Part-I

Chapter-2

A short review on dicoumarols
Dicoumarol

3,3'-Methylene-bis-4-hydroxycoumarin (120) is commonly known as dicoumarin or dicoumarol. In 1941 Campbell and Link first isolated dicoumarol from spoiled sweet clover hay (*Heliotropium alba*) and also from hays that killed cattle in agricultural practice. It was shown to be the agent responsible for the hemorrhagic 'sweet clover disease' of cattle. On a dry basis spoiled sweet clover hay contains approximately 0.003% of the hemorrhagic agent.

Dicoumarol, m.p. 288-289°, has the empirical formula C_{19}H_{12}O_{6}. There are two acidic -OH groups and the acidity falls between that of the phenols and the carboxylic acids. It has a low solubility in the ordinary organic solvents, insoluble in acids and readily forms salts with dilute alkali. It forms a crystalline dimethylether, m.p. 168-170°, which is physiologically inactive. It also forms a diacetate, m.p. 250-252° with
acetic anhydride$^{60}$. The treatment of dicoumarol with alkali under different conditions was observed by Stahmann et al$^{61}$. When fused with solid KOH, it gave salicylic acid in $93\%$ yield. When refluxed for 24 hrs, with 30% KOH in methanol, it gave salicylic acid and a phenolic diketone (121), m.p. 101-103°.

\[
\text{(121)} \xrightarrow{\text{Fused with}} \text{Salicylic Acid and Phenolic Diketone (121)}
\]

which was practically the only product when 10% NaOH was used in place of alcoholic KOH. 1,3-Bis-(2-hydroxy benzoyl) oxoace (121) formed a dimethylether, m.p. 86-88°. The structure (121) was finally confirmed by synthesis.

\[
\text{(121)} \xrightarrow{\text{OH}} \text{Dimethylether (121)}
\]

Dicoumarol on treatment with hot aniline gave quantitative amount of 4-anilinocoumarin (122).

\[
\text{(120)} \xrightarrow{\text{PhNH}_2} \text{4-Anilinocoumarin (122)}
\]
Dicoumarol was synthetically known long before it was isolated from a natural source. It can be readily prepared by condensing formaldehyde with two molecules of 4-hydroxycoumarin (123) in boiling ethanol.

\[
\begin{align*}
2 \text{OH} & \xrightarrow{\text{HCHO}} \text{HO} \\
(123) & \xrightarrow{\text{HCHO}} \text{HO} (123)
\end{align*}
\]

Dicoumarol is an anticoagulant drug. It is one of the commonly used oral anticoagulants and an effective drug against thrombosis. It controls the level of the prothrombin group of plasma proteins by inhibiting their production in the liver. The fall in prothrombin level below a critical value has been found to cause bleeding symptoms. The active material responsible for the reduction of prothrombin level has been found to be the white crystalline compound dicoumarol.

The structural requirement for anticoagulant activity is an intact 4-hydroxycoumarin residue, the 3-position being substituted by a carbon residue or a hydrogen atom. For high activity, a bis-4-hydroxycoumarin structure or a related type of structure having the similar 1,5-spatial relationship between the ethyl-OH-group of 4-hydroxycoumarin and a ketogroup is specifically required.
Dicoumarol showed inhibitory action on the growth of certain bacteria, e.g., *S. aureus*, *S. albus*, *S. pyogenes* and *B. anthracis*69,70.

$^{13}$CMR spectra of dicoumarol has been studied by Kirkiacharian et al71 and $^{13}$CMR spectrum of some bridged dicoumarols was studied by Convest et al72.

Gerberinol

The only other derivative of dicoumarol was isolated by Sengupta and coworkers73, and it was named gerberinol. The structure elucidation of gerberinol is outlined below.

Sengupta et al73 isolated a new dimethyldicoumarol, m.p. 263-267°, named gerberinol (124) from the ethylacetate extract of an Indian plant *Gerbera lanuginosa* (compositae).

The compound showed negative color reactions in Lietermann-Burchard test, ferric chloride test and shinoda test. Microanalytical data showed the molecular formula of the compound to be $C_{21}H_{16}O_{6}$, which was in agreement with the molecular weight 364 determined from mass spectrometry. Ultraviolet absorption spectrum indicated the presence of one or more hydroxyl groups. Its infrared spectrum showed prominent peaks at 2970 cm$^{-1}$ to
3040 cm$^{-1}$ due to phenolic or enolic hydroxyl group and at 1600 cm$^{-1}$ for aromatic nucleous. It also showed the presence of unsaturated lactone group at 1645 cm$^{-1}$.

The structure of gerberinol (124) thus assigned was based mainly on the authors' interpretation of the NMR spectrum. The NMR spectra showed the presence of two phenolic hydroxyl groups at $\delta$ 12.00 which disappeared on D$_2$O exchange, six aromatic nuclei groups and one -CH$_2$- group of the Ar-CH$_2$-Ar system.

The total number of protons was found to be sixteen, which fact was in agreement with the proposed molecular formula.

$^{13}$C-NMR spectral data of gerberinol (124) are shown in Table-I.
Table I

[13C]CMR spectrum of gerberinol (124)

The spectra was taken in CDCl₃ and the shifts were recorded in δ-values.

<table>
<thead>
<tr>
<th>Carbon shift</th>
<th>Number of carbons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>168.3</td>
<td>2</td>
<td>Carbonyl</td>
</tr>
<tr>
<td>167.5</td>
<td>2</td>
<td>Oxygenated aromatic</td>
</tr>
<tr>
<td>153.6</td>
<td>2</td>
<td>- do -</td>
</tr>
<tr>
<td>138.3</td>
<td>2</td>
<td>Non protonated aromatic</td>
</tr>
<tr>
<td>131.5</td>
<td>2</td>
<td>Protonated aromatic</td>
</tr>
<tr>
<td>128.0</td>
<td>2</td>
<td>- do -</td>
</tr>
<tr>
<td>114.9</td>
<td>2</td>
<td>- do -</td>
</tr>
<tr>
<td>114.9</td>
<td>2</td>
<td>Non protonated aromatic</td>
</tr>
<tr>
<td>102.6</td>
<td>2</td>
<td>- do -</td>
</tr>
<tr>
<td>23.1</td>
<td>2</td>
<td>-CH₃</td>
</tr>
<tr>
<td>20.0</td>
<td>1</td>
<td>-CH₃</td>
</tr>
</tbody>
</table>

Gerberinol (124) on methylation with diazomethane in ether gave a mixture of isomeric dimethylethers.
Formation of two isomeric methylethers on methylation with diazomethane is characteristic of 4-hydroxycoumarin moity. Thus the formation of these two isomeric dimethylethers indicates the presence of two 4-hydroxycoumarin moities in gerberinol. NMR spectra of gerberinol (124) indicated the presence of an Ar-CH$_2$-Ar group. So it can be proposed that in gerberinol, the two 4-hydroxycoumarin moities are bridged by a -CH$_2$- group. As the two methyl groups are aromatic methyls, they must be attached to two carbons in the benzenoid rings of the 4-hydroxycoumarin moities. The other six benzenoid carbons must be substituted by hydrogen because there are six aromatic protons. So there is only C-3 of the 4-hydroxycoumarin moities left for attachment of the -CH$_2$- bridge. Moreover the absence of any NMR signal at $\delta$ 5.6 to $\delta$ 6.2 ppm indicates the absence of any proton of 4-hydroxycoumarin moities. So it can be concluded that the attachment of the -CH$_2$- bridge to two 4-hydroxycoumarin moities must be through C-3 of both the moities. Therefore a dicoumaro
type of skeleton (125) can be formulated for gerberinol (124) with the position of two symmetrical methyl groups to be decided.

Gerberinol (124) when refluxed with 10% aqueous KOH afforded a phenolic monoketone (126), m.p. 230-233°. Analytical data showed the molecular formula of monoketone was \( \text{C}_{20}\text{H}_{18}\text{O}_{5} \), which was confirmed from the mass spectrum where \( \text{M}^{+} \) appeared at \( m/z \) 338.

\[
\begin{align*}
\text{C}_{21}\text{H}_{16}\text{O}_{6} & \quad \text{---10\% ag. KOH---} \quad \text{C}_{20}\text{H}_{18}\text{O}_{5} \\
(124) & \quad \text{refluxed for 1 hr} \quad (126)
\end{align*}
\]

On the basis of spectral data of (126) its structure can be formulated in terms of a monoketone as shown below.
But dicoumarol itself on treatment with 10% aq NaOH is known to produce the diketone (121). The nonformation of the diketone but the formation of the monoketone can be explained on the basis of the structure (124) for gerberinol.

\[
\text{\textbf{Gerberinol (124)}}
\]

In gerberinol (124) the hydroxyl group is flanked by methyl and methylene groups. Therefore the opening up of the conjugated lactone group will be very difficult due to steric hindrance. So only one lactone moiety was opened up and the other did not.

Gerberinol (124) on treatment with acetic anhydride and pyridine furnished an anhydro compound (127) instead of an acetate. The structure of the anhydro compound (127) was established as

\[
\text{\textbf{Gerberinol (124)}}
\]

\[
\text{\textbf{Gerberinol (124)}}
\]
Formation of the anhydro compound is mechanistically shown below. Each phenolic hydroxyl group in gerberinol is flanked by methyl and methylene groups and is thus resistant to acetylation. However, the hydroxyl groups in gerberinol are in favourable position to eliminate water and then form a six membered ring.
Finally the structure of gerberinol (124) was confirmed to be 5,5'-dimethyldicoumarol by its synthesis from m-cresol (128).

\[ \text{phenol} + \text{glutaric acid} \rightarrow \text{gerberinol (124)} \]

\[ \text{C}_{6}\text{H}_{5}\text{CH}_{3} + \text{HOOCCH}_{2} \rightarrow \text{C}_{12}\text{H}_{12} \]

\[ \text{i) } \text{POCl}_{3}, \text{ ii) } \text{Anhyd } \text{ZnCl}_{2}, \text{ iii) Heated at 60-70°C} \]

\[ \text{Reaction scheme for the synthesis of gerberinol (124)} \]