihydroxy flavone was observed by Wessely and Moser who obtained 5,6,7,4'-tetrahydroxy flavone (CXVIIIa) (Scutellarein) from 7-hydroxy-5,8,4'-trimethoxy flavone (CXVIIa)

\[
\text{(CXVII)}
\]

(a) \( R_1 = \text{CH}_3 \); \( R_2 = \text{H} \); \( R_3 = \text{OCH}_3 \)
(b) \( R_1 = R_2 = R_3 = \text{H} \)
(c) \( R_1 = \text{CH}_3 \); \( R_2 = R_3 = \text{H} \)
(d) \( R_1 = R_2 = R_3 = \text{H} \); \( \text{CH}_3 = \text{H} \)

The view was later confirmed by Wessely and Källabö who found that contrary to the results of Nattori 5,7,3,4'-tetramethoxy flavone on treatment with HI suffered isomeric change to Scutellarein. This important type of rearrangement of a 5,6- to a 5,8- configuration during demethylation under the influence of hydroiodic acid is known as Wessely-Moser rearrangement and was first observed in flavones. The rearrangement is not
only confirmed to flavones but is exemplified by most of the compounds related to flavones eg. flavonols, chromones, chromonols, xanthones and isoflavones.

**Flavones:** It has been shown by Shah, Mehta and Wheeler\(^233\) that wogonin (CXVIIb) and its dimethyl ether (CXVIIc) undergo isomerisation to baicalin (6,6,7-trihydroxy flavone (CXVIIb) with HI in presence of acetic anhydride when heated under refluxing at 145-150° on an oil bath for an hour. It was also observed by the same authors that dimethyl ether of wogonin (CXVIIc) when treated with aluminum chloride in dry nitrobenzene undergoes normal demethylation to 5,7,8-trihydroxy flavone (CXVIIb) (see also Sastri and Seshadri\(^204\)). A similar observation was made in the demethylation of the primatin (5,3-dihydroxy) series of flavones and these give rise to the corresponding 5,6-dihydroxy flavones\(^236\). This isomeric change has been used for preparative work by Rao, Seshadri and Viswanadham\(^238\).

In certain cases the isomeric change takes place even under comparatively mild conditions eg. norwogonin to baicalin, completely even when boiled for six hours with a mixture of glacial acetic acid and concentrated hydrochloric acid (1:1)\(^239\) and also with other acids eg. 6-amino-5-hydroxy flavone and primatin were converted by boiling with 72% hydrochloric acid or 50-80% sulphuric acid into 6-amino compound and 5,3-dihydroxy flavone respectively.\(^240\)

**Flavonols:** All the above mentioned cases relate to flavones without a substituent in the 7-position. Though a number of flavonols (3-hydroxy compounds) having the 3,7,8-arrangement of hydroxyl groups had been synthesised and examined, they were not known to
undergo isomeric change during demethylation (See, gossypetin, herbacetin, hibiscetin and 8-hydroxy galangin). Briggs and Lockyer, however, found that the flavonols, melitertinin (CIX), 5,6-dimethoxy-7,3',4'-dimethyl-1,4'-dioxo-flavone, and ternatin (XX) (6,4'-dihydroxy-3,7,8,5'-tetramethoxy flavone) isolated from the bark of Helicopha terneta undergo rearrangement under the drastic conditions of demethylation by hydriodic acid and phenol at 150-160°C to querceagatin (XXI) (7,5,6,7,3',4'-hexahydroxy flavone) in conformity to the rearrangement of a 5,3'- to a 5,6'-configuration. Much unexpectedly a third flavonol, melitertinin (CXXI) (7,5,6,7,8-tetramethoxy-3',4'-dimethylene-dioxy flavone) isolated from the same source has been shown by Briggs and Lockyer not to undergo isomerisation but to furnish gossypetin (XXII) (7,5,7,6,3',4'-hexahydroxy flavone) under the same condition of demethylation as employed earlier.
In this connection, Seshadri, Varadarajan and Venkatswarlu further made a study of the simplest compounds having the requisite structure i.e. 3'-methoxy primatin and found that there was no isomeric change.

Another example of rearrangement of the above type is furnished by 3,3',4'-trimethyl ether of gossypetin (CXXIV). When this compound is demethylated under the conditions employed by Briggs and Locker, yields gossypetin (CXXIII) and only a trace of quercetagetin (CXXI). Under sufficiently drastic conditions i.e. treatment with hydrochloric acid and phenol at 180-190° under pressure the compound (CXXIV) rearranges to (CXXI). It is clear therefore, that 5,8-dihydroxy flavonol may rearrange when treated with HCl under sufficiently drastic conditions. Compound (CXXIV) with AlCl₃ in benzene yielded gossypetin (CXXIII).

2'-Hydroxy(methoxy) compounds: The possibility of a new type of ring isomeric change involving the 2'-hydroxy(methoxy) group of a flavone was suggested by the work of Cullinane, Algar and Ryan. A recent study of the synthesis of 5,7,2'-trihydroxy and 5,7,2',4'-tetrahydroxy flavones has shown that under ordinary conditions of demethylation isomeric change does not occur. Flavonols of this type also do not under go isomeric change. However, Gallagher et al. have recorded that certain 2'-methoxy flavones rearrange during
demethylation with hydriodic acid under sufficiently drastic conditions to give the related 2'-hydroxy flavones in which the 2-phenyl group and the fused aromatic ring of the original flavone are interchanged. Demethylation by aluminum chloride does not produce rearrangement. The more stable of each pair of related 2'-hydroxy flavone investigated is that in which the 2-phenyl side chain contains the smaller number of hydroxyl groups. Philbin and Wheeler pointed out earlier that some 2'-methoxy flavones (CXXV) when demethylated by hydriodic acid under sufficiently drastic conditions yield by rearrangement the corresponding 2'-hydroxy flavones (CXXVI) with presumably intermediate formation of a di-ketone of the disalicyloyl-methane type (CXXVII). The methoxy flavones corresponding to (CXXVII) did not rearrange under similar conditions.

More examples in this connection are furnished by the work of Duporte et al. Both 5,8,2'-trimethoxy flavone (CXXVIII, R=Me) and the 2',3',6'-isomer (CXXIX, R=Me) yield 5,6,2'-trihydroxy flavone (CXXXI, R=H) on demethylation by hydriodic acid. This result confirm previous views (See Gallagher et al.251) that the diketone produced by decyclisation eg. (CXXX) is intermediate in the rearrangement of both 5,8-dihydroxy and 2'-hydroxy flavones. The presence of hydrogen ion is necessary for the 5,8-→ 5,6-rearrangement of flavone. Similarly 7,2',4',6'-tetramethoxy flavone (CXXXIII, R=Me) rearranges to form (CXXXII, R=H) on treatment with hydriodic acid.
Flavanones: The flavanones are capable of ring fission into chalcones even in weak acid medium. At the same time when there are two hydroxyl groups in the 2- and 3- positions the chalcone structure becomes unstable and the corresponding flavanone tends to be produced. Consequently the ring isomeric change should be highly facile in this group. The earliest study of this related to the hydrolysis by dilute acid of carthamin, a chalconic glycoside, giving rise to a mixture of isomeric flavanone, carthamin and iso-carthamin. It was recorded by Kuroda that the former (5,7,8,4'-tetrahydroxy flavanone), when heated with water in a sealed tube or in the presence of animal charcoal, undergoes change into the latter (5,6,7,4'-tetrahydroxy flavanone). Recently efforts to prepare 7-methoxy-5,6-dihydroxy flavanone using partial demethylation of (CXXXV), with hydrobromic acid have yielded only
5,6-dihydroxy-7-methoxy flavanone (CXXXVI). The change does not take place if aluminum chloride is used as a demethylating agent.

Flavilium salts: No isomeric change was observed when 5,3,4'-trimethoxy-7-hydroxy flavilium chloride was subjected to demethylation with hydriodic acid. Earlier, 5,6,7,4'-tetramethoxy flavilium chloride was studied by Chapman, Perkin, and Robinson who showed that even in this case there was no change. Obviously the pyrylium ring is stable in acid solutions.

Coumarins: The possibility of similar isomeric change in polyhydroxy coumarins has also been examined using the methyl ethers of 4-methyl-5,7,3'- and 4-methyl-5,6,7-trihydroxy coumarins. They suffer smooth demethylation with boiling hydriodic acid without isomeric change.

Chromones: The important observation of an extraordinary nature and the change, just the opposite what usually happens is recorded by Schmid who found that 2,8-dimethyl-5-hydroxy-7-methoxy chromone (eugenitin)(CXXXVII) on demethylation with hydriodic acid undergoes isomeric change to isoeugenitol (2,8-dimethyl-5,7-dihydroxy chromone)(CXXXVIII). Further, isoeugenitin methyl ether (2,8-dimethyl-5,7-dimethoxy chromone) (CXXXIX) is demethylated to isoeugenitol (CXXXIX) without undergoing any isomeric change.

These findings are confirmed by Zakerjee, Vardarajan and Geshadri.
5,8-dihydroxy chromonols on treatment with hydriodic acid under sufficiently drastic conditions undergo an isomeric change (Wessely-Koser rearrangement) to form the corresponding 5,6-dihydroxy compounds. Chakravarty, Chakarjee, Murty and Seshadri concluded that derivatives of 5,8-dihydroxy chromonols (CXL, R₁=R₂=R₃=H) did not undergo the well-known Wessely-Koser rearrangement to the corresponding 1,6-dihydroxy chromonols (CXXIII) on treatment with hydriodic acid. It has now been found that on demethylation by hydriodic acid under pressure 5,7,8-trihydroxy-β-methoxy-2-methyl chromone (CXLIII, R₂=H, R₁=OCH₃) gives 3,6,7-trihydroxy-2-methyl chromonol (CXLIV, R₂=H, R₁=OCH₃).
Xanthones: 1,4-dihydroxy-7-methoxy xanthone (CXLII; $R^1=\text{Me}$) when treated with hydriodic acid under pressure rearranged to form 1,2,7-trihydroxy xanthone (CXLIV). Nao and Seshadri\textsuperscript{264} found that 1,4-dihydroxy-7-methoxy xanthone (CXLII; $R=\text{Me}$) when treated with hydriodic acid did not undergo the isomeric change but gave the normal product 1,4,7-trihydroxy xanthone (CXLII; $R=\text{H}$). It was now been found\textsuperscript{265} that compound (CXLII; $R^1=\text{Me}$) when demethylated by hydriodic acid under sufficiently drastic conditions yield, by rearrangement the corresponding 1,2,7-trihydroxy xanthone (CXLIV) with, presumably, intermediate formation of the diaryl ketone (CXLIII). This is in agreement with the mechanism proposed for this type of rearrangement in flavones\textsuperscript{265}.

![Chemical Structures]

Isoflavones: The arrangement of 5,8- to 5,6-dihydroxy flavones and chromones under the conditions of demethylation with hydrogen bromide or iodide is well established and is due to the hydrolytic opening of the pyrone ring and closure in the alternative direction involving the hydroxyl group initially in position 5. As expected such changes occur with the isoflavones, e.g. 5,7,8-trihydroxy-2-methyl isoflavone (CXLIV: $R=\text{OH}$, $R^1=\text{Me}$, $Ar=\text{Ph}$) when boiled for 8 hours with hydrobromic and acetic acids gives the 5,6,7-isomer (CXLIV: $R=\text{OH}$, $R^1=\text{Me}$, $Ar=\text{Ph}$)\textsuperscript{267}. It has now been found\textsuperscript{268} that demethylation
of 5,7-dihydroxy-3,3',4',5'-tetramethoxy isoflavone (CXLV; \( R=\text{OMe}, R''=\text{H} \), \( \text{Ar}=3,4,5\text{-trimethoxy phenyl} \)) gives 5,6,7,3',4',5'-hexahydroxy isoflavone (irigenol) (CXLVI; \( R=\text{OH}, R''=\text{H} \), \( \text{Ar}=3,4,5\text{-trihydroxy phenyl} \)). The conditions of the reaction are the deciding factor in controlling whether or nor the rearrangement occurs. Thus 5,7,8-trimethoxy isoflavone and its 2'-methyl derivative have been demethylated with hydriodic acid without change of orientation.

Another problem of orientation has been encountered in the new isoflavone synthesis in which ethoxyl chloride is used as a condensing agent. Here the ethyl formate method gives rise to compounds with the 5,8,7-orientation; the ethoxyl chloride process gives the 5,7,8-orientation. Whalley observed that 3' and 4'-methoxy isoflavones under the influence of hydriodic acid undergo demethylation normally eg. 4',5,7-trimethoxy-3-methyl, 4',7-dihydroxy-5-methoxy-3-methyl isoflavones are demethylated to 4',5,7-trihydroxy-3-methyl isoflavone and 5-hydroxy-4',7-dimethoxy-5-methyl isoflavone is demethylated to 4',5,7-trihydroxy-6-methyl isoflavone.

It was also postulated that 2'-hydroxy isoflavones can not be obtained by demethylation of 2'-methoxy compounds because rapid decomposition occurs even under the most mild circumstances. This may be due to the opening of chromone ring under the influence of acid reagents across the dotted line (a,b).
An improved technique for the demethylation of 2'-methoxy isoflavones was put forward by Whalley. 5,7,2'-trimethoxy isoflavone by the use of stabilised hydriodic acid or better aluminium chloride (good quality) furnished 5,7,2'-trihydroxy isoflavone, though in poor yield.

A new type of isomerism in the flavone series has been independently observed by Baker and his colleagues. 5,7,2'-trimethoxy 8-methyl isoflavone under the influence either of stabilised hydriodic acid or aluminium chloride in dry benzene furnishes a mixture of 5,7,2'-trihydroxy-8-methyl and 5,7,2'-trihydroxy-6-methyl isoflavones. Similarly demethylation of 5,7,4'-trimethoxy-8-methyl isoflavone gives a mixture of 5,7,4'-trihydroxy-8-methyl and 5,7,4'-trihydroxy-6-methyl isoflavones.

Doenally, Philbin and Wheeler have suggested that, as no rearrangement has yet been observed during demethylation of 5,8-dimethoxy flavones by aluminium chloride, the production of some 5,7,2'-trihydroxy-6-methyl isoflavone from 5,7,2'-trimethoxy-8-methyl isoflavone by this reagent might be ascribed to the direct migration of methyl residue rather than to ring opening followed by a ring closure in an alternative position.
The rearrangement, under the influence of hydroiodic acid of 5-hydroxy-$\alpha$-substituted compounds of type (CXLIX) to 5-hydroxy-$\beta$-substituted compounds of type (CL) has been observed for flavones, flavonoids, chromones, chromonols, and isoflavones etc. The reverse change i.e., the rearrangement of compounds of type (CI) to those of type (CXLIX) has not yet been reported in any series of such compounds with the exception of only one example in chromone series. Here the demethylation with hydroiodic acid of 5,7-dimethoxy-2,6-dimethyl chromone (type CXLIX) furnished the corresponding dihydroxy chromone $^{261}$ without rearrangement, 5-hydroxy-$7'$-methoxy-2,6-dimethyl chromone (type CL) yields under similar conditions, the isomeric 5,7-dihydroxy-2,6-dimethyl-chromone (type CXLIX).$^{260-273}$

![CXLIX] $^{(CXLIX)}$

![CL] $^{(CL)}$

The reverse type of change i.e., rearrangement from type CL to type CXLIX has for the first time been reported$^{274}$ in isoflavone series. 5-hydroxy-$7',4'$-dimethoxy-$8$-methyl isoflavone (CI) on demethylation with HCl furnished two distinct products isolated in the crystalline form after methylation i.e., (a) 5-hydroxy-$7',4'$-dimethoxy-$8$-methyl isoflavone (CL) and (b) 5-hydroxy-$7',4'$-dimethoxy-$8$-methyl isoflavone (CLII, $R=H$).
Isomeric change in alkaline medium: In all the above discussion isomeric change took place in acid media. Alkaline solutions could not be used because decomposition sets in. A special case was observed where this change took place to some extent in alkaline medium. Recently it was shown that 5-hydroxy-7,8-dimethoxy isoflavone (ClIII) undergoes fission with alcoholic alkali satisfactorily and when the resulting phenyl benzyl ketone (ClIV) is resubjected to isoflavone condensation the alternative structure with 5,6,7-arrangement (5-hydroxy-6,7-dimethoxy isoflavone (ClV) is produced. In the course of this study it was noted that even during the alkaline fission a small quantity of the isomeric isoflavone was formed. This constitutes an example of isomeric change in alkaline medium.
(Sodium) and the insoluble part recrystallised twice with dilute alcohol gave yellow needles m.p. 251-52°. Further recrystallisations did not raise the melting point (Fig. 1). The solution of the product in alcohol did give an olive green colouration with ferric nitrate.

Fig. 1

Of the yellow form thus crystallised and recrystallised with alcohol with yellow needles m.p. 251-52°, further recrystallisation from a mixture of solvents did not raise the melting point. The alcoholic solution on being taken in boiling alcohol and on the addition of cold iced acetate solution gave an off-white precipitate of a yellowish brown precipitate which was filtered and rejected. The clear filtrate on treatment with alcohol formed an acetate solution formed a bright yellow granular precipitate which was filtered. The precipitate on being worked up the usual way gave yellow needles m.p. 212-14°. These were
The filtrate when worked up showed that only glucose was present. The aglycone when treated with sodium amalgam followed by acidification developed a pink colouration thereby establishing the presence of a flavone. The alcoholic solution of the flavone gave an olive green colouration with alcoholic ferric chloride.

**Local Hydrolysis:** The product m.p. 281-285°C was also hydrolysed in one step and yielded only the product m.p. 370-371°C.

The sugars were chromatographically examined along with synthetic samples of glucose and found to be the only sugars. Thus the flavone m.p. 251-252°C carries two sugars; glucose and glucose and the aglycone m.p. 370-371°C.

**Characterization of aglycone m.p. 370-371°C:** This aglycone was identified as luteolin by (a) its melting point and analysis, (b) by the melting points and analyses of its acetyl, benzoyl and methyl derivatives and finally confirmed by (i) a cineole.
with luteolin obtained according to the method of Hutchins and Wheeler (1969) and (iii) the chromatographic examination of the sample alongside with the sample of luteolin synthesised according to Allen-Robinson procedure (cf. experimental).

**Location of the position of the sugar moiety:** The methylation of the glycoside m.p. 251-52°C went smoothly with dimethyl sulphate and potassium carbonate (anhydrous) in dry acetone as solvent. The methylated product could not be crystallised and was therefore directly hydrolysed when hot and separated. This en epimethylation from diisopropyl ether gave yellow crystals m.p. 258-52°C. This melting point is the same as recorded for 7-hydroxy-3,4',4'-trimethoxy flavone (277, 277). The shape of the crystals under the microscope was like a convex lens. This result made no doubt that the carbohydrate moiety (glucose and arabinose) is present as a disaccharide in the position and hence that the glycoside m.p. 251-52°C is luteolin 7-arabinosyl-glucoside (named as luteosylflavone). The various steps of synthesis are shown systematically below.

![Chemical structures](image-url)
Examination of the product m.p. 214-16\(^\circ\): The product m.p. 214-16\(^\circ\) was subjected to similar treatments as luteolin-7-apisosyl-glucoside (Graviobioside A). The acetate of the product was found to melt at 244-45\(^\circ\). Regulated hydrolysis (first stage) gave anise and a product in the form of light yellow needles m.p. 274-75\(^\circ\). The second stage of the hydrolysis yielded glucose and a product melting at 270-73\(^\circ\) (cf. Loevy, Robinson et al. \(^{279}\) m.p. 300-31\(^\circ\)) (cf. aglycone from the glycoside m.p. 251-52\(^\circ\) obtained earlier). The total hydrolysis of the product also gave a yellow product which, after crystallisation with pyridine melted at 216-17\(^\circ\) (Fig. 3). The mixed melt of this product with the aglycone obtained earlier from the glycoside m.p. 251-52\(^\circ\) showed them to be different (mixed melt of the two melted at 216-23\(^\circ\)). The identification of the sugars through osazones and chromatographic examination established them to be anise and glucose, as in the case of Graviobioside A.
The attachment of sugar residue was determined by methylation followed by hydrolysis. The product obtained was found to be 4-methoxy-5,8',4'-trimethoxy flavone m.p.245-36° as in the case of Gracilliberisa x (cf. Yamasura et al. 277 and Sionda 273). The results led to the conclusion that the same disaccharide residue (fructose and glucose) is present in the 7-position of the product m.p.245-36°.

Characterisation of aglycon. The aglycon was identified as 3'-methyl ether of luteolin (chrys CSA) by its melting point 330-34° and mixed melting point 330-34° with an authentic sample of chrysoselin prepared according to Allen-Robinson synthesis. 279 The analytical values showed the presence of one methoxyl group (Ziessel's method). In comparison of various derivatives such as acetate m.p.90.5-91° (cf. Robinson et al. 273), m.p.92.5-93° (Fig. 4), benzoate m.p.203-41° (Fig. 5) and methyl ether m.p.141-53° (cf. Gesserle and Vezenrov 270, m.p.141-92°) (Fig. 6) and their analytical values gave positive support to its identity as chrysoselin.

Fig. 4 & 5

Fig. 6
The chrysoeriol has also been synthesised by a different route (cf. following discussion) and was chromatographed along with the natural sample. Both the samples were found to be identical.

The above results prove that the product m.p.214-215 is chrysoeriol-7-glucosyl glucoside, a new glucoside named as 

\[
\text{Granichloride II.}
\]

The various steps of the hydrolysis may be represented as: 

\[
\text{Apisyl glucosyl-7-} \rightarrow \text{CHO} \quad \text{C(H)OH} \quad \text{CH}_2\text{CH}_2\text{OH} \\
\text{(CLX) \rightarrow (CLXI)}
\]

\[
\text{HCCH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \\
\text{(CLXII)}
\]

\[
\text{OH} \quad \text{CH}_3 \\
\text{(CLXIII) + C}_6\text{H}_{12}O_6}
\]
SYNTHESIS OF LUTEOLIN

There are a number of syntheses available in the literature for the preparation of luteolin. The one synthesis easily accessible being from dimethyl-ether of phenacetophenone (CLXIV) and veratraldehyde (CLXV) and therefore was taken up for its preparation. The luteolin (CLXIV) thus obtained was recrystallised from alcohol in yellow needles m.p. 28\(^\circ\) (cf. Hutchins and Wheeler \(^{160,231}\), m.p. 25-26\(^\circ\)). This sample of luteolin was further purified by acetylation followed by deacetylation and two recrystallisations from dilute alcohol when it was obtained in fine yellow needles m.p. 30-31\(^\circ\). A mixed melt of this luteolin with the one obtained from Graviobioside A showed no depression in the melting point. This therefore lends synthetic support to the identity of luteolin from Graviobioside A.

The various steps involved in the synthesis and purification of luteolin are given below:
Luteolin was also prepared (for the first time) by fusing together an intimate mixture of phlor-acetophenone (CLXXXI) veratic anhydride (CLXXXIII) and sidum veratrate. The fusion was carried out at 180-250° (under reduced pressure). The product was worked in the usual manner and gave 3,7'-dihydroxy-3',4'-dimethoxy flavone (CLXXXIV). This was demethylated with hydriodic acid in presence of acetic anhydride when it yielded luteolin (yellow) m.p.223-229° (CLXXXV). The product was recrystallised from dilute alcohol in yellow needles and finally had m.p.229°. This sample of luteolin showed no depression in the melting point when admixed with either the luteolin obtained from Gratioside A or synthesised earlier. The various steps are shown below:
Preparation of Chryscerol

This flavone was synthesized from chlor-acetophenone (CLXXI), 2-benzyl vanillic anhydride (CLXXVII) and sodium-2-benzyl vanillate according to the method of Robinson et al. The crude product so obtained had m.p. 328-3000. The crude product was purified by acetylation followed by deacetylation and subsequent crystallization from dilute pyridine when chryscerol (CLXXIII) was obtained in the form of fine yellow needles m.p. 310-3100.

A comparison of this with the chryscerol obtained from the biological plant did not show any depression of the melting point.

The various stages of the synthesis are given below:
A new synthesis of Chrysoeriol.

Chrysoeriol is 3'-methyl ether of luteolin and as such is a partial methyl ether. Naturally a logical way to prepare it would have been to use selective methylation of hydroxy-flavones or demethylation of methoxy-flavones for its synthesis. Unfortunately this course of was not available on account of a difficulty arising from the order of relative rates of cleavage of methoxy groups in flavones (6' > 4' > 7') and the order of relative rates of methylation of hydroxy flavones (7' > 6' > 4').

Therefore, a new route involving the intermediate formation of a chalcone and a flavanone was adopted.

Chloroglucinol (CIVXII) and carbethoxy benzyl chloride (CIXXIII) were condensed together by the gradual addition of a solution of anhydrous aluminium chloride in ether. This gave a reddish brown oily product (chalcone) which developed a pink shade with sulphuric acid (conc) and a reddish brown colour with alcoholic ferric chloride solution. Carbomethoxychlorine solutions of the substance and antimony pentachloride when mixed together also developed pink shade. The chalcone (CIVXIV) obtained could not be crystallised despite various and numerous efforts. It was therefore directly cyclised with sulphuric acid (2N, boiling). The product obtained gave a positive test for a flavanone. All efforts to crystallise the flavanone failed and
therefore chromatographic purification using alumina column was tried. When it separated into three fractions.

Fractions 1 and 3 have not so far been identified, and are under investigation. The second fraction when recrystallised from methyl alcohol gave m.p. 220.5-222.5° (Corrected, Koffler's block) (CLXXXIV). Shinoda and Sato have recorded for homo-eriodictylo (CLXXXV) m.p. 224-225° while Mussel and Todd have reported m.p. 219-220° for it. It may be pointed out here that the Japanese workers synthesised could not be reproduced.

The oxidation of the flavanone was carried out in alcoholic solution, using sodium acetate and iodine as the use of selenium dioxide had to be avoided on account of the presence of three free hydroxyl groups. The product obtained could not be crystallised but an alcoholic solution of the substance gave
slime pink colour with sodium amalgam followed by the addition of hydrochloric acid thus showing the presence of a flavone. It also gave reddish brown colour with ferric chloride solution but the test for a flavanone gave negative results 101. It was therefore acetylated and the acetate on recrystallisation from ethyl acetate gave crystalline product m.p. 320-31°C (Fig. 7; compare with Fig. 4). Chrysosiel acetate-natural alone and also when admixed with an authentic sample of the chrysosiel acetate (G XIVVII). This confirmed the identity of the product as chrysosiel (G XIVVII).

Paper chromatographic examination of the product (G XIVVII) along with an authentic sample of chrysosiel (from Gravio-
biocide 9) fully supported the identity of the product as chrysosiel, (Fig. 8 & 9).
Fig. 8
BuOH:AcOH:H$_2$O (14:10:50)
$R_P = 0.91$

Fig. 9
AcOH: H$_2$O (60:40)
$R_P = 0.72$

$F =$ Front of solvent.

$N =$ Chrysoeri nature

$S =$ Chrysoeri synth
Parsley, *Petroselinum sativum*, Moench, (Apium petroselinum L.)

A biennial Umbellifer is wild in the south of Europe, from Spain to Turkey. It has also been found at Tlemcen in Algeria and Lebanon. Dioscorides and Pliny speak of it under the names of *Petroselinon* and *Petroselinum*, but only as a wild medicinal plant. Later on it was cultivated. English gardeners received it in 1548.

It is nowadays a common garden herb much utilised in flavouring and garnishing. Parsley oil and extracts from parsley fruit have been described in dysmenorrhoea and as a diuretic.

A hot aqueous extract of parsley seeds or plant yields on cooling a gelatinous material which was mentioned by Linné. Later Bracornat who studied it, gave it the name of apiin on account of its jelly forming tendency like pectin. Planta and Wallace obtained apiin as colourless mass m.p. 180° and assigned to it the formula C\textsubscript{24}H\textsubscript{26}O\textsubscript{13}. The brown product obtained from apiin on acid hydrolysis was represented by the formula C\textsubscript{24}H\textsubscript{20}O\textsubscript{9}. It was Lindenborn who obtained apiin for the first time in a crystalline form (under the microscope) m.p. 228°. He found that on acid hydrolysis apiin yielded glucose and apigenin m.p. 133°.

He gave to apiin the formula C\textsubscript{12}H\textsubscript{14}O\textsubscript{7} and suggested that the acid hydrolysis of apiin probably proceeds as under:
Later on von Gerichten investigated apiin (Merck's) and showed that on alkaline fusion it gave phloroglucinol and an acid not closely examined; but from which under more energetic conditions of reaction protocatechuic acid and some oxalic, formic and p-hydroxyacetic acids were obtained. As a result of this investigation it considered apigenin to be represented by $C_{15}H_{10}O_7$ and stilbyl by $C_{27}H_{30}O_9$, yielding apigenin and glucose on acid hydrolysis as shown below:

$$C_{27}H_{30}O_9 + H_2O = C_{15}H_{10}O_7 + 2 C_6H_{12}O_3$$

After twenty-one years the subject again received attention from Pankin who boiled apigenin (source not mentioned) with strong aqueous solution of potassium hydroxide and isolated two products, phloroglucinol and p-hydroxyacetophenone. The fusion of apiin with caustic potash gave the above substances and also protocatechuic acid. The presence of this acid was puzzling; and Pankin suggested that it might have been obtained through the secondary excision of the p-hydroxyacetophenone which was produced in the above hydrolysis. Later investigations showed that protocatechuic acid may have come from petroselinin, the second glycoside present in apiin. The aglycone, $C_{27}H_{30}O_9$ of petroselinin was obtained by von Gerichten from the hydrolytic product of apiin. He showed that this was a mono-acetyl ether of luteolin. He further collected evidence which made it probable that the substance was 5,7,3'-trihydroxy-
authentic sources of apiin and apiosucone were needed in connection with the work on Karaffa's seeds in these laboratories and the work done by parsley seeds was done from Coopers, Poona, who started two new following two articles on the glycosides from parsley seeds; plants grown in India appeared. The first one was from Becker et al. who obtained the parsley seeds from Coopers, Poona (Carter's Champion Moss Curled variety). Then they used the parsley of the seeds in Delhi. Whether they used Coopers or Poona or obtained them from the raised plants is not mentioned. They described that the only anthocyanidin component they were able to obtain from parsley seeds and plant was apiin as apiin. This result is surprising in view of the findings of Palm (protocatechuic acid) and von Carichten (apiin and apiosucone) in parsley. The second paper from "Norstrom et al." who used paper chromatographic technique for the separation of glycosidic components of apiin (so called; and apparently the most prevalent) mentions the presence of four components in apiin. Three of them are glycosides and the fourth component
has been tentatively identified as a derivative of naringenin. Of the three glycosides, they have identified one as apiin (CLXLI) and the second as luteolin-7-apiosyl-glucoside (CLXLII) and the third as apigenin glucoside (tentatively). The naringenin derivative and apigenin glucoside occurred in the above as minor components.

\[
\begin{align*}
\text{Apiosyl-} & \quad \text{Apiosyl-} \\
\text{glucosyl-O} & \quad \text{glucosyl-O} \\
\text{OH} & \quad \text{OH} \\
\text{CO} & \quad \text{CO} \\
\end{align*}
\]

This account shows that there is no finality either about the parsley glycosides or the exact nature of apiin yet. This gave added interest to the work on the glycosides of parsley seeds.

**NEW WORK**

The powdered seeds after thorough defatting with light petroleum ether (40-60°C) followed by treatment with boiling carbon tetrachloride were extracted by refluxing with rectified spirit (90°C). The solvent was recovered under diminished pressure and the gelatinous mass left was triturated with ether in order to remove remaining chlorophyll, fatty matter and any free flavones. The crude apiin thus obtained in the form of yellow powder despite numerous and varied efforts could not be
crystallised in the solution, on cooling, always resulted in an almost transparent jelly. Therefore it suggested itself to use lead acetate method (for the first time in parsley) for the effective separation and purification of parsley glycosides.

To a boiling aqueous solution of crude apiin, a little quantity of a hot solution of neutral lead acetate (10%) was added dropwise when a dirty brownish precipitate was obtained. This was filtered hot. The filtrate (still hot) was again dropwise treated with an excess of hot lead acetate solution but only gave an insignificant quantity of a brownish precipitate. It was filtered and the precipitate rejected. The filtrate on treatment with basic lead acetate (excess) gave a large amount of a yellow precipitate. The bulky yellow precipitate after hot dilution was suspended in alcohol and decomposed by passing hydrogen sulphide gas. The solvent was recovered under diminished pressure to dryness and the dry mass on crystallisation from alcohol gave a product m.p. 226-228°. The product was crystallised (vapour) with dry acetone and then twice recrystallised from alcohol gave a light yellow crystalline acetaate of apiin m.p. 272-274° (Fig 10) (cf. von Gerichten92,93,94.

Goshadri et al.20, m.p.239-242° and Shishichiro Nakaochi et al.20, from leaves of Vicia hirsuta, m.p.232-233°).
Fig. 10

The product m.p. 235-37° was subjected to regulated hydrolysis according to the procedure of von Gerichten\footnote{22} and the sugar characterized by the formation of neoisozyme m.p. 185-37°. The total hydrolysis of the product with cooling aqueous sulphuric acid \footnote{27} over four hours yielded yellow needles m.p. 343-44°. These were several times recrystallised and finally came down as fine yellow needles m.p. 34° (cf. Bashor et al\footnote{29}, m.p. 343-44°; Konstand et al\footnote{30} m.p. 343° (synthetic); Baker, Dening and Ulstein\footnote{31} m.p. 343-45°) (Fig. 11).
The only other sugar on the hydrolysis of the partial glucoside which could be ascertained was glucose. Thus it appears that according to the definition the product m.p. 236-37° is apiin.

The aglucone was further characterised as apigenin by the formation of the acetate m.p. 181-82° (Kostanecki et al. m. p. 181-82°). The analytical values of the acetate also support it to be apigenin acetate.

Dimethyl ether of apigenin was also prepared through the action of diazomethane and gave m.p. 174-75° (cf. Baner and Dietrich m. p. 174-75°).

The apiin m.p. 236-37° was subjected to chromatographic separation (ascending technique) using Whatman filter paper No. and butanol:acetic acid: water (60:10:30) as solvent. The spots were revealed under U.V. light and in presence of ammonia vapour. The spots were also revealed by spraying with (a) sodium carbonate solution and (b) alcoholic ferric chloride solution (Fig 12).

F= Front of the solvent
A= Apiin.

Fig. 12
This examination shows that there are at least three components in this apiin m.p. 236-37° (two major and one minor*). One of the major components was probably identical with apigenin-apiosyl glucoside (Rf value 0.91) giving support to the findings of Nordstrom et al. The second component does not correspond with the luteolin-7-apiosyl glucoside because when a chromatogram of this compound was run alongside with an authentic sample of luteolin-7-apiosyl glucoside (Graviobioside A) the two differed in Rf values and colour reactions (Fig. 13). The third component was in insignificant amount.

\[ \text{F = Front of solvent} \]
\[ \text{A = Apiin} \]
\[ \text{A = Luteolin-7-apiosyl glucoside (Graviobioside A)} \]

---

Fig. 13
In order to obtain some quantity of the various component of aspirin, chromatographic separation using the earlier described method was adopted. The only change was in the method of the application of aspirin solution (streaks instead of spots). The chromatograms were dried and examined and the different spots revealed were marked in U.V. light. The spots were carefully cut and the two major components extracted separately by refluxing them with 70% alcohol. On the recovery of the solvent under diminished pressure a small amount of residue was left in each case. These residues were dissolved in alcohol. The fractions on further chromatographic examination showed that they were practically pure samples.

The two fractions were separately hydrolysed with aqueous sulphuric acid (72%) refluxing time (24 hours) and the aglycones obtained were chromatographically along side with the authentic samples of eugenine, luteolin5, chrysoeriol6, and diosmetin9. The spots were revealed in U.V. light, U.V. light and ammonia vapor along with aqueous sodium carbonate and alcoholic ferric chloride solutions. (Fig. 14).

* The sample of eugenine was isolated by the hydrolysis of glucosides of parsley.
+ Luteolin and chrysoeriol samples were obtained from celery glycosides (Galeobiobioside A and B).
8 The author wishes to place on record his grateful thanks to Dr. R. M. Horowitz for his gift of an authentic sample of diosmetin.
Fig. 18.

Fig. 18 refers to the chromatogram of flavonoids. The components are identified as Apigenin and the other as Chryseisol. The diagram illustrates the findings of von Gerich. 29
CONCLUSION
CONCLUSIONS

The main conclusions to be drawn from the experimental work on Karaffs (Apium graveolens) and Parsley (Apium petroselinum) seeds described in the thesis are:

A. Karaffs Seeds (Apium graveolens):

1. Karaffs seeds and husk (Indian equivalent of English Celery-Apium graveolens) both carry the same two glycosides m.p. 250-51°C and m.p. 214-16°C. Being new glycosides they have been named Graviobioside A and Graviobioside B.

2. The two glycosides carry the same sugars, apiose and glucose. These sugars occur as a disaccharide.

3. The position of the attachment of the sugar residue in both the glycosides has been determined (position 7).

4. The aglycone obtained from Graviobioside A has been identified as 5,7,3',4'-tetra-hydroxy flavone (luteolin) and that from Graviobioside B as 5,7,4'-trihydroxy-3'-methoxy flavone (Chrysoeriol).

5. Graviobioside A has been shown to be luteolin-7-α-L-apiosyl-glucoside while Graviobioside B as chrysoeriol-7-α-L-apiosyl-glucoside.
6. Two glucosides m.p. 255-57 and m.p. 234-35° have been obtained on the controlled hydrolysis of the gravibioside A and Graviobioside B respectively. The Glucoside m.p. 256-57° has been identified as 7-glucoside of luteolin. The Glucoside m.p. 234-35° which is a new one has been characterised as 7-glucoside of chrysocariol.

7. Chrysocariol and Luteolin have been synthesised by new routes also.

B. Parsley Seeds (Apium petroselinum):

1. The glycosidic fraction on paper chromatographic examination showed it to be made up of atleast three components.

2. The melting point 236-37° of the glycoside named apiin (in literature) is considerably higher than the melting points recorded for it. (von Gerichten 92, 291 m.p. 228°; Gupta and Seshadri 234, m.p. 230-32°; Nakao et al 295, m.p. 232-33°).

3. Two of the above components have been tentatively identified as Apigenin-7-apiosyl-glucoside and diosmetin-7-apiosyl-glucoside.

4. Both the above glycosides carry the same two sugars, apiose and glucose, and like the case of Karaff Glycosides carry the sugars in the form of disaccharides.