ISOLATION AND CHARACTERIZATION
OF NATURAL PRODUCTS

SUMMARY

THESIS
SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy
IN
CHEMISTRY

By
OMER AHMED M. BASUDAN

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
2002
SUMMARY

The present thesis entitled “Isolation and Characterization of Natural Products”, consists of six chapters. The first chapter includes a critical review of the chemistry of coumarins, triterpenic and flavonoidic compounds and high-lighted the recent advances in the analytical techniques applied to the isolation and structure elucidation of natural products.

The chapters second to sixth deal with the isolation and characterization of the naturally occurring compounds from the following five medicinally important plants.

A. *Garcinia mannii* (Guttiferae), (Leaves).
B. *Rhus alata*, (Anacardiaceae), (Bark).
C. *Bergenia ligulata*, (Saxifragaceae), (Roots).
D. *Ficus lyrata*, (Moraceae), (Leaves).
E. *Cassia siamea*, (Fabaceae), (Leaves).

**Chapter-II**

Following four compounds have been isolated and characterized from *Garcinia mannii*

1. **2,4-Dimethoxy-6-hydroxy acetophenone**
   - M.F. C_{10}H_{12}O_{4}
   - M.P. 149 °C.
   - M.W. 196
   - Crystallized from benzene-chloroform as white shining crystals.
   - Structure elucidation is based on the basis of chemical reaction, IR, UV, $^{1}H$-NMR, $^{13}C$-NMR and MS.

2. **Quercetin**
   - M.F. C_{15}H_{10}O_{7}
   - M.P. 311-12 °C.
   - M.W. 302
   - Crystallized from chloroform-methanol as yellow shining crystals.
   - $\text{Ac}_2\text{O}/\text{Pyridine (mild)} \rightarrow \text{pentaacetate}$
M.P. 194-95 °C
Crystallized from ethylacetate as cream coloured crystals.
Me₂SO₄/ acetone (mild) → pentamethyl ether
M.P. 151-52 °C
Crystallized from methanol-ethylacetate as colourless needles.
Structure elucidation is based on the basis of chemical reaction and spectral studies, IR, UV, \(^1\)H-NMR and MS.

3. 5,7,4′-Trihydroxyflavone (Apigenin)
\[
\text{M.F. } C_{15}H_{10}O_5
\]
M.P. 352 °C.
M.W. 270
Crystallized from benzene-acetone as yellow crystals.
Ac₂O/Pyridine (mild) → triacetate
M.P. 183-84 °C
Structure elucidation is based on the basis of chemical reaction and spectral studies, IR, UV, \(^1\)H-NMR and MS.

\( \text{(new compound)} \)
Me₂SO₄/ acetone (mild) → octamethyl ether
\[
\text{M.F. } C_{38}H_{34}O_{12}
\]
M.P. 189-90 °C.
M.W. 682
Crystallized from chloroform-methanol as white crystals.
Structure elucidation is based on the basis of chemical reaction and spectral studies, IR, UV, \(^1\)H-NMR, \(^13\)C-NMR and MS.
Chapter-III

Following nine compounds have been isolated and characterized from Rhus alata.

1. Dimethylester of terephthallic acid.
   M.F. C_{10}H_{10}O_{4}
   M.P. 138 °C
   M.W. 194
   Crystallized from chloroform-methanol as white needles.
   Structure elucidation is based on the spectral studies, IR, UV, \(^1\)H-NMR and MS.

2. β-Amyrin
   M.F. C_{30}H_{50}O
   M.P. 198 °C
   M.W. 426
   \([\alpha]_D^{19} +88.4 \, ^o\) (CDCl\(_3\))
   Crystallized from chloroform-methanol as white needles.
   Ac\(_2\)O/Pyridine (mild)→monoacetate
   M.P. 241-42 °C
   \([\alpha]_D^{23} +68.9 \, ^o\) (CDCl\(_3\))
   Crystallized from chloroform-methanol as colourless crystals.
   Structure elucidation is based on chemical reaction and spectral studies, IR, \(^1\)H-NMR and MS.

3. Freidelin
   M.F. C_{30}H_{49}O
   M.P. 262-64 °C
   M.W. 426
   \([\alpha]_D^{23} -29.4 \, ^o\) (CDCl\(_3\))
   Crystallized from chloroform-methanol as white needle-shaped crystals.
   Structure elucidation is based on the spectral studies, IR and \(^1\)H-NMR and MS.
4. **Lupeol**

M.F. C\textsubscript{30}H\textsubscript{50}O  
M.P. 214-15 °C  
M.W. 426  
\([\alpha]^{20}_{D} + 23.64^\circ \text{ (CHCl}_3\text{)}\)

Crystallized from methanol-chloroform.

\(\text{Ac}_2\text{O/Pyridine (mild)} \to \text{monoacetate}\)

M.P. 218-20 °C

Crystallized from chloroform-methanol as colourless flakes.

Structure elucidation is based on the chemical reaction and spectral studies, IR and \(^1\text{H-NMR}\).

5. **β-Sitosterol**

M.F. C\textsubscript{29}H\textsubscript{50}O  
M.P. 136-37 °C  
M.W. 414  
\([\alpha]_D^{\text{I}} -32.1^\circ \text{ (CDCl}_3\text{)}\)

Crystallized from chloroform-methanol as white needle-shaped crystals.

\(\text{Ac}_2\text{O/Pyridine (mild)} \to \text{monoacetate}\)

M.P. 114-15 °C  
\([\alpha]_D^{\text{I}} -48.5^\circ \text{ (CHCl}_3\text{)}\)

Crystallized from chloroform-methanol as colourless flakes.

Benzoyl chloride/Pyridine (mild) \(\to \) Benzoate.

M.P. 145-46 °C

\([\alpha]_D^{\text{I}} -7.52^\circ\)

Crystallized from methanol.

Structure elucidation is based on the basis of chemical reactions and spectral studies, IR, \(^1\text{H-NMR}\) and MS.
6. Oleanolic acid
   M.F. C_{30}H_{48}O_{3}
   M.P. 299-300 °C
   Ac_2O/Pyridine (mild) → monoacetate
   M.P. 258-60 °C.
   Solid crystals from methanol.
   Diazomethane → monomethylester
   M.P. 220-21 °C.
   Crystallized from methanol-chloroform as colourless shining plates.
   Structure elucidation is based on the chemical and spectral studies, IR and \(^1\)H-NMR.

7. Taraxerone
   M.F. C_{30}H_{48}O
   M.P. 240 °C
   M.W. 424
   Crystallized from chloroform-methanol
   as colourless needles.
   Structure elucidation is based on the spectral studies, IR, \(^1\)H-NMR and MS.

8. Ethyl gallate
   M.F. C_{9}H_{10}O_{5}
   M.P. 155 °C
   M.W. 195
   Crystallized from methanol as reddish buff needles.
   Structure elucidation is based on the spectral studies, \(^1\)H-NMR and MS.

9. (2 E)-3-(4-hydroxy-5, 7-dimethylbenzo[3, 4-b] furan-6-yloxy)-prop-2-enoic acid.
   (new compound)
   M.F. C_{13}H_{12}O_{5}
   M.P. 160-62 °C
M.W. 248
Crystallized from chloroform-methanol as white crystals.
Structure elucidation is based on the spectral studies, IR, \(^{1}\text{H}-\text{NMR}, {^{13}\text{C}-\text{NMR}}\) and MS.

Chapter IV
Following six compounds have been isolated and characterized from *Ficus lyrata*

1. \(\beta\)-Sitosterol-D-glucoside
   M.F. C\(_{35}\)H\(_{60}\)O\(_{6}\)
   M.P. 282 °C.
   Crystallized from chloroform-methanol as colourless crystals.
   Ac\(_{2}\)O/Pyridine (mild) \(\square\) tetraacetate
   M.P. 166 °C
   Crystallized from methanol-chloroform as colourless crystals.
   Structure elucidation is based on the basis of chemical reaction and spectral studies, IR, \(^{1}\text{H}-\text{NMR}\) and MS.

2. 4-Methoxychalcone
   (new compound)
   M.F. C\(_{16}\)H\(_{14}\)O\(_{2}\)
   M.P. 59-60 °C.
   M.W. 238
   Crystallized from chloroform-methanol as light cream crystals.
   Structure elucidation is based on the basis of IR, UV, \(^{1}\text{H}-\text{NMR}, \text{MS and GC-MS}.\)

3. 7, 4'-Dimethoxy apigenin
   M.F. C\(_{17}\)H\(_{14}\)O\(_{5}\)
   M.P. 185-87 °C.
M.W. 298
Crystallized from chloroform-
methanol as white crystals.
Structure elucidation is based on the basis of IR, UV, $^1$H-NMR and MS.

4. **5, 7, 4′-Trihydroxy-2′, 3′, 6′-trimethoxyisoflavone**

M.F. C$_{18}$H$_{16}$O$_8$
M.P. 198-200°C.
M.W. 360
Crystallized from chloroform-
methanol as pale yellow plates.
Ac$_2$O/Pyridine (mild) $\rightarrow$ triacetate
M.P. 132 °C
Crystallized from aq. ethanol as colourless needles.
Me$_2$SO$_4$/ acetone (mild) $\rightarrow$ hexamethyl ether
M.P.156 °C.
Crystallized from ethylacetate as colourless shining crystals.
Structure elucidation is based on the basis of chemical reaction, IR, UV, $^1$H-NMR, $^{13}$C-NMR and MS.

5. **Acacetin-7-glucoside**

M.F. C$_{22}$H$_{22}$O$_{10}$
M.P. 255 °C.
Crystallized from chloroform-
methanol as cream coloured crystals.
Ac$_2$O/Pyridine (mild) $\rightarrow$ pentaacetate.
M.P. 204 °C
Crystallized from chloroform-methanol as colourless needles.
Structure elucidation is based on basis of chemical reaction and spectral studies, IR, UV and $^1$H-NMR.
6. **Acacetin-7-O-neohesperidoside**  
M.F. C$_{28}$H$_{32}$O$_{14}$  
M.P. 266-68 °C.  
Crystallized from chloroform-methanol as white crystals.  
Ac$_2$O/Pyridine (mild) → heptaacetate.  
M.P. 216-20 °C  
Crystallized from methanol as light cream crystals.  
Structure elucidation is based on the basis of chemical reactions, IR, UV,  
$^1$H-NMR and MS.

**Chapter-V**

Following six compounds have been isolated and characterized from  
**Bergenia ligulata**

1. **Lanost-7(8)-en-3β-formyloxy-6β-acetoxy-11β, 15β-diol-26-heptanoxy-27-oic acid.**  
M.F. C$_{40}$H$_{64}$O$_{10}$  
M.P. 161-62 °C  
M.W. 702  
Crystallized from  
chloroform-methanol  
Ac$_2$O/Pyridine (mild) → diacetyl product.  
M.P. 110 °C.  
M.F. C$_{44}$H$_{69}$O$_{1}$  
Crystallized from chloroform-methanol as white needles.  
CH$_3$N$_2$ / acetone → monomethylester  
M.P. 114-15 °C.  
M.F. C$_{41}$H$_{66}$O$_{10}$  
Crystallized from chloroform-methanol as colourless needles.
Structure elucidation is based on the basis of chemical and spectral studies, IR, UV, $^1$H-NMR and FABMS.

2. Urs-12-en-3β-22α-diol-15β-formyl-21β-glycoside

M.F. C$_{37}$H$_{10}$O$_{10}$
M.P. 190-91 °C.
M.W. 664
Crystallized from chloroform-methanol as white needles.
Ac$_2$O /Pyridine (mild) $\rightarrow$ peracetylated product
M.P. 125 °C.
Crystallized from chloroform-methanol as white needles.
Structure elucidation is based on chemical and spectral studies, IR, UV, $^1$H-NMR and FABMS.

3. 9, 11-Seco-lanost-20 (22)-en-3β-formyl-18-phenoxyoate-12β-D-glucose

M.F. C$_{43}$H$_{66}$O$_{10}$
M.P. 210-11 °C.
M.W. 742
Solid crystals from methanol.
Structure elucidation is based on
Spectral studies, IR, UV, $^1$H-NMR and FABMS.

4. Sumatrol

M.F. C$_{23}$H$_{20}$O$_5$ (OMe)$_2$
M.P. 190-92 °C.
Crystallized from acetone as colourless needles.
Hydroxylamine/ HCl (mild)$\rightarrow$ Oxime.
M.P. 245-46 °C.
Crystallized from alcohol as colourless needles.
Structure elucidation is based on chemical and spectral studies, IR, UV and $^1$H-NMR.

5. **5'-Methyleriodictyol-3'-O-β-D-galactopyranosyl (1 → 4) - α-L-rhamno-pyranose.**
   
   M.F. C$_{28}$H$_{34}$O$_{15}$
   
   M.P. >250 °C.
   
   Crystallized from ethanol as pale yellow needles.
   
   Ac$_2$O /Pyridine (mild)$\rightarrow$ nonaacetate
   
   M.P. 85-86 °C
   
   Crystallized from chloroform-methanol as colourless needles.
   
   Structure elucidation is based on the basis of chemical and spectral studies, IR, UV, $^1$H-NMR and MS.

6. **Bergenin**
   
   M.F. C$_{13}$H$_{15}$O$_9$
   
   M.P. 237-38 °C.
   
   Crystallized from methanol as white crystals.
   
   Ac$_2$O /Pyridine (mild)$\rightarrow$ pentaacetate product.
   
   M.P. 207- 08 °C.
   
   Crystallized from methanol as needle-shaped crystals.
   
   Structure elucidation is based on the basis of IR and UV.

**Chapter-VI**

Following five compounds have been isolated and characterized from **Cassia alata**

1. **Bergapten**
   
   M.F. C$_{12}$H$_8$O$_4$
   
   M.P. 190-91 °C.
   
   M.W. 216
Crystallized from chloroform-methanol as light yellow crystals.
Structure elucidation is made on the basis of IR, UV, $^1$H-NMR and MS.

2. **Seslin**

M.F. C$_{14}$H$_{12}$O$_3$
M.P. 119-20 °C.
M.W. 228

Crystallized from chloroform-methanol as light yellow crystals.
Structure elucidation is based on the basis of IR, UV, $^1$H-NMR and MS.

3. **5-Methoxy-4'-hydroxy-6,7-methylenedioxyisoflavone**

M.F. C$_{17}$H$_{12}$O$_6$
M.P. 258-60 °C.
M.W. 312

Crystallized from chloroform-methanol as white crystals.

Ac$_2$O/Pyridine (mild) $\rightarrow$ monoacetate

M.F. C$_{19}$H$_{14}$O$_7$
M.P. 189 °C

Crystallized from aq. ethanol as colourless needles.
Structure elucidation is based on the basis of IR, UV, $^1$H-NMR and MS.

4. **5, 4'-Dihydroxy-6, 7-methylenedioxyisoflavone**

M.F. C$_{16}$H$_{10}$O$_6$
M.P. 236-37 °C.
M.W. 298

Crystallized from chloroform-methanol as cream coloured crystals.

Ac$_2$O/Pyridine (mild) $\rightarrow$ diacetate

M.F. C$_{20}$H$_{14}$O$_8$
M.P. 187 °C

Crystallized from ethanol as colourless needles.
Structure elucidation is based on the basis of chemical reaction and spectral studies, IR, UV, $^1$H-NMR and MS.

5. 5, 7, 4'-Trihydroxy-6, 3'-dimethoxyisoflavone

![Chemical structure of 5, 7, 4'-Trihydroxy-6, 3'-dimethoxyisoflavone](image)

M.F. C$_{17}$H$_{14}$O$_{7}$
M.P. 255-56 °C.
M.W. 330

Crystallized from chloroform-methanol as cream coloured crystals.

Ac$_2$O/Pyridine (mild) → triacetate
M.F. C$_{23}$H$_{20}$O$_{10}$
M.P. 135 °C

Crystallized from ethanol as colourless needles.

Structure elucidation is based on the basis of chemical reaction and spectral studies, IR, UV, $^1$H-NMR and MS.
ISOLATION AND CHARACTERIZATION OF NATURAL PRODUCTS

THESIS
SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy
IN
CHEMISTRY

By
OMER AHMED M. BASUDAN

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
2002
CERTIFICATE

This is to certify that the work described in the thesis entitled "ISOLATION AND CHARACTERIZATION OF NATURAL PRODUCTS" is the original work carried out by Mr. Omer Ahmad M. Basudan under my supervision and is suitable for submission for the award of Ph.D. degree in Chemistry.

(Dr. M. Mushfiq)
ACKNOWLEDGEMENT

Praise be to Allah, the Lord of the World, who says in his glorious book, “There has come to you from Allah a light and a plain book” and peace and blessings of Allah be upon the noblest of the prophets and messengers, our Prophet Mohammed who has said, “You should insist on acquiring knowledge even if you have to travel up to China” And “seek knowledge from cradle to grave.”

In the name of Almighty Allah, who favoured me to achieve this work I thought unattainable To whom belong might and majesty, I bring all the praises and commendation for providing me with strength during the work of my research which helped me to overcome the troubles and difficulties in the toilsome journey and who well fixed me whenever I decline in vigor or vitality Praise is to him.

I wish to express my profound gratitude to Prof. M. Mushfiq, supervisor, for his excellent guidance, constant support and trust throughout the course of my research work. I am highly indebted to Prof. M. Ilyas, Chairman, Department of Chemistry, Aligarh Muslim University, for his persistent inspiration, vital encouragement and vigilant supervision and for the freedom he gave me in carrying out my research.

I wish to express my deep sense of gratitude to Dr. (Miss) M. Parveen, for her kind help, valuable suggestion and encouragement during the course of these studies.

I am also thankful to ex Prof. W. Hussain, Botany Department, A M U Aligarh, for the identification of the plants.

I am grateful to Dr. Atta Ahmed, and Dr. Bashir, CDRI, Lucknow, for cooperation and help in providing the spectral studies from time to time. Also, to Dr. Rafiuddin, Chemistry Department, A M U Aligarh, for providing some spectral facilities.
I express my deep sense to my parents whose blessings have always served as sheet anchor against the mundane odds and inspired me to complete my mission. I am equally holding to my brothers, Eng. Salem, Mr. Abdull-Rahman, Mr. Zaki, Mr. Waleed and to my lovely sister Ms. Haifa.

I duly acknowledge the kind cooperation, constant encouragement of my friends who helped me to overcome nostalgia and homesickness whenever I felt lonely. How fortunate I was to be associated with Dr. Hasan M. H. Muhaisen, Dr. Shafiullah, Dr. Jallat Lajyum, Dr. Osama Edely, Dr. Manssour Satakeb, Dr. Wail Darwish, Dr. Hassan Adnan, Dr. Izz Amayyreh, Mr. Saleh Baballaib, Mr. Ali Radwan, Mr. Wail Radwan, Mr. Adli Radwan, Mr. Abedalelah Bani Khalf, Mr. Firas Samarab.

My deep thankful extended to my all relatives in front of them Mr. Salibi Ali and Mr. Fahed Omer.

Last but not the least, for neat and efficient laser typing of this thesis and for punctuality as well, my regards and thank are really extended to Mr. M. Kalamuddin.

OMER AHMED M. BASUDAN
CONTENTS

CHAPTER-I
INTRODUCTION---------------------------------------------- 1-23
REFERENCE--------------------------------------------------- 24-28

CHAPTER-II        Garcinia mannii (Guttiferae)
DISCUSSION------------------------------------------------- 29-35
EXPERIMENTAL----------------------------------------------- 36-41
REFERENCE--------------------------------------------------- 42

CHAPTER-III       Rhus alata (Anacardiaceae)
DISCUSSION------------------------------------------------- 43-59
EXPERIMENTAL----------------------------------------------- 60-67
REFERENCE--------------------------------------------------- 68

CHAPTER-IV        Ficus lyrata (Moraceae)
DISCUSSION------------------------------------------------- 69-88
EXPERIMENTAL----------------------------------------------- 89-100
REFERENCE--------------------------------------------------- 101-102

CHAPTER-V         Bergenia ligulata (Saxifragaceae)
DISCUSSION------------------------------------------------- 103-116
EXPERIMENTAL----------------------------------------------- 117-127
REFERENCE--------------------------------------------------- 128

CHAPTER-VI        Cassia siamea (Fabaceae)
DISCUSSION------------------------------------------------- 129-138
EXPERIMENTAL----------------------------------------------- 139-144
REFERENCE--------------------------------------------------- 145-146
CHAPTER 1
INTRODUCTION
Plants have always been common source of medication either in the form of traditional preparations or as pure active principles. In a survey done by WHO it has been estimated that 80% of more than 4000 million inhabitants of the World rely chiefly on traditional medicines for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts of their active principles. In the developed countries too, plants derived drugs are important. In USA, for example, 25% of all prescriptions dispensed from community pharmacies, contain plant extracts or active principles prepared from higher plants.

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

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

1. **Isoflavone**

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

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

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

\[\text{II: } R_1 = H; R_2 = H; R_3 = H; R_4 = OH; R_5 = \text{Prenyl}.\]

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

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

3. **Isoflavone glycoside:**

F.N. Ngounou et al.\(^1\), have isolated a new isoflavone glycoside, Pentandrin glucoside (V), from methanolic extract of the stem barks of *Ceiba Pentandra*.
Salwa F. Farag et al.\textsuperscript{20}, have reported two new isoflavonoids, tectorigenin 4’-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (VI), iristectorigenin B 7-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (VII) from rhizomes of Iris carthaliniae.

\begin{center}
\includegraphics[width=0.5\textwidth]{structure_vi.png}
\end{center}

VI: \( R_1 = \text{H}; R_2 = \text{Glc-Glc}; R_3 = \text{H} \)

VII: \( R_1 = \text{Glc-Glc}; R_2 = \text{H}; R_3 = \text{OMe} \)

4. **Bi and tetraflavonoids:**

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

\begin{center}
\includegraphics[width=0.5\textwidth]{structure_viii.png}
\end{center}

VIII
5. Methylaurones:

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

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

Yoshihisa Asada et al.\(^{23}\), have isolated a new prenylated biaurone (XV) from the hairy root culture of *Glycyrrhiza glabra* named as licoagrone.

![Diagram of XV](image)

7. **Coumarin:**

Maryam H. Al Yousuf et al.\(^{24}\), have isolated four coumarins from acetone extract of *Leucas inflata* roots.

![Diagram of XVI-XIX](image)

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

8. **Dicoumarin glycoside:**

Nisar Ullah et al.\(^{25}\), have isolated dicoumarin glycoside from methanolic extract of whole plant of *Daphne oleoides*. Its structure was established as 6, 7-dihydroxy-3-
methoxy-8-[2-oxo-2H-1-benzopyran-7-(O-β-D-glucopyranosyl)-8-yl]-2H-1-benzopyran-2-one (XX).
In the present study, we have tried to carry out systematic chemical investigations of some important medicinal plants with a view to characterise their chemical components preferably flavonoids, which could be the starting point for chemists who are mainly concerned with pharmacological and clinical aspects of the herbal drugs.

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

1. **Infra-Red Spectroscopy:**

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

   In case of furanocoumarins (psoralens) (XXI) two or three bands of weak to medium intensity have been observed in the region 3025-3175 cm$^{-1}$. These absorptions are due to the C-H stretching vibrations of the pyrone, benzene and furan rings.$^{26,27}$ The pyrone-carbonyl stretching frequency of coumarin is usually found in the region 1700-1750 cm$^{-1}$.

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

   ![XXI](image)

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

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

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

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

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

The infrared spectra of triterpenes have got much resemblance with the spectra of the steroids. However in C-3 ketones of the series of steroids, the C-2 and C-4 methylene groups absorb near 1420 cm\(^{-1}\) while in the corresponding 3-oxo triterpenes, the C-2, methylene group absorbs near 1430 cm\(^{-1}\), a C-11 methylene in 12-oxo steroids absorbs at 1434 cm\(^{-1}\), whereas the same group in 12-oxo-triterpenes absorbs close to 1420 cm\(^{-1}\). Cole and coworkers have summarised the positions of carbonyl bonds, ethylenic double bonds and the equatorial or axial nature of the hydroxyl groups, in triterpenic compounds in the IR region.
As a result of infrared spectroscopic studies it might be possible to make a
distinction between tertiary equatorial (3613 cm\(^{-1}\)) and tertiary axial (3617 cm\(^{-1}\)) hydroxyl
groups, on this basis the band at 3629 cm\(^{-1}\) (CCl\(_4\)) in methyl melaleuca\(te^{37}\) (XXII) has
been assigned as equatorial secondary, while its 3-\(\alpha\) epimer, obtained by oxidations of
the alcohol (XXII) and subsequent reduction gives a band at 3636 cm\(^{-1}\) due to axial
secondary nature of OH group.

![Chemical Structure](XXII)

2. **Ultra-Violet Spectroscopy:**

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

Psoralens mono-oxygenated at C-5 or C-8 gave spectra which are different from
those of 5, 8-dioxygenated psoralens. Psoralens mono-oxygenated at C-5 or C-8 show
maxima at \(\sim\) 260 nm and minima at \(\sim\) 277 nm.\(^{26}\), which are absent in spectra of
dioxygenated compounds.

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

![Chemical Structure](XXIII)
UV spectroscopy has become a major technique for the structure analysis of flavonoids for two main reasons. The first is that only a small quantity of pure material is required. The second reason is that the structural information obtained about flavonoids from UV is considerably enhanced by the use of specific reagents which react with one or more functional groups on the flavonoid nucleus. The addition of these reagents separately to an alcoholic solution of the flavonoid, induces structurally significant shifts in the UV spectrum. The commonly used shifts reagents\textsuperscript{39} are sodium methoxide (NaOMe), sodium acetate (NaOAc), sodium acetate / boric acid (NaOAc / H\textsubscript{3}BO\textsubscript{3}), aluminium chloride (AlCl\textsubscript{3}) and aluminium chloride/hydrochloric acid (AlCl\textsubscript{3}/HCl).

The UV spectra of most flavonoids consist of two major absorption maxima, one of which occurs in the range 240-285 nm (band II) associated with ring-A benzoyl system (XXIV) and second at a higher wave length (band I), occurs in the range of 320-380 nm associated with ring-B cinnamoyl absorption (XXV).\textsuperscript{39}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figures/UV_Spectra}
\caption{UV Spectra of Flavonoids}
\end{figure}

Substitution in ring-B specially at 4' stabilizes the cinnamoyl chromophore resulting in a red shift of band I whereas, substitution in ring-A has a similar effect on the position of band II. The presence of a free hydroxyl group at C-5 and C-3 positions is established by measuring the spectra in the presence of AlCl\textsubscript{3}\textsuperscript{39}. Compounds having a free 5-hydroxyl group absorb at higher wave length and methylation of this hydroxyl group brings about a blue shift of 1-15 nm of both bands. The hydroxyl groups at C-7 and 4' positions are more acidic than others and their occurrence is established by red shifts of band I & band II on the addition of fused sodium acetate.\textsuperscript{39} The presence of hydroxyl group at 4' position is also confirmed by a large red shift in band I without a decrease in intensity on the addition of sodium methoxide\textsuperscript{40}. The presence of ortho-dihydroxy groups in ring-A and ring-B is indicated by a red shift in band I in the presence of AlCl\textsubscript{3}/HCl and sodium acetate/boric acid respectively.
In flavanones and isoflavones, due to the absence of cinnamoyl chromophore the high wave length band is either totally absent or present only as an inflection. Thus it is difficult to distinguish between flavanones and isoflavones with the help of uv spectrum alone. The **ultra-violet** spectra of biflavonoids are very similar to those of monoflavonoids with the only difference that the molecular extinction coefficient (\(\varepsilon\)) of the biflavonoids is approximately double as compared to the corresponding monoflavonoids. This demonstrates the presence of two isolated chromophores of flavonoids per molecule of biflavonoids.

### 3. Nuclear Magnetic Resonance (\(^1\text{H-nmr}\)) Spectroscopy:

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

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

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

![Diagram of XXVI](image)

Since flavonoid compounds contain, in general, very few protons, nuclear magnetic resonance spectroscopy is a useful tool in the structural elucidation of this class of compounds. By the use of \(^1\text{H-nmr}\) studies of silyl derivatives,\(^{45}\) double irradiation techniques\(^{46a}\), solvent induced shift studies\(^{46b-48}\) and lanthanide induced shift studies\(^{49}\) (LIS), one can come to the structure of flavonoids without tedious and time consuming chemical degradation and synthesis.
The valuable contributions in this field have been made by Batterham \& Hightet$^{40}$, Mabry$^{31}$, Massicot$^{32a, b}$, Clark-Lewis$^{33}$, Kawano$^{40, 54}$, Pelter and Rahman$^{53-57}$ \textsuperscript{1}H-nmr spectroscopy is highly helpful in determining the substitution pattern of flavonoids. The most commonly occurring hydroxylation pattern in natural flavonoids is 5, 7, 4'-trihydroxy system (XXVII)

\textsuperscript{1}H-NMR signals in trimethyl silylated flavonoids$^{39}$ normally occur between 0 and 9 ppm. The chemical shifts of the protons of ring-A \& B prove to be independent of each other but are affected by the nature of ring-C.

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

(i) \textbf{H-5, H-6 and H-8 signals in 7-oxygenated flavonoids:}

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

(ii) \textbf{H-6 and H-8 signal in 5,7-dioxygenated flavonoids:}

The two ring-A protons, H-6 and H-8 give rise to two doublets ($J=2.5$ Hz) in the range $\delta$ 5-7-6.9 in flavones, flavonols, and isoflavones. The H-8 doublet occurs consistently downfield than the signal for H-6. The doublets for H-8 and H-6 are also clearly distinguished from each other by their widely different paramagnetic induced shifts. Depending upon the nature of the substituents, the chemical shifts may vary.
accordingly. For instance when a sugar is attached to the oxygen at C-7, the signal for both H-6 and H-8 are shifted downfield.

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

$^1$H-NMR provides the requisite information for differentiating 6 or 8 substituted isomers of 5,7,8 / 5,6,7-trisubstituted flavonoids with a high degree of surety. Horowitz & Gentili\(^{58}\) were able to fix up the structure for the two isomers of vitexin, viz. vitexin and isovitexin. The H-6 proton signal appears at about $\delta$ 0.2-0.3 upfield than H-8 signal.

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

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

With the introduction of 4'-hydroxyl group, the ring-B protons appear as a typical four peaks pattern of two doublets called A2B2 pattern (J=8 Hz, each). The H-3' and H-5' doublet always occurs upfield as compared to the H-2',6' doublet. This is attributed to shielding effect of the oxygen substituent and to the deshielding influence of ring-C functions on H-2' and H-6'. The position of H-2' and H-6' signal also depends to some extent on the oxidation level of ring-C.

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

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

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

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

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

The C-2 proton in dihydroflavonols appears near $\delta 4.9$ as a doublet ($J=11$ Hz) coupled to the C-3 proton which appears at about $\delta 4.2$ as doublet.\(^{59}\)

**Hydroxy protons:**

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

**Sugar protons:**

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

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

In flavonoid diglycosides, the C-1" proton of the terminal sugar (H-1") being relatively remote from the flavonoid nucleus, resonates upfield from H-1". The extent, however, can vary depending upon the position of attachment of terminal sugar.\textsuperscript{61} Methylated\textsuperscript{40} and acetylated\textsuperscript{40, 61-62} derivatives have also been used for disaccharide linkage determinations.

**Acetoxyl and Methoxyl protons:**

In the $^1H$-nmr spectra of acetylated flavonoids (CDCl$_3$), the position of methyl signals of acetyl groups can also give useful information about the position of acetyl group by which the position of the hydroxyl groups can be confirmed. The methyl signals of 4' and 7-O-acetyl groups appear in the range of $\delta 2.30-2.35$. While the methyl signal of a 5-O-acetyl group appears at about $\delta 2.45$. The aliphatic acetoxyl signals of sugars generally appear in the range of $\delta 1.65-2.10$. The position of the aliphatic acetoxyl groups of sugars also help in the location of sugar moiety in C-glycoxyyl flavonoids.\textsuperscript{39} Within the aliphatic acetoxyl group signals, the 2"-O-acetyl signal appears at $\delta 1.70-1.75$ in 8-C-glycosylflavonoids and $\delta 1.80-1.83$ in 6-C-glycosylflavonoids and 6"-O-acetyl signal in 8-C-glycoxyyl flavonoids appears at $\delta 1.90-1.95$ while in 6-C-glycosylflavonoids it appears between $\delta 1.98-2.04$. Methoxyl protons signals\textsuperscript{33-52a}, with few exceptions appear in the range of $\delta 3.5-4.1$.

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

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

The $^{13}$C-nmr spectra of flavonoids and their glycosides\textsuperscript{63} are of some interest in the context of compounds isolated during the course of this work. The spectra can be analysed by reference to those of simple compounds such as acetophenones\textsuperscript{63-64} and cinnamic acids\textsuperscript{64} which possess structural features characteristic of flavonoids.

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

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

The chemical shifts of flavones, substituted flavones and isoflavones are reproduced in the following table.
Chemical shift (in δ downfield from T.M.S.)

<table>
<thead>
<tr>
<th>Carbon Number</th>
<th>Flavone</th>
<th>7-methoxy flavone</th>
<th>5-hydroxy flavone</th>
<th>5,7,3',4'-tetrahydroxy flavones</th>
<th>7-methoxy isoflavone</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>163.2</td>
<td>162.6</td>
<td>164.07</td>
<td>165.07</td>
<td>152.4</td>
</tr>
<tr>
<td>3.</td>
<td>107.6</td>
<td>107.2</td>
<td>105.61</td>
<td>103.94</td>
<td>125.1</td>
</tr>
<tr>
<td>4.</td>
<td>178.4</td>
<td>177.4</td>
<td>182.90</td>
<td>182.63</td>
<td>175.3</td>
</tr>
<tr>
<td>5.</td>
<td>125.2</td>
<td>126.7</td>
<td>155.85</td>
<td>158.24</td>
<td>127.6</td>
</tr>
<tr>
<td>6.</td>
<td>125.2</td>
<td>114.1</td>
<td>107.22</td>
<td>94.90</td>
<td>114.6</td>
</tr>
<tr>
<td>7.</td>
<td>133.7</td>
<td>163.7</td>
<td>135.61</td>
<td>164.34</td>
<td>163.8</td>
</tr>
<tr>
<td>8.</td>
<td>118.1</td>
<td>100.2</td>
<td>110.83</td>
<td>99.91</td>
<td>100.0</td>
</tr>
<tr>
<td>9.</td>
<td>156.3</td>
<td>157.7</td>
<td>159.82</td>
<td>161.56</td>
<td>157.7</td>
</tr>
<tr>
<td>10.</td>
<td>124.0</td>
<td>117.6</td>
<td>110.13</td>
<td>104.2</td>
<td>118.3</td>
</tr>
<tr>
<td>1'</td>
<td>131.8</td>
<td>131.6</td>
<td>130.54</td>
<td>123.06</td>
<td>127.9</td>
</tr>
<tr>
<td>2'</td>
<td>126.3</td>
<td>125.8</td>
<td>126.39</td>
<td>114.38</td>
<td>128.2</td>
</tr>
<tr>
<td>3'</td>
<td>129.0</td>
<td>128.7</td>
<td>128.91</td>
<td>145.95</td>
<td>128.8</td>
</tr>
<tr>
<td>4'</td>
<td>131.6</td>
<td>131.1</td>
<td>131.97</td>
<td>149.84</td>
<td>131.8</td>
</tr>
<tr>
<td>5'</td>
<td>129.0</td>
<td>128.7</td>
<td>128.91</td>
<td>117.05</td>
<td>128.8</td>
</tr>
<tr>
<td>6'</td>
<td>126.3</td>
<td>125.8</td>
<td>126.39</td>
<td>120.14</td>
<td>127.9</td>
</tr>
</tbody>
</table>

(5) Mass Spectrometry

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

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

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

**Flavones:**

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

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

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

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

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

e) Doubly charged ions are frequently present.

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

Flavonoid O-glycosides:

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

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


40 R M Horowitz and B Gentilli, *Chem. and Ind.*, (London), 625 (1966)
51 T J Mabry, J, Kagan and H Rostel, Monograph, *'NMR analysis of flavonoids'* , Univ of Texas, Publication No 6418 (1964)


CHAPTER 2
DISCUSSION
Chemical constituents of *Garcinia mannii* (Guttifereae)

*Garcinia* is a large genus of evergreen trees or shrubs distributed in tropical Asia, Africa and Polynesia. About thirty species are found in India\(^1\). The genus *Garcinia* is known as a source of xanthonoids, biflavonoids and benzophenones\(^2\text{-}^4\).

*Garcinia mannii* is a rain forest tree of west Africa of Cameroon. The twigs and small adventitious roots have been observed to be used as chew-sticks and the dried, powdered root-bark, is reported to be a cure for sever diarrhoea and dysentery\(^5\).

The leaves of *Garcinia mannii* (2.5 kg) procured from Nigeria, were exhaustively extracted with acetone. The acetone extract was concentrated under reduced pressure. The acetone concentrate was successively extracted with light petroleum ether (60-80 °C), benzene and ethylacetate. The ethylacetate extract gave positive test for flavonoids\(^6\). On TLC examination, the ethylacetate extract showed four major spots in TEF and BPF solvent systems. Repeated column chromatography over silica gel followed by fractional crystallization and preparative TLC, afforded four crystalline TLC homogenous substances marked as GM-1, GM-2, GM-3 and GM-4.

**GM-1**

GM-1 was obtained from the column by benzene-ethylacetate (9:1) mixture, and was crystallized from benzene-chloroform as white shining crystals (140 mg). m.p. 149 °C. It gave greenish colour with alcoholic solution of ferric chloride. The elemental analysis agreed with molecular formula C\(_{10}\)H\(_{12}\)O\(_4\). The ir spectrum showed the characteristic bands at 1460 cm\(^{-1}\) (C=C), 1660 cm\(^{-1}\) (C=O) and 3300 cm\(^{-1}\) (OH) and uv spectrum gave \(\lambda_{\text{max}}\) at 312 nm.

The \(^1\)H-nmr spectrum (Table-1), exhibited a sharp singlet integrating for three protons at \(\delta\) 2.0 assigned to CH\(_3\). Two independent singlets of one proton each at \(\delta\) 6.38 and \(\delta\) 7.10 were ascribed to H-3 and H-5 protons respectively. Two singlets at \(\delta\) 3.16 and \(\delta\) 3.41 integrating for three protons each, indicated the presence of two methoxyl groups. On the basis of elemental analysis, ir, uv and \(^1\)H-nmr spectroscopy, the compound GM-1 was identified as dimethyl ether of phloroacetophenone (I).
The assigned structure was further supported by $^{13}$C-nmr spectrum (Table-2) which showed the presence of three oxygenated carbons at 166.2, 165.6 and 163.4, a carbonyl carbon at 180.1 and a methyl group at 16.5 ppm. The mass spectrum showed the molecular ion peak at m/z 196.

In the light of above results, GM-1 was characterized as 2, 4-dimethoxy-6-hydroxy acetophenone (dimethylether of phloroacetophenone)$^7$(I).

![Chemical structure of GM-1](image)

Table-1

$^1$H-nmr data of GM-1, values on $\delta$- scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>2.0 (s)</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>3</td>
<td>3.16 (s)</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>3</td>
<td>3.41 (s)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.38 (s)</td>
</tr>
<tr>
<td>H-5</td>
<td>1</td>
<td>7.10 (s)</td>
</tr>
<tr>
<td>OH</td>
<td>1</td>
<td>12.5 (s)</td>
</tr>
</tbody>
</table>

$s=$ singlet, spectrum run in CDCl$_3$ at 300 MHz, using TMS as internal standard.
Table-2

$^{13}$C-nmr data of GM-1, values on δ- scale

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>104.8</td>
</tr>
<tr>
<td>C-2</td>
<td>166.2</td>
</tr>
<tr>
<td>C-3</td>
<td>91.2</td>
</tr>
<tr>
<td>C-4</td>
<td>165.6</td>
</tr>
<tr>
<td>C-5</td>
<td>94.1</td>
</tr>
<tr>
<td>C-6</td>
<td>163.4</td>
</tr>
<tr>
<td>C=O</td>
<td>180.1</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>16.5</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>55.3</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>56.1</td>
</tr>
</tbody>
</table>

GM-2

GM-2 was eluted from the column by benzene-ethyl acetate (8:2) mixture. It was crystallized from chloroform-methanol as yellow shining crystals (100 mg), m.p. 311-12 °C. It was identified as quercetin (IIa) by m.p., m.m.p. and co-chromatography with an authentic sample of quercetin. It gave an acetate m.p. 194-95 °C and pentamethyl ether m.p. 151-52 °C. Its identity as quercetin was further confirmed by comparing its spectral data with those of an authentic sample of quercetin$^8$.

The $^1$H-nmr spectrum of its acetate GM-2 (Ac) (IIb) showed the signals due to five acetoxy groups in the range of δ 2.35-2.38. The meta-coupled doublets at δ 6.79 (J=2.5 Hz) and δ 7.21 (J=2.5 Hz) were assigned to C-6 and C-8 protons of A-ring respectively. The B-ring protons showed the ABX pattern, two doublets at δ 7.73 (J=2.5 Hz) for H-2' and δ 7.27 (J=9.0 Hz) for H-5' and quartet at δ 7.67 (J$_1$= 2.5 Hz, J$_2$=9.0 Hz) for H-6'.

In the light of the above results, the compound GM-2 was assigned the structure as 3, 5, 7, 3', 4'-pentahydroxy flavone (quercetin) (IIa), supported by the mass spectrum which showed molecular ion peak at m/z 302.
GM-3

GM-3 was eluted from the column by benzene-ethyl acetate (7:3) mixture. It was characterized as apigenin (III), by comparison with an authentic sample (Rf-value, m.p., m.m.p. and co-chromatography). The identity of the compound, GM-3, as apigenin (III, 5, 7, 4'-trihydroxy flavone) was further confirmed by $^1$H-nmr studies of its acetate, m.p. 183-84 °C (Table-3).
Table-3

$^1$H-nmr data of GM-3 (Ac), values on $\delta$- scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.76 (d, J= 8.6 Hz)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.20 (d, J= 8.6 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.89 (d, J= 2.5 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.74 (d, J= 2.4 Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.58 (s)</td>
</tr>
<tr>
<td>OAc-5</td>
<td>3</td>
<td>2.44 (s)</td>
</tr>
<tr>
<td>OAc-4',7</td>
<td>6</td>
<td>2.35 (s)</td>
</tr>
</tbody>
</table>

$s=$ singlet, $d=$ doublet, spectrum run in CDCl$_3$ at 300 MHz, using TMS as internal standard.

GM-4

The fraction obtained by the elution of the column by benzene-ethylacetate (1:1), on TLC examination was found to be an intricate mixture of several compounds, with one of them as the major one. Repeated column chromatography followed by preparative-TLC failed to separate the mixture. However, by PTLC, most of the minor fractions were removed. The major fraction along with some very minor impurities was analysed by $^1$H-nmr spectrum. As the compound was not absolutely pure, the spectrum could not be successfully interpreted. However, the presence of OCH$_3$ group was ruled out by the absence of any peak in the $^1$H-nmr spectrum.

The mixture was methylated with dimethyl sulphate and major band was separated by PTLC. It was purified by crystallization (165 mg), m.p. 189-90 °C. The UV spectrum and colour test$^6$ showed it to be a flavone. Elemental analysis gave the molecular formula as C$_{38}$H$_{34}$O$_{12}$, corresponding to the molecular ion peak at m/z 682. The molecular weight indicated it to be a methyl ether of biflavone. The structure of the methyl ether was established by $^1$H-nmr, $^{13}$C-nmr and mass spectroscopy.

The $^1$H-nmr spectrum of GM-4 (Me) (Fig-1, Table-4), showed the presence of eight methoxyl groups by four independent singlets of six protons each at $\delta$ 3.68, 3.85, 3.91 and 3.94 respectively. The A-ring protons appeared as two singlets for two protons each at $\delta$ 6.16 ascribed to H-I-6 and H-II-6 and at $\delta$ 6.20 attributed to H-I-8 and
H-II-8. The B-ring protons showed typical ABX pattern consisting of an ortho-coupled doublet at δ 7.32 (J= 8.1 Hz) assigned to H-I-6' and H-II-6' and a double doublet for four protons in the range of δ 6.09-6.17 (J1= 8.0 Hz, J2= 2.0 Hz) corresponded to H-I-3', 5' and H-II-3', 5' protons. Hence, it indicated that H-I-2' and H-II-2' are involved in inter flavonoid linkage which was further proved by the fragment ions at m/z 475, 460, 444, 416, 295, 268 in the mass spectrum (Fig-2, Scheme-1). The RDA fragments appeared at m/z 180 and 502. Other fragments are rationalized from the Scheme-1.

The above assigned structure was also supported by the 13C-nmr spectrum (Fig-3, Table-5) of GM-4 (Me) in which both the carbonyl groups were observed at 188.8 ppm. It also showed a downfield shift for I and II-C-2', further confirming the inter flavonoid linkage between I-C-2' and II-C-2'.

In view of the above facts GM-4 (Me) was assigned the structure I-3, II-3, I-5, II-5, I-7, II-7, I-4', II-4'-Octamethoxy [I-2', II-2'] biflavone (IVb). The parent compound, GM-4, was therefore, assigned the structure as I-3, II-3, I-5, II-5, I-7, II-7, I-4', II-4'-Octahydroxy [I-2', II-2'] biflavone (IVa) which is being reported for the first time.

(IV)

a) R=H
b) R=Me
Table-4

$^1$H-nmr data of GM-4 (Me), values on δ- scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x OMe</td>
<td>6</td>
<td>3.68 (s)</td>
</tr>
<tr>
<td>2 x OMe</td>
<td>6</td>
<td>3.85 (s)</td>
</tr>
<tr>
<td>2 x OMe</td>
<td>6</td>
<td>3.91 (s)</td>
</tr>
<tr>
<td>2 x OMe</td>
<td>6</td>
<td>3.94 (s)</td>
</tr>
<tr>
<td>H-I-6, H-II-6</td>
<td>2</td>
<td>6.16 (s)</td>
</tr>
<tr>
<td>H-I-8, H-II-8</td>
<td>2</td>
<td>6.20 (s)</td>
</tr>
<tr>
<td>H-I-3',5' and H-II-3', 5'</td>
<td>4</td>
<td>7.09-7.17 (dd, $J_1= 8.0$ Hz, $J_2= 2.0$ Hz)</td>
</tr>
<tr>
<td>H-I-6', H-II-6'</td>
<td>2</td>
<td>7.32 (d, $J= 8.1$ Hz)</td>
</tr>
</tbody>
</table>

$s=$ singlet, $d=$ doublet, dd= double doublet, spectrum run in CDCl$_3$ at 300 MHz, using TMS as internal standard.
Table-5

$^{13}$C-nmr data of GM-4 (Me), values on $\delta$- scale

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$8 \times$ OCH$_3$</td>
<td>55.64</td>
</tr>
<tr>
<td></td>
<td>55.99</td>
</tr>
<tr>
<td></td>
<td>56.10</td>
</tr>
<tr>
<td></td>
<td>56.28</td>
</tr>
<tr>
<td>I, II-C-2</td>
<td>160.6</td>
</tr>
<tr>
<td>I, II-C-3</td>
<td>105.8</td>
</tr>
<tr>
<td>I, II-C-4</td>
<td>188.8</td>
</tr>
<tr>
<td>I, II-C-5</td>
<td>159.6</td>
</tr>
<tr>
<td>I, II-C-8</td>
<td>98.4</td>
</tr>
<tr>
<td>I, II-C-7</td>
<td>162.6</td>
</tr>
<tr>
<td>I, II-C-6</td>
<td>98.6</td>
</tr>
<tr>
<td>I, II-C-9</td>
<td>151.6</td>
</tr>
<tr>
<td>I, II-C-10</td>
<td>108.8</td>
</tr>
<tr>
<td>I, II-C-1'</td>
<td>119.9</td>
</tr>
<tr>
<td>I, II-C-2'</td>
<td>129.8</td>
</tr>
<tr>
<td>I, II-C-3', 5'</td>
<td>113.4</td>
</tr>
<tr>
<td>I, II-C-4'</td>
<td>165.0</td>
</tr>
<tr>
<td>I, II-C-6'</td>
<td>128.0</td>
</tr>
</tbody>
</table>
Scheme 1
EXPERIMENTAL
The melting points were taken on a Kofler block and are uncorrected. All ultraviolet spectra were measured on Beckmann Model DU and Pye Unicam PU-8800 spectrophotometers in methanol / ethanol. Infrared spectra were taken on Shimadzu IR-408 Perkin Elmer 1800 (FTIR). The mass and $^1$H-nmr spectra were obtained from different institutes in the country and outside. The mass spectra were mostly measured in E.I. mode on Jeol D-300 while, the $^1$H-nmr spectra were usually recorded on Varian EM-360 L (60 MHz), 270 MHz, JEOL 4H-100 MHz, Perkin Elmer R-32 (90 MHz), Brucker dpx 200 MHz, DRX 300 MHz and WM 400 MHz in CDCl$_3$ / DMSO-d$_6$ using TMS as internal standard.

The silica gel used for different chromatographic purposes, was obtained from E. Merck (India), E. Merck (Germany) and SRL (India). TLC solvent systems used were benzene-pyridine-formic acid (BPF, 36:9:5), toluene-ethylformate-formic acid (TEF, 5:4:1), ethylacetate-ethylmethylketone-acetic acid-water (EtOAc-EtMeCO-AcOH-H$_2$O, 5:3:1:1; 20:3:1:1; 30:3:1:1), ethylacetate-methanol-water (EtOAc-MeOH-H$_2$O, 8:1:1), petrol-benzene (2:8) n-butanol-acetic acid-water (BAW, 4:1:5), n-butanol-water-ethanol (BEW, 60:28.5:16.5).

Alcoholic ferric chloride, iodine vapours and aniline hydrogen phthalate solutions were used as spray reagents for visualization of spots on TLC and on paper chromatograms.
GM-1

GM-1 was obtained from the column by benzene-ethylacetate (9:1) mixture. It was crystallized from benzene-chloroform as white shining crystals (140 mg), m.p. 149 °C. It gave greenish colour with alcoholic solution of ferric chloride.

Analysed for C_{10}H_{12}O_{4}.
Calcd.  C, 61.22; H, 6.12%
Foud    C, 61.19; H, 6.09%

$^{KBr}$IR $\nu_{\text{max}}$ cm$^{-1}$
3300 (OH), 1660 (C=O), 1460 (C=C).

UV $\lambda_{\text{max}}$ (MeOH) nm
312

$^1$H-nmr (300 MHz, CDCl$_3$), values on $\delta$-scale
2.0 (3H, s, CH$_3$), 3.16 (3H, s, OMe), 3.41 (3H, s, OMe), 6.38 (1H, s, H-3), 7.10 (1H, s, H-5), 12.5 (1H, s, OH).

$^{13}$C-nmr (300 MHz,CDCl$_3$), values on $\delta$-scale
16.5 (CH$_3$), 91.2 (C-3), 94.1 (C-5), 104.8 (C-1), 163.4 (C-6), 165.6 (C-4), 166.2 (C-2), 180.1 (C=O), 55.3 (OCH$_3$), 56.1 (OCH$_3$).

Mass m/z (rel. Intensity)

GM-2

GM-2 was eluted from the column by benzene-ethyl acetate (8:2) mixture. It was crystallized from chloroform-methanol as yellow shining crystals (100 mg), m.p. 311-12 °C.
Analysed for $C_{15}H_{10}O_7$.
Calcld. C, 59.60; H, 3.31 %
Foud C, 59.64; H, 3.33 %

**UV data with shift reagents $\lambda_{max} \text{ nm}**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>258, 272 sh, 300 sh, 371</td>
</tr>
<tr>
<td>NaOAc</td>
<td>258 sh, 275, 390</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>263, 301, 389</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>273, 304 sh, 335, 457</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>264, 358, 429</td>
</tr>
<tr>
<td>NaOMe</td>
<td>248 sh, 321</td>
</tr>
</tbody>
</table>

**Acetylation of GM-2**

GM-2 (20 mg) was heated with pyridine (1 ml) and acetic anhydride (2 ml) on a water bath for about two hours. After cooling, the mixture was poured on crushed ice and left over night. The solid obtained was collected, washed with water and dried. On crystallization from ethylacetate, it gave cream coloured crystals (15 mg), m.p. 194-95 °C.

**$^1$H-nmr (60 MHz, CDCl$_3$), values on $\delta$-scale**

2.38 (3H, s, OAc), 2.35 (12H, m, 4x OAc), 6.79 (1H, d, J=2.5 Hz, H-6), 7.21 (1H, d, J=2.5 Hz, H-8), 7.27 (1H, d, J=9.0 Hz, H-5'), 7.67 (1H, q, $J_1$=2.5 Hz, $J_2$=9.0 Hz, H-6'), 7.73 (1H, d, J=2.5 Hz, H-2').

**Methylation of GM-2**

Crystalline GM-2 (25 mg), dry acetone (30 ml), dimethylsulphate (1 ml) and anhydrous potassium carbonate (0.5 g) were refluxed for 24 hours. The reaction mixture was filtered and the inorganic residue washed several times with hot acetone. On distilling off the solvent, a colourless semi-solid mass was left behind. It was washed with hot petroleum ether to remove the excess of dimethyl sulphate. The solid residue on crystallization from methanol-ethylacetate gave colourless needles (20 mg), m.p. 151-52° C.
**GM-3**

The fraction obtained from the elution of the column with benzene-ethylacetate (7:3) mixture gave a yellow solid mass which was crystallized from benzene-acetone as yellow needles (180 mg), m.p. 352°C.

Analysed for C_{15}H_{10}O_5.
Calcd. C, 66.66; H, 3.70 %
Foud C, 66.78, H, 3.74 %

**UV data with shift reagents** \( \lambda_{\text{max}} \text{ nm} \)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>MeOH</th>
<th>NaOAc</th>
<th>NaOAc/H_3BO_3</th>
<th>AlCl_3</th>
<th>AlCl_3/HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>266, 298 sh, 339</td>
<td>279, 304, 377</td>
<td>268, 301 sh, 338</td>
<td>280, 302, 342,392</td>
<td>280, 301, 342, 391</td>
</tr>
<tr>
<td>NaOAc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOAc/H_3BO_3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlCl_3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlCl_3/HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Acetylation of GM-3**

GM-3 (40 mg) was heated with pyridine (1 ml) and acetic anhydride (2 ml) on water bath for about two hours. On usual work-up, the solid obtained was crystallized from chloroform-methanol as colourless needles (35 mg), m.p. 183-84 °C.

**\(^1\text{H-nmr (300 MHz, CDCl}_3\), values on } \delta \text{-scale}**

2.35 (6H, s, OAc-4’,7), 2.44 (3H, s, OAc-5), 6.58 (1H, s, H-3), 6.74 (1H, d, J=2.4 Hz, H-6), 6.89 (1H, d, J=2.5 Hz, H-8), 7.20 (2H, d, J=8.6 Hz, H-3’,5’), 7.76 (2H, d, J=8.6 Hz, H-2’,6’).

**GM-4**

The fraction obtained by the elution of the column by benzene-ethylacetate (1:1), on TLC examination was found to be an intricate mixture of several compounds, one of them was the major one. Repeated column chromatography followed by preparative-TLC failed to separate the mixture. However, by PTLC using TEF (5: 4: 1) system as the developing solvent most of the minor fractions were
removed. The mixture was methylated with dimethyl sulphate and major band was separated. It was purified by crystallization (160 mg), m.p.189-90 °C. The uv spectrum and colour test showed it to be a flavone. Elemental analysis gave the molecular formula as C_{38}H_{34}O_{12}. Further confirmed by the molecular ion peak at m/z 682.

**Methylation of GM-4**

Crystalline GM-4 (150 mg), dry acetone (30 ml), dimethylsulphate (2 ml) and anhydrous potassium carbonate (0.5 g) were refluxed for 24 hours. After usual work-up the solid obtained was crystallized from chloroform-methanol as white crystals (165 mg), m.p.189-90 °C.

Analysed for C_{38}H_{34}O_{12}

Calcd. C, 66.86; H, 4.98%

Found C, 66.90; H, 5.01%

**IR \nu_{\text{max}} \text{ cm}^{-1}\**

3200-3400 (br, OH), 1680 (C=O), 1600, 1510, 1440

**UV data with shift reagent \lambda_{\text{max}} \text{ nm}\**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>\lambda_{\text{max}} nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>258 sh, 268, 294 sh, 323 sh, 368</td>
</tr>
<tr>
<td>NaOAc</td>
<td>276, 304, 387</td>
</tr>
<tr>
<td>NaOAc/H_3BO_3</td>
<td>277, 304, 388</td>
</tr>
<tr>
<td>AlCl_3</td>
<td>262 sh, 278, 303 sh, 351, 424</td>
</tr>
<tr>
<td>AlCl_3/HCl</td>
<td>263 sh, 279, 303 sh, 351, 424</td>
</tr>
<tr>
<td>NaOme</td>
<td>279, 316, 416 (dec.)</td>
</tr>
</tbody>
</table>

**\^1H-nmr (300 MHz, CDCl_3), values of \delta-scale\**

3.68 (6H, s, 2 x OMe), 3.85 (6H, s, 2 x OMe), 3.91 (6H, s, 2 x OMe), 3.94 (6H, s, 2 x OMe), 6.16 (2H, s, H-I-6, H-II-6), 6.20 (2H, s, H-I-8, H-II-8), 7.09-7.17 (4H, dd, J_1=8.0 Hz, J_2=2.0 Hz, H-I-3^\prime,5^\prime, H-II-3^\prime, 5^\prime), 7.32 (2H, d, J=8.1 Hz, H-I-6^\prime, H-II-6^\prime).
$^{13}$C-nmr (300 MHz, CDCl$_3$), values of $\delta$-scale

55.64, 55.99, 56.10, 56.28 (8 x OMe), 160.6 (I-II-C-2), 105.8 (I-II-C-3), 188.8 (I-II-C-4), 159.6 (I-II-C-5), 98.4 (I-II-C-8), 162.6, (I-II-C-7), 98.6 (I-II-C-6), 151.6 (I-II-C-9), 108.8 (I-II-C-10), 119.9 (I-II-C-1'), 129.8 (I-II-C-2'), 113.4 (I-II-C-3',5'), 165.0 (I-II-C-4'), 128.0 (I-II-C-6').

Mass m/z (rel. int.)

682 [M$^+$] (2.5), 502 (2.1), 475 (2.0), 474 (3.8), 473 (8.5), 460 (5.4), 459 (4.2), 444 (8.1), 416 (2.9), 342 (2.0), 341 (2.9), 327 (2.2), 312 (2.0), 295 (2.8), 268, 240 (2.6), 238 (3.6), 181 (18.3), 180 (100), 165 (2.7), 153 (3.8), 151 (18.7), 150 (2.6).
REFERENCES
CHAPTER 3
DISCUSSION
Chemical constituents of *Rhus alata*  
*(Anacardiaceae)*

The family *Anacardiaceae* consists of 73 genera and about 600 species. The genus *Rhus* one of the largest genera (about 50 species) of trees, shrubs and climbers is chiefly distributed in the warm temperate region of both the hemispheres extending in the tropical and cold temperate region. About a dozen species occur in India\(^1\), find extensive use in folk medicines for the treatment as spasmodytic, antiviral and anticancer\(^2\). Some species may cause dermatitis in sensitive people\(^3\). *Rhus* species have been reviewed by Khan\(^4\).

Earlier report from this plant is the isolation and characterization of biflavones\(^5\) and triterpenes\(^6\) from the leaves.

The present discussion deals with the isolation and characterization of the following compounds from the bark of *Rhus alata*

1) Dimethylester of terephthalic acid.  
2) β-Amyrin  
3) Friedelin  
4) Lupeol  
5) β-Sitosterol  
6) Oleanolic acid  
7) Taraxerone  
8) Ethyl gallate  
9) (2E)-3-(4-hydroxy-5,7-dimethylbenzo[3,4-b]furan-6-yloxy)-prop-2-enoic acid.

The barks of *Rhus alata* were collected from Pucchunga University College, Aziaul, Mizoram. After defatting with petroleum ether (40-60°C), they were refluxed exhaustively with benzene, chloroform and ethylacetate respectively. Benzene and chloroform extracts responded positively to terpenoid test\(^7\) and showed similar spots on TLC plates. Hence, they were mixed together. After removal of the solvents under reduced pressure, a gummy mass was obtained. The gummy mass was chromatographed over silica gel column, using different solvent systems namely, petrol, petrol-benzene (9:1-1:1), benzene, benzene-chloroform (9:1-1:1) and ethylacetate
respectively. The fractions obtained from petrol-benzene (9:1-1:1) showed four spots on TLC plates with some minor impurities. Repeated column chromatography followed by fractional crystallization with chloroform-methanol gave four compounds labelled as RA-1, RA-2, RA-3 and RA-4. Benzene and benzene-chloroform (9:1) elutes were found to contain two same substances, which was separated by repeated column chromatography followed by fractional crystallization and labelled as RA-5 and RA-6. Benzene-chloroform elutes (8:2-1:1) were found to be a mixture of three compounds which were separated by repeated column chromatography and labelled as RA-7, RA-8 and RA-9.

RA-1

RA-1 was obtained from the column with petroleum ether-benzene (9:1) mixture. It was crystallized from chloroform-petroleum ether as white needles (100 mg), m.p. 138°C. The elemental analysis agreed with molecular formula C_{10}H_{10}O_{4}, further supported by the molecular ion peak [M⁺] at m/z 194.

The ir spectrum of the compound RA-1 showed a carbonyl band at 1720 cm⁻¹ along with bands at 1500, 1435 cm⁻¹ characteristic of an aromatic compound. The uv spectrum showed maxima absorption at 265 nm along with weak absorption at 340 nm.

The ¹H-nmr spectrum showed a sharp singlet at δ3.97 integrating for six protons, corresponded to two methoxy groups. A sharp singlet at δ8.13 integrated for four protons was assigned to H-2, H-3, H-5 and H-6 of phthalic acid.

The mass spectrum was quite typical for aromatic methyl esters. The base peak was observed at m/z 163 [M⁺-OCH₃] and other fragments were observed at m/z 179 [M⁺-CH₃], 164 [M⁺-2 x CH₃], 135 [M⁺-COOCH₃] and 76 [M⁺-2 x COOCH₃] respectively.

On the basis of above results, the RA-1 was identified as dimethylester of terephthalic acid. (I).
RA-2

RA-2 was eluted from column with petroleum ether-benzene (7:3) mixture and crystallized with chloroform-methanol as white needles (190 mg), m.p. 198 °C, $[\alpha]_{D}^{19} +88.4^\circ$ (CDCl$_3$). It gave positive Leibermann-Burchard test. The IR spectrum showed the bands at 3360 cm$^{-1}$ (OH), 2960 cm$^{-1}$, 2880 cm$^{-1}$, 1650 cm$^{-1}$, 1465 cm$^{-1}$, (C=C) 1040 cm$^{-1}$ and 980 cm$^{-1}$ indicating the presence of hydroxyl group and olefinic bond. The molecular ion peak of RA-2 at m/z 426 along with elemental analysis agreed with molecular formula C$_{30}$H$_{50}$O. The $^1$H-nmr data are given in (Table-1).

From the above data and their direct comparison with an authentic sample, RA-2 was identified as $\beta$-Amyrin$^8$ (II).

![Chemical structure of RA-2](image)

### Table-1

$^1$H-nmr data of RA-2, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 $\times$ CH$_3$</td>
<td>24</td>
<td>0.78 (s, 3H), 0.83 (s, 3H), 0.88 (s, 6H), 0.95 (s, 3H), 0.98 (s, 3H), 1.0 (s, 3H), 1.14 (s, 3H).</td>
</tr>
<tr>
<td>-CH$_2$ and -CH protons of cyclic system</td>
<td>23</td>
<td>1.83, 2.01, 3.01 (dd, $J$=9.0 Hz and 7.0 Hz)</td>
</tr>
<tr>
<td>$\alpha$- to OH</td>
<td>1</td>
<td>4.88 (s, br)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.21 (m)</td>
</tr>
</tbody>
</table>

$s$= singlet, $d$= doublet, $m$= multiplet, spectrum run at 100 MHz, using TMS as internal standard.
RA-3

RA-3 was obtained from the column with petroleum ether-benzene (6:4) mixture and crystallized with chloroform-methanol as white needle-shaped crystals (150 mg), m.p. 262-64 °C, $[\alpha]_D^{23} -29.4^\circ$ (CDCl$_3$). It gave positive Leibermann-Burchard test and Nollers test indicating RA-3 to be a triterpene. The elemental analysis and molecular ion peak at m/z 426 agreed with molecular formula C$_{30}$H$_{50}$O. Its identity as freidelin (III) was established by comparison of its spectral data including $^1$H-nmr data (Table-2), ir and mass spectra and co-chromatography, m.p. and m.m.p. with that of authentic sample of Friedelin.

![Diagram of RA-3](image)

Table-2

$^1$H-nmr data of RA-3 values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.72</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.87</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.89</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.92</td>
</tr>
<tr>
<td>2 x CH$_3$</td>
<td>(6H, s)</td>
<td>0.95</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>1.05</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>1.18</td>
</tr>
<tr>
<td>-CH$_2$ and -CH protons</td>
<td>(23H, m)</td>
<td>1.25, 1.34, 1.45, 1.52, 1.58</td>
</tr>
<tr>
<td>C$_2$- 2H and C$_4$- 1H</td>
<td>(3H, m)</td>
<td>2.26- 2.41</td>
</tr>
</tbody>
</table>

$s=$ singlet, $m=$ multiplet, spectrum run at 200 MHz in CDCl$_3$, using TMSi as internal standard
RA-4

RA-4 was obtained from the column with petroleum ether-benzene (1:1) mixture and crystallized from methanol-chloroform (170 mg), m.p. 214-15 °C, [α]D20 +23.64° (CHCl₃). It gave positive Leibermann-Burchard test, Nollers test and gave yellow colour with tetranitromethane. The elemental analysis agreed with molecular formula C₃₀H₅₀O. IR showed bands at 3360 cm⁻¹ and 1030 cm⁻¹ (OH), 1645 cm⁻¹ (C=C) and 1385 cm⁻¹ (geminal dimethyl), 885 cm⁻¹ (terminal methylene). Mass spectrum of the triterpene alcohol gave [M⁺] m/z 426 (11.0%) with other principal ions at m/z 411 [M-CH₃] (6.0%), 207 (34.0%), 189 (77.0%) and a base peak at m/z 95. It afforded an acetate m.p. 218-20 °C. IR spectrum of the acetate revealed the presence of terminal methylene by a band at 875 cm⁻¹. Some other important bands were observed at 1245 cm⁻¹ (acetate), 1640 cm⁻¹ (C=C) and 1730 cm⁻¹ (C=O). ¹H-nmr spectrum gave the signals at δ 0.82, 0.87, 0.94, 1.04 (CH₃-protons), 1.27, 1.41, 1.46, 1.47 (CH₂-protons), δ 2.03 (OCOCH₃) and multiplets in the range of δ 4.28-4.77 (>CHOAc), δ 4.59-δ 4.67 (J=8.0 Hz, >C=CH₂).

On the basis of the above physico-chemical data for RA-4 and its derivatives, RA-4 was identified as Lupeol (IV).

RA-5

RA-5 was obtained from the column with benzene only and crystallized from chloroform-methanol as white needle-shaped crystals (150 mg), m.p. 136-37°C, [α]D -32.1° (CDCl₃). It gave positive Leibermann-Burchard test and responded to tetranitromethane colour test. The ir spectrum showed the presence of gem-dimethyl groups, hydroxyl group and olefinic double bond and showed bands at 3340 cm⁻¹ (OH), 1055 cm⁻¹, 1655 cm⁻¹, 1460 cm⁻¹, 840 cm⁻¹ (C=C), 1375 cm⁻¹ (C-Me₂). RA-5 gave an acetate with acetic
anhydride and pyridine, m.p. 114-16 °C, [α] D <sup>17</sup> -48.5° (CHCl<sub>3</sub>) and benzoate m.p. 145-46 °C. The <sup>1</sup>H-nmr data are given in (Table-3). The mass spectrum showed the molecular ion peak at m/z 414.

From the above data and direct comparison with an authentic sample<sup>8</sup>, RA-5 was characterized as β-Sitosterol (V).

![Structure of RA-5](image)

**Table-3**

<sup>1</sup>H-nmr data on RA-5, values on δ-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.70 (s, 3H)</td>
</tr>
<tr>
<td>29-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.80 (d, J=6.8 Hz, 3H)</td>
</tr>
<tr>
<td>26,27-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.88 (d, J=6.5 Hz, 6H)</td>
</tr>
<tr>
<td>21-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.92 (d, J=6.5 Hz, 3H)</td>
</tr>
<tr>
<td>19-CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.02 (s, 3H)</td>
</tr>
<tr>
<td>3-ax-H</td>
<td>3.56 (m, 1H)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>5.36 (m, 1H)</td>
</tr>
<tr>
<td>-CH&lt;sub&gt;2&lt;/sub&gt; and -CH protons of cyclic system and side chain</td>
<td>1.07-2.34</td>
</tr>
<tr>
<td>OH</td>
<td>4.25 (m, 1H)</td>
</tr>
</tbody>
</table>

s= singlet, d= doublet, m= multiplet, spectrum run in CDCl<sub>3</sub> at 90 MHz, using TMS as internal standard

**RA-6**

Elution of the column with benzene-chloroform (9:1) afforded a fraction which on TLC examination showed the presence of one major spot along with some minor
impurities. It was purified by crystallization into pure component RA-6 (225 mg) m.p. 299-300 °C. It responded positively to Leibermann-Burchard test.

Its ir spectrum showed the characteristic bands at 3200 (OH), 2750-2610 (COOH), 1655 (C=O), 1210 cm⁻¹. The ¹H-nmr spectrum of RA-6 (Table-4) indicated the presence of seven tertiary methyl groups at δ 0.71 (3H), 0.82 (6H), 0.84 (3H), 0.89 (6H) and 1.10 (3H). The two unresolved multiplets at δ 4.39 and 5.20 are ascribed to one proton α-to hydroxyl group and one olefinic proton. Acetylation of RA-6 with acetic anhydride and pyridine afforded a monoacetate RA-6 (Ac). The ir spectrum of RA-6 (Ac) displayed bands at 1724, 1686 and 1250 cm⁻¹ characteristic of the acetyl function. The ¹H-nmr spectrum of the acetate RA-6(Ac) showed seven tertiary methyls as four independent singlets at δ 0.75 (3H), 0.85(6H), 0.94 (9H) and 1.12 (3H) and one singlet for an acetyl function at δ 2.02, an unresolved multiplet at δ 4.50 for one proton α- to the acetoxyl and another multiplet at δ5.25 for the olefinic proton. These data further support to the β-amyrin skeleton of the compound with one acetyl function present. Formation of its methyl ester (m.p. 198 °C) with diazomethane and not with methanolic hydrochloric acid, coupled with difficulty of its hydrolysis suggested the attachment of carboxyl group at tertiary carbon atom (C-17). Its ¹H-nmr spectrum showed one singlet of methyl ester at δ 3.58 and seven tertiary methyls as singlets at δ 0.70 (3H), 0.84 (6H), 0.86 (3H), 0.90(6H) and 1.10 (3H). Two unresolved multiplets centered at δ4.50 and δ5.30 can be attributed to one proton α- to methyl ether and one olefinic proton respectively.

On the basis of above spectral data and comparison of m.p., m.m.p. with an authentic sample confirmed the identity of RA-6 as oleanolic acid (VI).
Table-4

'H-nmr data of RA-6, values on δ- scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td>3</td>
<td>0.71 (s)</td>
</tr>
<tr>
<td>2 x CH₃</td>
<td>6</td>
<td>0.82 (s)</td>
</tr>
<tr>
<td>CH₃</td>
<td>3</td>
<td>0.84 (s)</td>
</tr>
<tr>
<td>2 x CH₃</td>
<td>6</td>
<td>0.89 (s)</td>
</tr>
<tr>
<td>CH₃</td>
<td>3</td>
<td>1.10 (s)</td>
</tr>
<tr>
<td>H-α to hydroxyl group</td>
<td>1</td>
<td>4.39 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.20 (m)</td>
</tr>
</tbody>
</table>

s= singlet, m= multiplet, spectrum run at 60 MHz in CDCl₃, using TMS as internal standard.

RA-7

RA-7 was eluted from the column with benzene-chloroform (8:2) mixture. It was crystallized from chloroform-ethanol as colourless needles (170 mg), m.p. 240°C. The elemental analysis agreed with molecular formula C₃₀H₄₈O. It gave positive Leibermann-Burchard test. The ir spectrum showed a strong carbonyl band at 1690-1700 cm⁻¹.

The 'H-nmr spectrum of RA-7 (Table-5) showed five singlets attributed to the protons of eight methyl groups at δ 0.83 (3H), 0.93 (6H), 0.97 (3H), 1.06 (9H) and 1.12 (3H). Methylene and methine protons appeared as multiplets at δ 1.32 and δ 1.50-1.90. The multiplets at δ 2.28-62.52 integrating for two protons were assigned to methylene protons at C-2 which is adjacent to carbonyl group at C-3. A double doublet at δ 5.56 integrating for one proton is attributed to olefinic proton.

These data compare well with Taraxerone¹⁴, therewith support its structure (VII). The mass spectrum also supported the above structure, showed a molecular ion peak at m/z 424 and the base peak at m/z 300.
Table-5

$^1$H-nmr data of RA-7, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.83 (s)</td>
</tr>
<tr>
<td>2 x CH$_3$</td>
<td>6</td>
<td>0.93 (s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.97 (s)</td>
</tr>
<tr>
<td>3 x CH$_3$</td>
<td>9</td>
<td>1.06 (s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.12 (s)</td>
</tr>
<tr>
<td>-CH and -CH$_2$ protons</td>
<td>21</td>
<td>1.32 (m), 1.5-1.9 (m)</td>
</tr>
<tr>
<td>-CH$_2$ protons at C-2</td>
<td>2</td>
<td>2.28-2.52 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.56 (dd)</td>
</tr>
</tbody>
</table>

$s=$singlet, $dd=$double doublet, $m=$multiplet, spectrum run in CDCl$_3$ at 60 MHz, using TMS as internal standard.

RA-8

RA-8 was eluted from the column by benzene-chloroform (7:3) mixture. It was crystallized from methanol as reddish buff needles (120 mg), m.p. 155$^\circ$C. It gave blue colour with alc. ferric chloride solution and brown spot under uv light. It was characterized as ethyl gallate by m.p., m.m.p., spectral studies and co-chromatography with an authentic sample.

The $^1$H-nmr spectrum of RA-8 (Table-6), showed a triplet at $\delta$ 1.31 integrating for three protons and a quartet at $\delta$ 4.24 integrating for two protons corresponded to methyl and methylene protons respectively.

On the basis of above results, RA-8 was characterized as ethyl gallate (VIII).
Table-6

$^1$H-nmr data of RA-8, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.36 (t, $J=7.0$ Hz)</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>2</td>
<td>4.24 (q, $J=7.0$ Hz)</td>
</tr>
<tr>
<td>H-2, 6</td>
<td>2</td>
<td>7.11 (s)</td>
</tr>
</tbody>
</table>

s= singlet, t=triplet, q=quartet, spectrum run in DMSO-d$_6$ at 100 MHz, using TMS as internal standard.

RA-9

RA-9 was eluted from the column by benzene-chloroform (1:1) mixture. It was crystallized from chloroform-methanol as white crystals (190 mg), m.p.160-162°C. Homogeneity of the compound was established by TLC examination using different solvent systems indicating it to be a pure compound. It showed blue fluorescence under uv light. Elemental analysis and the molecular ion peak at m/z 248 agreed with the molecular formula as C$_{13}$H$_{12}$O$_5$.

The ir spectrum (Fig. 1) was helpful in identifying two functional groups present as COOH and OH groups. In the hydroxyl regions the absorption was evident between 3500 cm$^{-1}$ and 2100 cm$^{-1}$ with prominent peaks appearing at 3496, 3429, 3110 and 2817 cm$^{-1}$. The O-H stretching of the carboxyl group is reported to appear as a broad band with a series of minor peaks between 3000 and 2500 cm$^{-1}$ with the main peak around 3000 cm$^{-1}$ and a satellite peak near 2650 cm$^{-1}$.$^{15a}$ The low frequency region of the hydroxylic absorption can thus be attributed to the O-H stretching in the carboxyl. The peaks with high frequency region 3496 and 3429 cm$^{-1}$, therefore can be attributed to a hydroxyl function.
A corresponding peak at 1669 cm\(^{-1}\) in the carbonyl region can thus be attributed to the carbonyl group of the carboxyl, either aromatic or more likely of \(\alpha, \beta\)-unsaturated character. A band in the range 1440-1375 cm\(^{-1}\) has been said to arise out of C-O vibration of the carboxyl group\(^{15b}\). A band of fairly high intensity appearing at 1394 cm\(^{-1}\) in this instance can be reasonably assumed to confirm the presence of a carboxyl function in RA-9. Another band appearing between 1320-1210 cm\(^{-1}\)\(^{15c}\) and found to be the most intense appearing between 1600-700 cm\(^{-1}\) has also been attributed to a carboxyl function. The band appearing at 1276 cm\(^{-1}\) in the ir spectrum of RA-9, can thus be assumed to arise only due to the presence of a carboxyl function.

Another band appearing at 910 cm\(^{-1}\) can now be attributed with O-H deformation vibration of the COOH group, which has been reported to appear at 935 ± 15 cm\(^{-1}\)\(^{15d}\).

Another feature which stands out in the ir spectrum is a band appearing at 1606 cm\(^{-1}\) equal in intensity of the carbonyl of the carboxylic group appearing at 1669 cm\(^{-1}\). This band based on its frequency and intensity can be reasonably attributed to a conjugated \(-\text{C} = \text{C}-\) system or to an aromatic system.

RA-9, can therefore be tentatively concluded to possess either the system IX or X, preferably X as it has already concluded to carry a carboxyl function.

\[
\begin{align*}
\text{(IX)} & : \quad \text{C}=\text{C} - \text{C}=\text{C} \\
\text{(X)} & : \quad \text{C}=\text{C} - \text{OH}
\end{align*}
\]

Additional information can be had from the \(^1\)H-nmr and the \(^13\)C-nmr spectra of RA-9. The \(^1\)H-nmr spectra (Fig.2, Table-7), shows in all, the presence of 12 protons. The spectrum was determined in a mixture of CDCl\(_3\) and DMSO-d\(_6\) and hence the peak appearing at \(\delta2.39\) was excluded from consideration. The peak appearing at the highest field at \(\delta2.71\) from its integral is found to represent six protons and from its sharp nature can be concluded to represent two methyl groups situated in identical environments. Two sets of signals integrating for two protons each appeared in the region of \(\delta6.8\) and \(\delta7.4\). These signals when integrated with the postulated partial structure (X) for RA-9, can lead to the conclusion that one proton each from these sets can represent hydrogen each attached to \(\alpha, \beta\)-carbons represented in (X), leading to the partial structure (XI).
RA-9 has already been found to have the molecular formula C_{13}H_{12}O_{5}, out of which XI accounts for three carbons, three hydrogens and two oxygens. Additionally two methyl groups and a hydroxyl group have been concluded to be present in RA-9, on the basis of its $^1H$-nmr and ir spectra. This information take together with XI, now accounts for five carbons, ten hydrogens and three oxygens (XI, $C_3H_3O_2 + 2 \times CH_3 + 1 \times OH = C_5H_{10}O_3$). The partial structure for RA-9 can therefore now be expanded to (XII).

\[
\begin{array}{c}
\text{CH}_3 \times 2 \\
\text{OH} \times 1 \\
\text{C}_8\text{H}_2\text{O}_2
\end{array}
\text{--CH=CH--CO}_2\text{H}
\]

(XII)

The signals for two methyl groups in RA-9 appear in the region of aromatic methyls. Assuming thus RA-9 to be aromatic in character, six carbons are accounted for bearing two carbons, two hydrogens and two oxygens yet to be accounted for. The two sets of relatively deshielded signals representing two hydrogens each, appearing in the region of $\delta$6.8 and 7.4 came in handy in further expanding the partial structure (XII). One hydrogen each from these two signals at $\delta$6.81 ($J=9.0$ Hz) and $\delta$7.43 ($J=9.0$ Hz) have been assigned to hydrogens attached to the $\alpha,\beta$-unsaturated carbons of the acid moiety (XI) coupling constant of which indicate the hydrogens to be cis-oriented.

Chemical shifts of one proton each from the two sets, $\delta$7.41 ($J=3.0$Hz) and $\delta$ 6.83 ($J=3.0$ Hz) correspond to the well documented values of the $\alpha'$- and $\beta'$- protons of a furan ring in benzofurans at $\delta$ 7.3 and $\delta$6.6 respectively\(^6\).

Thus RA-9 is concluded to have a benzofuran moiety, two carbons, two hydrogens and one oxygen are further accounted for, thus enabling the partial structure for RA-9 to be expanded as (XIII).
It can be noted that all the thirteen carbons are accounted for in (XIII) and hence appendages have to be attached to the eight carbons of the benzofuran nucleus. However, the furan ring, as evidenced by the presence of both the $\alpha$ and $\beta$- hydrogens in the $^1$H-nmr spectrum is unsubstituted. Hence the groups to be accounted further in formulation of the structure for RA-9 have to be appended to four aryl carbons, leading to the partial structure (XIV). However, one more oxygen is to be accounted for.

Considering that there is no further locations it can be attached to, or any other atom which it can carry, it has to be present as an ether function. This further limits the location of this oxygen as present between an aryl carbons and the acid unit. The other alternative is automatically excluded in the absence of an aromatic methoxyl signal in the $^1$H-nmr spectrum. RA-9 can therefore be represented by (XV) or any of its regiomers.

The $^1$H-nmr spectrum of RA-9, still requires further assignments. The signal appearing at $\delta6.06$ and broadish in nature can be assigned to the phenolic proton
considering that DMSO-d$_6$ is used as a co-solvent. The most deshielded of the signals which appears at $\delta 9.89$ should arise only out of the carboxylic proton.

The final structure of RA-9 has now to be one of the possible regioisomers (XVI $\rightarrow$ XXVII).

\[ \text{H}_3\text{C} \quad \text{O-CH=CH-CO}_2\text{H} \]
\[ \text{H}_3\text{C} \quad \text{OH} \]
\[ \text{CH}_3 \quad \text{O-CH=CH-CO}_2\text{H} \]
\[ \text{CH}_3 \quad \text{OH} \]
\[ \text{H}_3\text{C} \quad \text{O-CH=CH-CO}_2\text{H} \]
\[ \text{H}_3\text{C} \quad \text{OH} \]
\[ \text{CH}_3 \quad \text{O-CH=CH-CO}_2\text{H} \]
\[ \text{CH}_3 \quad \text{OH} \]
Of the above structures, the one that can be assigned to RA-9 should have some features present to enable the methyl signals in both the $^1$H-nmr and $^{13}$C-nmr to coincide and have identical chemical shifts. Three oxygens are directly attached to the phenyl ring and these are the one which can cause shielding as well as deshielding around the ring and more particularly on the methyl groups. Analyzing all the possible structures (XVI→XXVII), the structures XVII, XVIII, XXIV and XXV emerge as the possible structure for RA-9.
Of the above (XVIII) appears to be the structure which can correspond to RA-9, not only with respect to situations of oxygen, but also with respect to the side chain which is capable of having similar effect on both the methyl groups. RA-9 can therefore be assigned the structure (XVIII).

The signals in the $^{13}$C-nmr spectrum (Fig.3) can be assigned as below by employing additive factors $^{17}$.

The mass of RA-9 is compatible with the proposed structure (XVIII) as has been detailed in the scheme (I).

On the basis of above discussion, RA-9 has been characterized as (2E)-3(4-hydroxy-5,7-dimethylbenzo[3,4-b]furan-6-yloxy-prop-2-enoic acid named as alatic acid (XVIII) which is being reported for the first time.
**Table-7**

$^1$H-nmr data of RA-9, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2 \times \text{CH}_3$</td>
<td>6</td>
<td>2.71 (s)</td>
</tr>
<tr>
<td>$\alpha$-H of the acid moiety</td>
<td>1</td>
<td>6.81 (d, $J=9.0$ Hz)</td>
</tr>
<tr>
<td>$\beta$-H of the acid moiety</td>
<td>1</td>
<td>7.43 (d, $J=9.0$ Hz)</td>
</tr>
<tr>
<td>4-OH</td>
<td>1</td>
<td>6.06 (br s)</td>
</tr>
<tr>
<td>OH of acid moiety</td>
<td>1</td>
<td>9.89 (br s)</td>
</tr>
<tr>
<td>H-2 ($\alpha'$-H of furan ring)</td>
<td>1</td>
<td>7.41 (d, $J=3.0$ Hz)</td>
</tr>
<tr>
<td>H-3 ($\beta'$-H of furan ring)</td>
<td>1</td>
<td>6.83 (d, $J=3.0$ Hz)</td>
</tr>
</tbody>
</table>

$s=$ singlet, br $s=$ broad singlet, $d=$ doublet, spectrum run at 300 MHz using TMS as internal standard.
EXPERIMENTAL
RA-1
Elution of the column with petroleum ether-benzene (9:1) mixture and crystallized from chloroform-petrol as white needles (100 mg), m.p. 138°C.
Analysed for C_{10}H_{10}O_{4}
Calcd.: C, 61.85; H, 5.15%
Found: C, 61.79; H, 5.12%

UV data  \( \lambda_{\text{max}} \) (CHCl \(_{3} \)) nm
265, 340.

IR  \( \nu_{\text{max}} \) cm\(^{-1} \)
1720, 1500, 1435.

\(^{1}\)H-nmr (100 MHz, CDCl \(_{3} \)) values on \( \delta \)-scale
3.97 (6H, s, 2x OCH\(_{3}\)), 8.13 (4H, s, Ar-H).

Mass m/z
194 [M\(^{+}\)], 163[M\(^{+}\)-OCH\(_{3}\)] (100%), 179 [M\(^{+}\)-CH\(_{3}\)], 164 [M\(^{+}\)-2 x CH\(_{3}\)], 135 [M\(^{+}\)-COOCH\(_{3}\)], 76 [M\(^{+}\)-2 x COOCH\(_{3}\)].

RA-2
Elution of the column with petroleum ether-benzene (7:3) mixture and crystallized from chloroform-methanol as white needles (190mg) m.p. 198°C, R\(_{f}\)= 0.63 (benzene-chloroform, 8:2). It gave positive Leibermann-Burchard test.

IR  \( \nu_{\text{max}} \) cm\(^{-1} \)
3360 (OH), 2960, 2880, 1650, 1465 (C=C), 1040 and 980.

\(^{1}\)H-nmr (100 MHz, CDCl \(_{3} \)) values on \( \delta \)-scale
0.78 (3H, s), 0.83 (3H, s), 0.88 (6H, s), 0.95 (3H, s), 0.98 (3H,s), 1.0 (3H, s), 1.14 (3H, s), 1.83, 2.01 (22H, -CH\(_{2}\) and -CH protons of cyclic system), 3.01 (1H, dd, J=9.0 Hz and 7.0 Hz), 4.88 (1H, br s, \( \alpha \)- to OH ), 5.21 (1H, m, olefinic proton).

Mass m/z
426 [M\(^{+}\)].
**Acetylation of RA-2**

Crystalline RA-2 (30 mg) was treated with acetic anhydride (2 ml) and pyridine (0.5 ml) and allowed to stand overnight at room temperature and then heated on a water bath for four hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid obtained was separated, washed well with water and dried. On crystallization from chloroform-methanol, it gave colourless needles (25 mg), m.p. 241-42°C, [α]$_D^{23}$ +68.9°.

**IR V$_{max}$ cm$^{-1}$**

1722 and 1240 (OAc), 1635, 812.

**$^1$H-nmr (100 MHz, CDCl$_3$) values on δ-scale**

0.84 (3H, s, H-28), 0.89 (12H, s, CH$_3$-23, 24, 29, 30), 0.96 (6H, s, CH$_3$-25, 26), 1.14 (3H, s, CH$_3$-27), 2.08 (3H, s, OAc), 4.54 (1H, dd, J = 6.0 Hz, H-3α), 5.20 (1H, t, J = 3.5 Hz, H-12).

**RA-3**

Elution of the column with petroleum ether-benzene (6:4) and crystallized from chloroform-methanol as white needle-shaped crystals (150 mg), m.p. 262-64°C.

Analysed for C$_{30}$H$_{50}$O

Calcd.: C, 84.50; H, 11.30 %

Found: C, 84.47; H, 11.10 %

**IR V$_{max}$ cm$^{-1}$**

2900 (CH, str.), 1705 (>C=O), 1455, 1385, 1355, 1170, 1070.

**$^1$H-nmr (200 MHz, CDCl$_3$) values on δ-scale**

0.72 (3H, s, CH$_3$), 0.87 (3H, s, CH$_3$), 0.89 (3H, s, CH$_3$), 0.92 (3H, s, CH$_3$), 0.95 (6H, s, 2 x CH$_3$), 1.05 (3H, s, CH$_3$), 1.18 (3H, s, CH$_3$), 1.25, 1.34, 1.45, 1.52, 1.58 (23 H, m, -CH$_2$ and CH- protons), 2.26-2.41 [3H, m, (C$_2$-2H and C$_4$-1H)].
RA-4

Elution of the column with petroleum ether-benzene (1:1) and purified by repeated crystallization from methanol-chloroform afforded RA-4 as crystalline solid (170 mg) m.p. 214-15°C, [α]D+23.64° (CHCl3). It gave positive Leibermann-Burchard, Noller's test and yellow with tetranitromethane.

IR (KBr, Vmax cm⁻¹)

3360, 1030 (OH), 1645 (C=C), 1385 (geminal dimethyl), 885 (terminal methylene).

Analysed for C30H50O
Calcd.:  C, 84.95; H, 11.50 %

Found:   C, 84.93; H, 11.45 %

Acetylation of RA-4

Crystalline RA-4 (30 mg) was treated with acetic anhydride (2 ml) and pyridine (0.5 ml) and allowed to stand over night at room temperature. After usual work-up the solid obtained was crystallized from chloroform-methanol as colourless flakes (25 mg), m.p. 218-20°C.

IR (KBr, Vmax cm⁻¹)

875 (terminal methylene), 1245 (acetate), 1640 (C=C), 1730 (C=O).

1H-nmr (100 MHz, CDCl3) values on δ–scale

0.82, 0.87, 0.94, 1.04 (21H, s, 7 x CH3), 1.27, 1.41, 1.46, 1.47 (m, –CH and –CH2 protons), 2.03 (OCOCH3), 4.28–4.77 (1H, m, α- to acetate), 4.59–4.67 (2H, d, J=8.0 Hz, olefinic protons).

Analysed for C32H52O2
Calcd.:  C, 82.05; H,11.11 %

Found:   C, 82.14; H,11.17%
RA-5

Elution of the column with benzene only and crystallized from chloroform-methanol as white needle-shaped crystals (150mg) m.p. 136-37°C.

\[ \text{KBr IR } V_{\text{max}} \text{ cm}^{-1} \]

3340 (OH), 1055, 1655, 1460, 840 (C=C), 1375 (C-Me2).

\[ \text{H-nmr (90 MHz, CDCl}_3\text{) values on } \delta\text{-scale} \]

0.70 (3H, s, 18-Me), 0.80 (3H, d, J=6.8 Hz, 29-Me), 0.88 (6H, d, J=6.5 Hz, 26,27-Me), 0.92 (3H, d, J=6.5 Hz, 21-Me), 1.02 (3H, s, 19-Me), 3.56 (1H, m, 3-ax-H), 5.36 (1H, m, olefinic proton), 1.07-2.34 (-CH2 and -CH protons of cyclic system and side chain), 4.25 (1H, m, OH).

\[ \text{Mass } m/z \]

414 [M+].

Acetylation of RA-5

Crystalline RA-5 (30 mg) was treated with acetic anhydride (2 ml) and pyridine (0.5 ml) and allowed to stand over night at room temperature and then heated on water bath for two hours. After cooling, the mixture was poured on crushed ice and left over night. The solid was collected, washed well with water and dried. On several crystallization from chloroform-methanol, it gave colourless flakes (20 mg), m.p.114-15°C, \([\alpha]^{17}_D - 48.5^\circ\) (CDCl3).

Analysed for C31H52O.
Calcd.: C, 81.57; H,11.40%
Found : C, 81.52; H,11.37%

\[ \text{KBr IR } V_{\text{max}} \text{ cm}^{-1} \]

2930, 2850, 1730, 1660, 1460, 1380, 1260
Benzoate formation

The compound RA-5 (40 mg) was treated with benzoyl chloride (1 ml) and pyridine (0.5 ml) and the mixture was left over night at room temperature and then heated for six hours on a water bath. The reaction mixture was cooled and ice was added. The solid thus separated was filtered, washed with aqueous solution of potassium hydroxide (KOH) 2% and then with water. It was crystallized from methanol (25 mg), m.p. 145-46°C, \([\alpha]_D^{17} - 7.52^\circ\).

RA-6

Elution of the column with benzene-chloroform (9:1) afforded a fraction which on TLC examination showed the presence of one major spot alongwith some minor impurities. It was purified by crystallization into pure component RA-6 (225 mg), m.p. 299-300 °C. It responded positively to Leibermann-Burchard test.

\[
\text{nujol} \quad \text{IR } \nu_{\text{max}} \text{ cm}^{-1}
\]

3200 (OH), 2750-2610 (COOH), 1655 (C=O), 1210

\[^{1}H\text{-nmr (60 MHz, CDCl}_3\text{) values on } \delta\text{–scale}\]

0.71 (3H, s, CH₃), 0.82 (6H, s, 2 x CH₃), 0.84 (3H, s, CH₃), 0.89 (6H, s, 2 x CH₃), 1.10 (3H, s, CH₃), 4.39 (1H, m, \alpha- to hydroxyl group), 5.20 (1H,m, olefinic proton).

Acetylation of RA-6

Crystalline RA-6 (100 mg) was treated with acetic anhydride (1 ml) and pyridine (2 ml) and allowed to stand over night at room temperature. After usual work-up the solid obtained was crystallized several times from methanol to give RA-6 (Ac) (80 mg), m.p. 258-60°C.

\[
\text{nujol} \quad \text{IR } \nu_{\text{max}} \text{ cm}^{-1}
\]

1724, 1686 and 1250
$^1$H-nmr (60 MHz, CDCl$_3$) values on $\delta$-scale

0.75 (3H, s, CH$_3$), 0.85 (6H, s, 2 x CH$_3$), 0.94 (9H, s, 3 x CH$_3$), 1.12 (3H, s, CH$_3$),
2.02 (3H, s, OCOCH$_3$) 4.50 (1H, m, H-$\alpha$ to acetoxyl), 5.25 (1H, m, olefinic proton).

**Methylation of RA-6 (Ac)**

The acetate RA-6 (Ac) (40 mg) was dissolved in ether and treated with an excess
of ethereal solution of diazomethane. After leaving the contents at room temperature over
night, the ether was evaporated and the product crystallized from methanol-chloroform as
colourless shining plates (30 mg), m.p. 220-21 °C.

$^1$H-nmr (60 MHz, CDCl$_3$) values on $\delta$-scale

0.70 (3H, s, CH$_3$), 0.84 (6H, s, 2 x CH$_3$), 0.86 (3H, s, CH$_3$), 0.90 (6H, s, 2 x CH$_3$),
1.10 (3H, s, CH$_3$), 3.58 (3H, s, methyl ester), 4.50 (1H, m, H-$\alpha$ to methyl ether), 5.30
(1H, m, olefinic proton).

**RA-7**

RA-7 was eluted from the column with benzene-chloroform (8:2) mixture. It was
crystallized from chloroform-ethanol as colourless needles (170 mg), m.p. 240°C. It gave
positive Leibermann-Burchard test.

IR $\nu_{\text{max}}$ cm$^{-1}$

1690-1700 (C=O), 1655, 1055, 840 (C=C)

$^1$H-nmr (60 MHz, CDCl$_3$), values on $\delta$-scale

0.83 (3H, s, CH$_3$), 0.93 (6H, s, 2 x CH$_3$), 0.97 (3H, s, CH$_3$), 1.06 (9H, s, 3 x CH$_3$),
1.12 (3H, s, CH$_3$), 1.32, 1.50-1.90 (21 H, m, CH and CH$_2$ protons), 2.28-2.52 (2H, m,
$-$CH$_2$ protons at C-2), 5.56 (1H, dd, olefinic proton).
**Mass m/z**

424 \([M^+]\) (37.0%), 409 (18.3), 300 (100), 285 (45.4), 257 (9), 232 (7.3), 218 (16.5), 205 (62.6), 204 (95), 189 (10), 149 (10), 133 (25), 121 (12), 119 (10), 109 (15), 107 (10), 95 (17), 93 (10), 91 (8), 67 (15), 57 (15), 43 (12).

**RA-8**

RA-8 was eluted from the column by benzene-chloroform (7:3) mixture. It was crystallized from methanol as reddish buff needles (120 mg), m.p. 155°C. It gave blue colour with alc. ferric chloride solution and brown spot under uv light.

Analysed for C₉H₁₀O₅

Calcd.:  C, 54.9; H, 5.05 %

Found:  C, 54.7; H, 5.00 %

**¹H-nmr (100 MHz, DMSO-d₆), values on δ-scale**

1.31 (3H, t, J=7.0 Hz, CH₃), 4.24 (2H, q, J=7.0 Hz, CH₂), 7.11 (2H, s, H-2, 6).

**Mass m/z**

195 \([M^+]\) (45.0%), 183 (8.0%), 170 (20.0%), 153 (100.0%), 125 (20.0%), 107 (4.0%), 79 (9.0%), 51 (6.0%).

**RA-9**

RA-9 was eluted from the column by benzene-chloroform (1:1) mixture. It was crystallized from chloroform-methanol as white crystals (190 mg), m.p.160-162 °C.

Analysed for C₁₃H₁₂O₅

Calcd.:  C, 62.90; H, 4.83 %

Found:  C, 62.84; H, 4.79 %

**IR \(_{KBr}^V\), cm\(^{-1}\)**

3496 (OH), 3429, 1669 (C=O), 1606, 1440-1375 (C-O vib. of COOH), 1394, 1320-1210, 1276 (COOH), 910 (OH def. of COOH).
$^1$H-nmr (300 MHz, CDCl$_3$ + DMSO-d$_6$), values on δ-scale

2.71 (6H, s, 2 x CH$_3$), 6.81 (1H, d, J=9.0 Hz, α-H of acid moiety), 7.43 (1H, d, J=9.0 Hz, β-H of acid moiety), 6.06 (1H, br s, 5-OH), 9.89 (1H, br s, COOH), 6.83 (1H, d, J= 3.0 Hz, H-2 of furan ring), 7.41 (1H, d, J=3.0 Hz, H-3 of furan ring).

$^{13}$C-nmr (300 MHz, CDCl$_3$), values on δ-scale

161.57 (C$_3$-b), 161.02 (COOH), 155.07 (β-C), 152.87 (C-6), 152.85 (C-4), 125.40 (C-2), 113.02 (C-5), 112.45 (C-7), 110.7 (C$_{4+b}$), 102.97 (C-3), 162.97 (α-C), 18.45 (2 x CH$_3$).

Mass m/z (rel. int.)

248 [M$^+$] (90.4%), 233 [M−CH$_3$]$^+$ (9.2), 219 [M−CO−H]$^+$ (9.4), 217 [233−CH$_3$]$^+$ (1.5), 216 [217−H]$^+$ (13.8), 204 [203+H]$^+$ (17.5), 203 [M−COOH] (100), 202 [M−H$_2$O−CO] (9.8), 201 [202−H]$^+$ (17.2), 190 [2 x CH$_3$−CO] (15.8), 189 [204−CH$_3$] (23.6), 177 [M−CH=CH−CO$_2$H] (6.6), 176 [M−CO−CO$_2$] (22.6), 174 [189−CH$_3$] (8.3), 173 [174−H]$^+$ (16.4), 161 [M−O−CH=CH−CO$_2$] (7.0), 159 [177−H$_2$O] (5.6), 149 (10.5), 148 (26.9), 147 (23.5), 146 (7.1).
REFERENCES
4 H U Khan, in Recent Advances in Medicinal, Aromatic and Spice Crops (S P Raychaudhuri, ed), Today & Tomorrow's Printers and Publishers, New Delhi, Vol I, P 113 (1991)
5 M Parveen and N U Khan, Current Science, Vol. 50 (22), 1171 (1987)
8 T Inoue, Y Ishidata, M Fujita, M Kubo, M. Fukushima and M Nagai, Yakugake Zasshi, 98 (1), 41-46 (1978)
9 C R Nollers, R A Souith, G H Harris and J W Walker, J. Am. Chem. Soc., 64, 3027 (1962)
11 M Crawford, S W Hansan and M E S Kokar, Tetrahedron letters, 3099 (1975)
13 J S Chanbanand S K Srivastava, Phytochemistry, 1005 (1978)
14 a) A P Toloch, Lipids, 12, 233 (1977)
   a) 186,  b) 194,  c) 195,  d) 197
CHAPTER 4
DISCUSSION
Chemical constituents of Ficus lyrata (Moraceae)

Ficus is a large genus of trees, shrubs and often climbers, with milky juice, widely distributed throughout the tropics of both hemispheres but particularly abundant in South-East Asia and Polynesia. About 65 species are found in India. All species of Ficus yield latex containing caoutchouc. Many species of Ficus are medicinally important. Ficus lyrata is used for treatment of hypothermic, diuretic and CNS active.

Medicinal importance and scanty work on the plant accelerates our interest to carry out the comprehensive investigation of the plant Ficus lyrata.

The present study deals with the isolation and characterization of the following compounds from the leaves of Ficus lyrata.

1. β-Sitosterol-D-glucoside
2. 4-Methoxychalcone
3. 7, 4'-Dimethoxy apigenin
4. 5, 7, 4'-Trihydroxy-2, 3', 6'-trimethoxyisoflavone
5. Acacetin-7-glucoside
6. Acacetin-7-O-neohesperidoside

Leaves of Ficus lyrata were collected from Aligarh, A.M.U., Fort, India. They were dried under shade and powdered (2.5 Kg). The powdered leaves were thoroughly extracted with petroleum ether (60-80°C) (3 x 3L) on water bath (14 hours, periods) yielded fraction ‘A’ (50 + 20 + 10 gm), and twice with hot chloroform yielded fraction ‘B’ (80+20 gm). Finally, the defatted leaves were extracted twice with hot methanol, yielded fraction ‘C’ (80 +35 gm). The fraction ‘A’ was found to be a dark green oily mass of fatty nature, therefore was not further examined.

The fraction ‘B’, a green gummy mass, gave positive test of flavonoids. TLC examination of the fraction ‘B’ in different solvent systems [benzene-pyridine-formic acid (36:9:5) and toluene-ethyl formate-formic acid (5:4:1)] showed it to be a mixture of four major compounds along with some minor ones. Fraction ‘B’ was chromatographed over silica gel column using successively petroleum ether, petroleum ether-benzene mixtures, benzene, benzene-ethyl acetate mixtures, ethylacetate and methanol as eluting solvents. Appropriate fractions (ir spectra and TLC) were combined. Repeated column
chromatography of the fractions followed by fractional crystallization afforded four crystalline TLC homogenous substances, labelled as FL-1, FL-2, FL-3 and FL-4.

The methanol extract also gave positive test of flavonoids. TLC examinations of the extract in different solvent systems showed the presence of several compounds. Repeated column chromatography over silica gel and fractional crystallization afforded two crystalline compounds, labelled as FL-5 and FL-6.

**FL-1**

The compound FL-1 was eluted from the column by benzene and crystallized from chloroform-methanol as colourless crystals (150 mg), m.p. 282 °C. It gave positive Liebermann-Burchard test and Molisch test for glycosides. The elemental analysis agreed with the molecular formula C_{35}H_{60}O_{6}.

The $^1$H-nmr of FL-1 (Table-1) showed the presence of six methyl groups in the range of δ0.70 to 61.01. The signal at δ5.2 indicated the characteristic olefinic proton of steroid. The protons appeared as multiplet in the range of δ4.0 – 5.1 were assigned to sugar protons. An anomeric proton appeared at δ4.2 as a doublet (J=10.0 Hz). Hence, it was concluded that compound FL-1 is the monoglycoside of steroid.

Acetylation of FL-1 with acetic anhydride and pyridine gave a colourless acetyl derivative FL-1 (Ac), m.p. 166°C. $^1$H-nmr spectrum of FL-1 (Ac) (Table-2) showed six methyl groups in the range of δ0.71 – 1.04. The four independent singlets of three protons each at δ1.95, 1.99, 2.02 and δ2.04 supported the presence of four aliphatic acetoxyl groups of monoglycosidic acetate. There was a signal centered at δ5.4 characteristic of an olefinic proton of steroids. It showed a group of multiplets from δ4.2-δ5.3 for a protons α- to acetoxyl reminiscent of monoglycosidic acetate. From these data it could be concluded that the parent compound is a monoglycoside of sterol. Similar conclusions were drawn from the mass spectral data too. The mass spectrum of the glycoside acetate FL-1 (Ac) (Scheme-1) showed the fragmentation pattern characteristic of β-sitosterol.

Hydrolysis of FL-1 by Killiani’s mixture yielded glucose and an aglycone FL-1 (Agl). The aglycone having m.p. 137 °C was identified as β-sitosterol by m.p., m.m.p., co-TLC and comparison of the spectral data (ir, $^1$H-nmr and MS) with those of an authentic sample.
On the basis of above results, the compound FL-1 was characterized as β-sitosterol-D-glucose (I) ².

![Chemical Structure](image)

**Table-1**

$^1$H-nmr data of FL-1, values on δ-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H</td>
<td>0.70 (s)</td>
</tr>
<tr>
<td>3H</td>
<td>0.73 (s)</td>
</tr>
<tr>
<td>6H</td>
<td>0.75 (s)</td>
</tr>
<tr>
<td>3H</td>
<td>0.78 (s)</td>
</tr>
<tr>
<td>3H</td>
<td>1.01 (s)</td>
</tr>
<tr>
<td>CH$_2$-protons</td>
<td>1.10-1.23 (m)</td>
</tr>
<tr>
<td>H-1' (anomeric)</td>
<td>4.2 (d, J=10.0 Hz)</td>
</tr>
<tr>
<td>H-1',2',3',4',5',6'</td>
<td>4.0-5.1 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>5.2 (m)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet and m = multiplet, Spectrum run at 300 MHz in CDCl$_3$ using TMS as internal standard
Table 2

$^1$H-nmr data of FL-1 (Ac), values on δ-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 x CH$_3$</td>
<td>18</td>
<td>0.71-1.04</td>
</tr>
<tr>
<td>H-6 (Olefinic proton)</td>
<td>1</td>
<td>5.4 (m)</td>
</tr>
<tr>
<td>Sugar acetoxyls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 x Acetoxyl</td>
<td>12</td>
<td>1.95 (3H, s), 1.99 (3H, s)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.02 (3H, s)</td>
</tr>
<tr>
<td>H-1',2',3',4',5',6'</td>
<td>7</td>
<td>4.2-5.3 (m)</td>
</tr>
</tbody>
</table>

s = singlet and m = multiplet, Spectrum run at 300 MHz in CDCl$_3$ using TMS as internal standard

FL-2

It was obtained from the column with benzene-chloroform (1:1) mixture and crystallized with chloroform-methanol as light cream coloured crystals (165 mg), m.p. 59-60°C. The elemental analysis agreed with the molecular formula C$_{16}$H$_{14}$O$_2$. A red colour with conc. H$_2$SO$_4$ and orange to red colour with aq. NaOH suggested it to be a chalcone$^8,9$. The ir spectrum displayed the characteristic bands at 1661 cm$^{-1}$ (C=O) and 1475 cm$^{-1}$ (C=C). Its uv spectrum showed the maximum absorption at 345 nm and minimum absorption at 245 nm Analysis with the diagnostic shift reagents$^{10a}$ gave no shift in $\lambda_{max}$ indicated the absence of any hydroxyl group in the chalcone nucleus which was further supported by the negative ferric chloride test.

The $^1$H-nmr spectrum of FL-2 (Table-3, Fig.1) showed a singlet of three protons at δ 3.87 assigned to a methoxyl group. A pair of ortho-coupled doublets at δ 6.92 (J=8.7 Hz) and δ7.59 (J=8.7 Hz) integrating for two protons each were attributed to H-3, 5 and H-2, 6 protons respectively. Another pair of doublets at δ 7.39 (J=15 0 Hz) and δ 7.65 (J=15.0 Hz) were ascribed to α- and β-protons of chalcone. The 2', 6' protons appeared as an ortho-coupled doublet at δ7.90 (J= 8.4 Hz) while 3', 4',5' protons were resonating as a multiplet in the range of δ 7.52- 7.63.

The above assigned structure was further supported by the mass spectrum (scheme-II, Fig.2) which showed the molecular ion peak at m/z 238. The fragment ions
[M\(^+\)] absent

-glucose tetracetate

m/z 396

-C\(_{10}H_{21}\)

m/z 255

m/z 214

Scheme-1
at m/z 161 and 134 supported the presence of p-methoxyphenyl ring. The other fragments are rationalized from the Scheme-II. It was also supported by the GC-MS spectra which gave the retention time 16, 30 (Fig.3).

On the basis of above results, FL-2 was characterized as 4-methoxychalcone (II) which is being reported for the first time.

![Chemical structure](image)

**(II)**

**Table-3**

$^1$H-nmr data for FL-2, values on δ-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCH$_3$</td>
<td>3</td>
<td>3.87 (s)</td>
</tr>
<tr>
<td>H-3,5</td>
<td>2</td>
<td>6.92 (d, J=8.7 Hz)</td>
</tr>
<tr>
<td>H-2,6</td>
<td>2</td>
<td>7.59 (d, J=8.7 Hz)</td>
</tr>
<tr>
<td>H-α</td>
<td>1</td>
<td>7.39 (d, J=15.0 Hz)</td>
</tr>
<tr>
<td>H-β</td>
<td>1</td>
<td>7.65 (d, J=15.0 Hz)</td>
</tr>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.90 (d, J=8.4 Hz)</td>
</tr>
<tr>
<td>H-3',4',5'</td>
<td>3</td>
<td>7.52 – 7.63 (m)</td>
</tr>
</tbody>
</table>

$s$=singlet, $d$ = doublet, $m$ = multiplet, spectrum run at 300 MHz in CDCl$_3$, using TMS as internal standard.
Current Data Parameters
NAME proton
EXPANG 70
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 10:52
INSTRUM dpx300
PROBMD 5 mm 880 739
PULPROG zg
TD 32758
SOLVENT CDC13
NS 16
DS 0
SNH 8992.86 Hz
FDRES 0 274439 Hz
AD 1 8219500 sec
RG 812 7
DN 55.500 usec
DE 4.50 usec
TE 300 0 K
D1 5 0000000 sec
P1 7 50 usec
OE 4.50 usec
SF0J 300 1342010 MHz
NUC1 1H
PL1 -6.00 DS

F2 - Processing parameters
SI 32758
SF 300.130057 kHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 22.00 cm
F1P 10.000 ppm
F1 3001.30 Hz
F2P -0.500 ppm
F2 -150.36 Hz
PPMCM 0.47727 ppm/cm
HZCM 143 24395 Hz/cm
Current Data Parameters
NAME proton
EXPNO 70
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 10.52
INSTRUM dpb300
PROBHD 5 mm 990 Z9
PULPROG 2g
TD 32768
SOLVENT CDCl3
NS 16
BS 0
SKH 8992.005 Hz
FIDRES 0.274439 Hz
AQ 1.8219568 sec
AB 812.7
BK 55.600 ussec
DE 4.50 ussec
TE 300.0 K
DI 5.00000000 sec
P1 7.60 ussec
DE 4.50 ussec
SFO1 300.134201 MHz
NUC1 1H
PL1 -6.00 dB

F2 - Processing parameters
SI 32768
SF 300.1300007 MHz
MDM EM
SSB 0
LB 1.00 Hz
BB 0
PC 1.40

1D NMR plot parameters
CX 22.00 cm
F1P 4.474 ppm
F1 1342.64 Hz
F2P 1.225 ppm
F2 367.78 Hz
PPM CM 0.14704 ppm/cm
HZCM 44.31190 Hz/cm
Current Data Parameters
NAME proton
EXPCNO 70
PROCNO 1

F2 - Acquisition Parameters
Date_ 500000
Time 10.52
INSTRUM dpx300
PROSHD 5 mm BBO Z39
POLPfold 29
TD 32768
SOLVENT CDCl3
MS 16
GS 0
SW 8922.866 Hz
FIBES 0.274496 Hz
A0 1.821908 sec
RG 812.7
DM 55.600 usec
DE 4.500 usec
TE 300.0 K
D1 5.000000000 sec
P1 7.600 usec
DE 4.500 usec
SF01 300.1342018 MHz
NUCI 1H
PL1 -6.00 dB

F2 - Processing parameters
SI 32768
SF 300.1340000 MHz
MOD EHM
SSB 0
LB 1.00 Hz
SB 0
PC 1.40

1D NMR plot parameters
CX 22.30 cm
FP1 0.0 ppm
F1 2602.20 Hz
F2 6.006 ppm
F2 1802.59 Hz
PMCH 0.01210 ppm/cm
HZCM 36.34553 Hz/cm
$C_{10}H_{14}O_2$

$[M^+]$, m/z 238 (100)

- $m/z$ 210 (18.18) $\rightarrow$ $\text{CO}$
- $m/z$ 223 (10.60) $\rightarrow$ $\text{CH}_3$

**Scheme II**

- $m/z$ 78 (40.90) $\rightarrow$ C$_6$H$_6$
- $m/z$ 77 (1.5) $\rightarrow$ $\text{CH}_3$
- $m/z$ 161 (49.8) $\rightarrow$ C$_{10}$H$_5$O$_2$
- $m/z$ 134 (15.15) $\rightarrow$ C$_9$H$_4$O
- $m/z$ 105 (22.72) $\rightarrow$ C$_7$H$_5$O
The compound FL-3 was eluted from the column by benzene-ethylacetate (8:2) mixture. It was crystallized from chloroform-methanol as white crystals (180 mg), m.p. 185-87°C. The elemental analysis and the molecular ion peak at m/z 298 agreed with the molecular formula C₁₇H₁₄O₅. The flavonoidic nucleus was evidenced by positive Shinoda’s test. It gave greenish-brown colour with FeCl₃. The uv spectrum showed absorption maxima at 244 nm (band-II) and 369.5 nm (band-I) analysis with diagnostic reagents, gave a red shift of 32.5 nm with AlCl₃ indicating the presence of free hydroxyl group at 5-position.

IR spectrum of FL-3 showed a carbonyl band at 1669.38 cm⁻¹, phenolic hydroxyl group at 3429.19 cm⁻¹ and complex aromatic substitution pattern at 1507.94, 1464.34, 1383.70, 1270.73, 1162.82, 833.55, 818.35 cm⁻¹ along with two strong peaks at 2920.87 and 2850.18 cm⁻¹. The ¹H-nmr spectrum of FL-3 (Table-4) in CDCl₃ showed the presence of seven aromatic protons. A sharp singlet at δ 6.58 indicating the presence of C-3 proton of γ-pyrone ring. Two meta-coupled doublets at δ 6.36 (J=3.0 Hz) and δ 6.48 (J=3.0) integrating for one proton each were assigned to H-6 and H-8 respectively. Two ortho-coupled doublets at δ 7.00 (J=9.0 Hz) and δ 7.83 (J=9.0 Hz) integrating for two protons each corresponding to H-3’, 5’ and H-2’,6’ protons respectively. A sharp one proton singlet at δ 12.81 was assigned to a hydroxyl group at 5-position. Also a sharp six proton doublet at δ 3.88 (J=3.0 Hz) was ascribed to two methoxyl groups.

Acetylation of FL-3 with acetic anhydride and dry pyridine gave a monoacetate FL-3 (Ac) (IIIb), m.p. 153 °C. The ¹H-nmr showed an aromatic acetoxyl group as a singlet of three protons at δ 2.45 at C-5 position.

It was further supported by mass spectrum which showed the molecular ion peak [M⁺] at m/z 298. The fragment ions at m/z 283, 268 corresponding to the lose of methyl group from the molecular ion peak and from the fragment m/z 283. The fragment at m/z 270 showed the lose of carbonyl group from [M⁺].

On the basis of the above results, the compound FL-3 was characterized as 7,4’-dimethoxyapigenin (IIIa).
(III)
a) \(R=H\)
b) \(R=\text{Ac}\)

Table-4

\(^1\text{H-nmr} \) spectral data on FL-3 (values of \(\delta\)-scale)

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>1</td>
<td>12.81 (s)</td>
</tr>
<tr>
<td>2 x OCH\text{3}</td>
<td>6</td>
<td>3.88 (d, (J=3.0) Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.58 (s)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.36 (d, (J=3.0) Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.48 (d, (J=3.0) Hz)</td>
</tr>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.83 (d, (J=9.0) Hz)</td>
</tr>
<tr>
<td>H-3'-5'</td>
<td>2</td>
<td>7.00 (d, (J=8.7) Hz)</td>
</tr>
</tbody>
</table>

\(s=\) singlet, \(d=\) doublet, spectrum run at 300 MHz in CDCl\(_3\). TMS as internal standard.

FL-4

It was obtained by elution of the column with benzene-ethylacetate (7:3) and crystallized with chloroform-methanol as pale yellow plates (180 mg), m.p. 198-200\(^0\)C. The elemental analysis agreed with molecular formula as \(C_{18}H_{16}O_8\). The mass spectrum showed the molecular ion peak at \(m/z\) 360 in agreement with the molecular formula assigned to it. Active hydrogen determination showed the presence of three hydroxyl groups and methoxyl group estimation showed the presence of three-O-methyl groups. This was confirmed by the formation of a triacetate and hexamethylether.

A negative Durham test and absence of any colouration with \(\text{Mg/HCl}^+\) ruled out flavanone or flavone nucleus for FL-4. On the other hand, treatment with sodium
amalgam followed by acidification resulted in pink colouration, suggesting an isoflavone nucleus, further supported by uv absorption at 262 nm and an inflection at 329 nm. The colour reaction, uv spectral studies and a sharp singlet of one proton at δ 8.36 in 1H-nmr spectrum of FL-4 suggested it to be an isoflavone having three methoxyl and three hydroxyl groups.

The compound gave a dark green colour with ferric chloride and the presence of a chelated hydroxyl group was further indicated by the band at 3433.14 cm⁻¹ in its ir spectrum (Fig.4). A red shift of 12 nm was also observed in uv spectrum on addition of anhydrous AlCl₃. The presence of chelated 5-hydroxyl was further confirmed by the signal at δ 13.01 in the 1H-nmr spectrum of FL-4. Furthermore, a red shift of 10 nm with NaOAc and 1H-nmr signal at δ 10.32 indicated the phenolic hydroxyl at 7-position. The methanolic solution of the compound was not oxidised by pentamine cobalt trichloride, indicating the absence of adjacent phenolic hydroxyl groups. Thus, eliminating the possibility of the third hydroxyl in the same ring.

The 1H-nmr spectrum of FL-4 (Fig-5, Table-5) showed a sharp singlet at δ 8.36 indicating the presence of C-2 proton for γ-pyrene nucleus. A pair of meta-coupled doublets at δ 6.63 (J=3.0 Hz) and 6.68 (J=3.0 Hz) each indicating for one proton were assigned to C-6 and C-8 protons respectively. The C-6 proton was shielded by two ortho and one para oxygen. A sharp upfield singlet of one proton at δ 6.48 could be assigned to either C-3' or C-5' proton. The possibility of it being at any other position in B-ring was ruled out as the hexamethylether of FL-4 furnished 2, 3, 4, 6-tetramethoxy benzoic acid when subjected to oxidative degradation with alkaline H₂O₂. The identity of 2, 3, 4, 6-tetramethoxy benzoic acid was confirmed by co-TLC and m.m.p. with an authentic sample. Thus the singlet at δ 6.48 was assigned to C-5' proton which was shielded by two ortho and para oxygens. The presence of three methoxyl groups were indicated through three singlets at δ 3.67, 3.73 and 3.76 each integrating for three protons. The remaining hydroxyl group exhibited by a signal at δ 9.33 may be placed at C-3' or C-4'. However, the comparison of the 13C-nmr of the parent compound (IVA) with that of its acetate (IVb) (Table-6) ruled out the possibility of hydroxyl group at C-3' position. The signal of C-4' carbon of the acetate (IVb) moved upfield by 12.0 ppm, while the signal due to C-1', C-3' and C-5' moved down field by 6.9 ppm, 8.3 ppm and 8.4 respectively. The upfield shift of C-4' carbon of the acetate and downfield shift of carbons ortho and
para to it confirmed the presence of the hydroxyl group at C-4\(^\prime\)\(^b\).\(^c\) The signals due to meta carbons namely C-2\(^\prime\) and C-6\(^\prime\) remained almost unchanged in the acetate (IVb). This fixed up the position of the hydroxyl group at C-4\(^\prime\) position.

The \(^{13}\)C-nmr spectrum (Fig-6, Table-6) showed the C-2 carbon of the \(\gamma\)-pyrone at 153.27 ppm and C-3 at 126.09 ppm. In flavones the corresponding values are 163.2 and 103.1 ppm. As expected, the values for ring A and ring B carbons are about the same for flavones and isoflavones and chemical shifts depend upon only on site and degree of oxygenation of rings\(^{9c}\).

The mass spectrum (Fig-7, Scheme-III) showed \(M^+\) at m/z 360 and M-15, M-30, M-45 corresponding to the successive loss of three methyl groups at m/z 345, 330 and 315 respectively. The RDA cleavage was facile and led to the fragments of mass m/z 153, 152 and 151 of ring A and m/z 208, 207 and 178 of ring B which were indicative of the presence of two hydroxyls in ring A and one hydroxyl and three methoxyls in ring B. The significant fragment at m/z 329 [M-31, OMe] supported the presence of isoflavone with 2'-OMe\(^14\). The other fragments were rationalized from Scheme-III.

On the basis of above results, the compound FL-4 was assigned as 5, 7, 4'-trihydroxy-2', 3', 6'-trimethoxyisoflavone (IVa).
Table-5

$^1$H-nmr data on FL-4 ($\delta$-scale)

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2</td>
<td>1</td>
<td>8.36 (s)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.68 (d, J=3.0 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.63 (d, J=3.0 Hz)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>6.48 (s)</td>
</tr>
<tr>
<td>OCH$_3$-2'</td>
<td>3</td>
<td>3.67 (s)</td>
</tr>
<tr>
<td>OCH$_3$-6'</td>
<td>3</td>
<td>3.73 (s)</td>
</tr>
<tr>
<td>OCH$_3$-3'</td>
<td>3</td>
<td>3.76 (s)</td>
</tr>
<tr>
<td>OH-4'</td>
<td>1</td>
<td>9.33 (s)</td>
</tr>
<tr>
<td>OH-7</td>
<td>1</td>
<td>10.32 (s)</td>
</tr>
<tr>
<td>OH-5</td>
<td>1</td>
<td>13.01 (s)</td>
</tr>
</tbody>
</table>

s= singlet, d= doublet, spectrum run in DMSO-$d_6$ at 300 MHz using TMS as internal standard.
Table-6

\(^{13}\text{C-nmr data of FL-4}\)

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>Chemical Shift</th>
<th>(\text{Ia})</th>
<th>(\text{Ib})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>153.27</td>
<td>153.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>126.09</td>
<td>126.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>180.27</td>
<td>178.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>154.74</td>
<td>151.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100.05</td>
<td>113.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>157.68</td>
<td>152.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>93.94</td>
<td>110.2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>154.27</td>
<td>151.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>104.56</td>
<td>117.4</td>
<td></td>
</tr>
<tr>
<td>1'</td>
<td>121.75</td>
<td>128.6</td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>150.25</td>
<td>146.1</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>153.27</td>
<td>161.6</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>152.86</td>
<td>140.8</td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>119.82</td>
<td>128.2</td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>152.66</td>
<td>148.3</td>
<td></td>
</tr>
<tr>
<td>(\text{OCH}_3)</td>
<td>59.92</td>
<td>56.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55.82</td>
<td>56.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55.10</td>
<td>55.5</td>
<td></td>
</tr>
<tr>
<td>3 (\times) (\text{C}=\text{O}) (\text{of OAc})</td>
<td></td>
<td>168.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>168.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>169.4</td>
<td></td>
</tr>
<tr>
<td>3 (\times) (\text{CH}_3) (\text{of OAc})</td>
<td></td>
<td>20.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.1</td>
<td></td>
</tr>
</tbody>
</table>

Spectrum run at 300 MHz in CDCl\(_3\)
Current Data Range = 55
NAME: 43A rosc
ASSOCIATED
MACRO: 1

F2 ACQUISITION PARAMETERS
Date: 2023
Time: 15:45
MAGNUM: O2/3000
PROCEDURE: 5 or WIN
PLANTIGG: 50
TG: 37.56
SOLVENT: OEO
NS: 1
DS: 1
SM: 4107.27 MHz
FIDERS: 0.1461 MHz
AC: 1.20s/2092 set
TE: 1.20s
DI: 1
000000000 set

--- CHANNEL 1 ---
HFEI
1 M1
2 R1
3 R2
SFEI
011193530 MHz

--- Processing parameters ---
SQ: 16324
SF: 320 1000361 MHz
SM:
S:
C:

--- run parameters ---
CR: 20.00 (c)
1: 1.29 ppm
2: 2.05 ppm
3: 3.19 ppm
4: 4.95 ppm
5: 0.72 ppm
6: 7.15 ppm
7: 10.66 ppm

FIG-5
Current Data Parameters
NAME  C674 psi
EXPMO  
PROCNO  

F2 - Acquisition Parameters
Tr  0.009277
Vnmr  16.45
PO4AF  0.0000
PO4AF  5 min multi
POLHOG  15
TD  32768
SOLVENT  2950
DS  16
DW  120272 Hz
TDFS  64,40197 MHz
AG  1.58099 sec
DS  26
JL  0.4050 usec
tE  200 usec
tE  398.0 usec
tE  10000000 usec

------ CHANNEL 1 ------
NMI
xl  3.88 usec
R2  0.50 kHz
R01  300.131953 MHz

F2 - Processing parameters
SI  0.5624
SP  300.130000 MHz
DM  2
SSB  3.20 kHz
GB  0
PC  1.00

1D NMR alos parameters
C1  20.00 cm
FH  0.49 cm
F1  240.88 kHz
F2  3.33 MHz
F2  3061.37 kHz
PM  0.999999 kHz/ce
HFCF  121.80 kHz
CHF  121.80 kHz
Scheme-III
**FL-5**

The compound FL-5 was eluted from the column by benzene-ethylacetate (6:4) mixture. It was crystallized from chloroform-methanol as cream coloured crystals (150 mg), m.p.255°C. The elemental analysis agreed with the molecular formula C_{22}H_{22}O_{10}. The glycosidic nature of the compound was evidenced by positive Molisch test. It gave greenish colour with FeCl_3. The flavonodic nucleus was evidenced by positive Shinoda’s test and its uv spectrum showed \( \lambda_{\text{max}} \) at 268 nm (band II) and 321 nm (band I). Analysis with diagnostic reagents gave a bathochromic shift of 32 nm with AlCl_3 indicating the presence of free hydroxyl group at 5-position. The possibility of its being a flavanone glycoside was ruled out as it gave yellow colour with Willson-Boric acid reagent. Its ir spectrum showed a carbonyl group at 1656 cm\(^{-1}\), phenolic hydroxyl group at 3399 cm\(^{-1}\) and a complex aromatic substitution pattern at 1615, 1498, 1304, 1173, 1081 and 832 cm\(^{-1}\).

The \(^1\)H-nmr of FL-5 in DMSO-d_6 (Table-7), showed the presence of seven aromatic protons. A sharp singlet at \( \delta \) 6.96 indicating the presence of C-3 proton of \( \gamma \)-pyrone ring. Two meta-coupled doublets at \( \delta \) 6.45 (J=3.0 Hz) and \( \delta \) 6.85 (J=3.0 Hz) integrating for one proton each were assigned to H-6 and H-8 respectively. Two ortho-coupled doublets at \( \delta \) 7.12 (J=9.0 Hz) and \( \delta \) 8.05 (J=9.0 Hz) integrating for two protons each corresponding to H-3',5' and H-2',6' respectively. A one proton singlet at \( \delta \) 12.91 for hydroxyl group at 5-position. The sugar protons were appearing in the region \( \delta \) 5.06-5.41 and a sharp three protons singlet at \( \delta \) 3.87 was assigned to methoxyl group. The H-1' proton of sugar appeared as a doublet at \( \delta \) 5.06 (J=8.0 Hz) which is of glucose.

The total hydrolysis of FL-5 with 6% hydrochloric acid yielded glucose and an aglycone FL-5 (Agl) (Va). The sugar was identified as glucose by co-chromatography with an authentic sample.

The quantitative estimation of sugar by Somogyi’s copper-micro method, indicating the presence of one mole of sugar per mole of aglycone.

The uv spectrum of the aglycone (Table-8) showed a bathochromic shift of 28 nm in band-II with NaOAc which was absent in the glycoside, thus, suggesting that 7-position is involved in glycosylation.

The acetylation of the aglycone FL-5 (Agl) (Va) with acetic anhydride and pyridine gave a diacetate FL-5 (Ac) (Vb). The TLC examination and \(^1\)H-nmr spectrum
indicated it to be acacetin. The $^1$H-nmr spectrum of FL-5 (Ac) (Table-9) showed one methoxyl group at $\delta$ 3.88. The uv spectrum of FL-5 (Agl) is comparable with the spectrum of acacetin (Table-8). The $^1$H-nmr values of FL-5 (Ac) and acacetin diacetate are recorded in (Table-9).

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.96 (s)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.45 (d, J=3.0 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.85 (d, J=3.0 Hz)</td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>2</td>
<td>7.12 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>2</td>
<td>8.05 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1' (anomeric)</td>
<td>1</td>
<td>5.06 (d, J=8.0 Hz)</td>
</tr>
<tr>
<td>H-1'', 2'', 3'', 4'', 5'', 6''</td>
<td>7</td>
<td>5.06-5.41 (m)</td>
</tr>
<tr>
<td>OMe</td>
<td>3</td>
<td>3.87 (s)</td>
</tr>
<tr>
<td>OH-5</td>
<td>1</td>
<td>12.91 (s)</td>
</tr>
</tbody>
</table>

$s$ = singlet, $d$ = doublet, spectrum run in DMSO-$d_6$ at 300 MHz using TMS internal standard.

UV spectrum of FL-5 (Agl) gave $\lambda_{\text{max}}$ at 269 nm (Band II) and a pronounced inflection at 327 nm. AlCl$_3$ produces a 55 nm shift of band-I showing thereby a free 5-hydroxyl group. A shift of 31 nm in band-II with sodium acetate indicated the presence of a free 7-hydroxyl group$^{10b}$. The sodium acetate-boric acid spectrum of FL-5 (Agl) showed a blue shift of 9 nm in band-I relative to apigenin with a decrease in its relative intensity, showing thereby a protected 4'-hydroxyl group.

M.P., UV, and $^1$H-nmr spectra of FL-5 (Ac) were found to be identical with that of acacetin diacetate (Table-9). FL-5 was, therefore, assigned the structure as acacetin-7-glucoside$^{17}$ (VI).
Table-8

UV data on FL-5 (Agl) and acacetin

<table>
<thead>
<tr>
<th>Reagent</th>
<th>FL-5 (Agl)</th>
<th>Acacetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>269, 303 sh, 327</td>
<td>270, 303 sh, 328</td>
</tr>
<tr>
<td>NaOAc</td>
<td>276, 297 sh, 358</td>
<td>276, 298 sh, 357</td>
</tr>
<tr>
<td>NaOAc /H\textsubscript{3}BO\textsubscript{3}</td>
<td>269, 309 sh, 331</td>
<td>269, 309 sh, 331</td>
</tr>
<tr>
<td>AlCl\textsubscript{3}</td>
<td>259 sh, 277, 292 sh, 302, 344, 382</td>
<td>260 sh, 277, 291 sh, 301, 344, 381</td>
</tr>
<tr>
<td>AlCl\textsubscript{3} / HCl</td>
<td>260 sh, 279, 294 sh, 300, 338, 379</td>
<td>260 sh, 280, 294 sh, 301, 337, 380</td>
</tr>
<tr>
<td>NaOMe</td>
<td>276, 295 sh, 383</td>
<td>275, 295 sh, 363</td>
</tr>
</tbody>
</table>
Table-9

$^1$H-nmr data of FL-5 (Ac) and acacetin diacetate

<table>
<thead>
<tr>
<th>Assignment</th>
<th>FL-5 (Ac)</th>
<th>Acacetin diacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-8</td>
<td>7.83 (1H, d, J=4.0 Hz)</td>
<td>6.55 (1H, d, J=3.0 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>6.79 (1H, d, J=3.0 Hz)</td>
<td>6.20 (1H, d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>6.53 (1H, s)</td>
<td>6.37 (1H, s)</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>7.78 (2H, d, J=9.0 Hz)</td>
<td>7.80 (2H, d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>6.96 (2H, d, J=9.0 Hz)</td>
<td>6.95 (2H, d, J=9.0 Hz)</td>
</tr>
</tbody>
</table>

OMe / OAc

- 4'    | (3.88) (3H, s) | (3.86) (3H, s) |
- 5     | 2.38 (3H, s)  | 2.43 (3H, s)  |
- 7     | 2.32 (3H, s)  | 2.33 (3H, s)  |

s= singlet, d=doublet, spectrum run in CDCl$_3$ at 300 MHz, using TMS as internal standard. Numbers in parentheses show chemical shifts of methoxy protons.

**FL-6**

FL-6 was eluted from the column by benzene-ethylacetate (1:1) mixture. It was crystallized from chloroform-methanol as white crystals (270 mg), m.p.266-68°C. The elemental analysis agreed with the molecular formula C$_{28}$H$_{32}$O$_{14}$. The glycosidic nature of FL-6 was evidenced by positive Molisch test. It gave greenish-brown colour with FeCl$_3$. The flavonoidic nucleus was evidenced by positive Shinoda’s test and uv spectrum which showed $\lambda_{\text{max}}$ at 269 nm and 325 nm. Analysis with diagnostic reagents gave a red shift of 32 nm with AlCl$_3$ indicating the presence of free hydroxyl group at 5-position. The possibility of its being a flavanone glycoside was ruled out as it gave yellow colour with Wilson-boric acid reagent. Its ir spectrum displayed a carbonyl group at 1660.81 cm$^{-1}$, phenolic hydroxyl group at 3438.93 cm$^{-1}$ and a complex substitution pattern at 1578.88, 1496.47, 1300.50, 1245.15, 1184.79, 1070.72, 1033.70, 835.45 and 771.02 cm$^{-1}$. 

Total hydrolysis of FL-6 with 6% hydrochloric acid yielded equimolar mixture of rhamnose, glucose and an aglycone FL-6 (Agl), m.p. 230°C. The aglycone was characterized as acacetin by spectral, m.m.p. and co-chromatography with an authentic sample.

The uv spectra of the aglycone and the glycoside were almost similar except that the aglycone gave a shift of 20 nm in band-II with NaOAc (absent in glycoside), thus suggesting that the 7-position is involved in glycosylation. Partial hydrolysis of the glycoside (VIIa) with 1% H₂SO₄ yielded L-rhamnose (identified by PC, co-chromatography and GLC) and a partial glycoside (VIII) indicating rhamnose to be the terminal sugar. Partial glycoside on complete hydrolysis with almond emulsion yielded D-glucose. Quantitative estimation of the sugars by Somogyi’s copper-micro method and periodate oxidation ⁴ of the glycoside further confirmed that the sugar moiety is a disaccharide and both the sugars to be in pyranose form.

¹H-nmr spectrum of FL-6 in DMSO-d₆ (Table-10) showed the presence of seven aromatic protons. A sharp singlet at δ 6.45 indicated the presence of C-3 proton, of γ-pyrone ring. Two meta-coupled singlets at δ 6.79 and δ 6.95 integrating for one proton each were assigned to H-6 and H-8 respectively. Two ortho-coupled doublets at δ 7.14 (J=9.0 Hz) and δ 8.04 (J=9.0 Hz) integrating for two protons each corresponded to H-3', 5' and H-2',6' protons respectively. A sharp three protons singlet at δ 3.86 was assigned to methoxyl group. A doublet at δ 1.07 (J=6.0 Hz) was ascribed to rhamnosyl methyl. The anomic proton of glucose H-1" appeared as a doublet at δ 5.09 (J=11.0 Hz) while the anomic proton of rhamnose H-1'" as a doublet at δ 4.55 (J=2.0 Hz). The other sugar protons appeared in the range of δ 4.45-5.43.

Acetylation of FL-6 with acetic anhydride and dry pyridine gave a heptaacetate FL-6 (Ac), m.p. 216-20°C. The ¹H-nmr spectrum of the acetate (Table-11) exhibited a multiplet at δ 1.60-2.02 integrating for eighteen protons assigned to aliphatic acetoxyls while the solitary aromatic acetoxyl appeared as a singlet of three protons at δ 2.45. A singlet at δ 3.90 integrating for three protons was assigned to methoxyl group. It established A₂B₂ pattern of B-ring protons. 2', 6' and 3',5' protons appeared as ortho-coupled doublet (J=9.0 Hz each) at δ 7.81 and 7.09 respectively. The C-6 and C-8 protons of A-ring appeared as meta-coupled doublets (J=2.5 Hz each) centered at δ 6.00
and 6.72. A singlet at δ 6.25 of one proton was assigned to H-3 proton. The anomeric protons of rhamnose (H-1') and glucose (H-1") were centered at δ 4.98 (d, J=2.0 Hz) and 5.25 (d, J=11.0 Hz) respectively. The coupling constant of anomeric protons of rhamnose and glucose suggested α-L-rhamnose and β-D-glucose. The signals over the range of δ 3.35-5.26 account for twelve protons of glucose and rhamnose residue. The position of anomeric protons of rhamnosyl, glucosyl moieties and that of rhamnosyl methyl\textsuperscript{10e} and the absence of characteristic signal for 2"-O-acetyl\textsuperscript{19} at δ 1.70 suggested (1→2) inter sugar linkage (attachment of rhamnosyl residue at 2"-position of glucose, neohesperidoside). This was further confirmed by the identification of methylated sugars. Hydrolysis of the methylated glycoside (VIIc) with 0.2 N HCl gave 2, 3, 4- tri-O-methyl rhamnose and 3, 4, 6-tri-O-methyl glucose (identified by SiO\textsubscript{2}-TLC and paper chromatography according to Petek\textsuperscript{20}) and partially methylated aglycone (IXb) which was characterized as 7-hydroxy-5, 4'-dimethoxy flavone.

The above assigned structure was further supported by the mass spectrum of acetylated glycoside (VIIb) (Scheme-6). The molecular ion peak as expected was not observed. The presence of an acetylated deoxyhexopyranoside and a hexopyranoside were evidenced by the presence of fragment ions at m/z 237 and 289. The fragment ion at m/z 284 corresponded to aglycone. The retro-Diels-Alder cleavage resulted in the formation of ions at m/z 152 due to A-ring and at m/z 132 and 135 due to B-ring.

On the basis of the above results, the compound FL-6 was identified as acacetin-7-O-neohesperidoside\textsuperscript{21} (VIIa)
(VIII)

(IX)
a) R=H
b) R=Me
Table-10

$^1$H-nmr data of FL-6, values of δ-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$ (rhm)</td>
<td>3</td>
<td>1.07 (d, J=6.0 Hz)</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>3</td>
<td>3.86 (s)</td>
</tr>
<tr>
<td>H-1'' (rhm)</td>
<td>1</td>
<td>4.55 (d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-1'' (glu)</td>
<td>1</td>
<td>5.05 (d, J=6.5 Hz)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1', 2', 3', 4', 5', 6', 1'', 2'', 3'', 4'', 5'''</td>
<td>12</td>
<td>4.45-5.43 (m)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.45 (s)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.79 (s)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.95 (s)</td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>2</td>
<td>7.14 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>2</td>
<td>8.04 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>HO-5</td>
<td>1</td>
<td>12.91 (s)</td>
</tr>
</tbody>
</table>

s=singlet, d=doublet, m=multiplet, spectrum run in DMSO-$d_6$ at 300 MHz, using TMS as internal standard.
Table-11

\(^1\text{H-nmr data of FL-6 (Ac), values of } \delta\text{-scale}\)

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH(_3) (rhm)</td>
<td>3</td>
<td>1.15 (d, J=6.0 Hz)</td>
</tr>
<tr>
<td>OCH(_3)-4(^-)</td>
<td>3</td>
<td>3.90 (s)</td>
</tr>
<tr>
<td><strong>Aliphatic acetoxyls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 x OAc</td>
<td>18</td>
<td>1.60-2.02 (m)</td>
</tr>
<tr>
<td><strong>Aromatic acetoxyls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-OAc</td>
<td>3</td>
<td>2.45 (s)</td>
</tr>
<tr>
<td>H-1(^{\prime}) (rhm)</td>
<td>1</td>
<td>5.25 (d, J=11.0 Hz)</td>
</tr>
<tr>
<td>H-1(^{\prime\prime}) (glu)</td>
<td>1</td>
<td>4.98 (d, J=2.0 Hz)</td>
</tr>
<tr>
<td><strong>Sugar protons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1(^{\prime\prime}), 2(^{\prime}), 3(^{\prime}), 4(^{\prime}), 5(^{\prime}), 6(^{\prime}), 1(^{\prime\prime}), 2(^{\prime\prime}), 3(^{\prime\prime}), 4(^{\prime\prime}), 5(^{\prime\prime})</td>
<td>12</td>
<td>3.35-5.26 (m)</td>
</tr>
<tr>
<td><strong>Aromatic prototons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.25 (s)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.60 (d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.72 (d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-2(^{\prime}), 6(^{\prime})</td>
<td>2</td>
<td>7.81 (d, J=9.0 Hz)</td>
</tr>
<tr>
<td>H-3(^{\prime}), 5(^{\prime})</td>
<td>2</td>
<td>7.09 (d, J=9.0 Hz)</td>
</tr>
</tbody>
</table>

S=singlet, d=doublet, m=multiplet, spectrum run at 300 MHz in CDCl\(_3\) using TMS as internal standard.
EXPERIMENTAL
**FL-1**

The compound FL-1 was obtained from the column by benzene only. It was crystallized from chloroform-methanol as colourless compound (150 mg), m.p. 282 °C. It gave positive Liebermann-Burchard test and Molisch test.

Analysed for C_{35}H_{60}O_{6}

Calcd.: C, 72.91; H, 10.41%

Found : C, 72.87; H, 10.34%

**^1H-nmr data (300 MHz, DMSO-d_6) values on δ-scale**

0.70 (3H, s), 0.73 (3H, s), 0.75 (6H, s), 0.78 (3H, s), 1.01 (3H, s), 1.10-1.23 (2H, m, CH2-protons), 4.2 (1H, d, J=10.0 Hz, H-1'), 4.0-5.1 (7H, m, H-2', 3', 4', 5', 5' and 6'), 5.20(1H, m, olefinic proton).

**Killiani's hydrolysis of FL-1**

The compound FL-1 (30 mg) was taken in a thick walled test tube and 2.0 ml of Killiani’s mixture (glacial acetic acid-conc. hydrochloric acid-water 7:2:1) was added to it. The contents were heated on a boiling water bath for 3 hours, after tightly fixing a cork on the mouth of the test tube and tightening it further with the help of a thread. The reaction mixture on dilution with water gave a precipitate which was extracted with ether three times (3 x 25 ml) in a separating funnel. All the etheral extracts were combined together and washed well with water to remove acid. The etheral extract was then dried over anhydrous sodium sulfate and filtered to remove inorganic salts. Recovery of ether gave a residue, which on crystallization with methanol gave a colourless compound (10.0 mg), m.p. 137 °C. By co-TLC, petrol (60-80°C): toluene: ethylacetate (10: 5: 3) with an authentic sample of β-sitosterol, it was found to be identical with it, m.p. of FL-1 when mixed with an authentic sample of β-sitosterol did not show any depression (m.m.p. 137 °C).
Analysed for C_{29}H_{50}O

Calcd.: C, 84.05; H, 12.07%
Found: C, 84.02; H, 12.04%

**Identification of sugar**

The acidic filtrate left after removing the aglycone was extracted with ethylacetate to ensure the complete removal of any residual aglycone. The filtrate was neutralized by concentrating it to a syrup in vacuum dissicator over KOH pellets. The hydrolysate was chromatographed on Whatmann No. 1 paper using n-BuOH-AcOH-H_{2}O (5:4:1) and ethylacetate-pyridine-water (2:1:2) as developing solvents alongwith authentic sugars as check. The chromatograms were then sprayed separately with aniline phthalate and p-anisidine phosphate solutions and dried at 100-05 °C in an oven for fifteen minutes. One coloured spot was developed on the chromatograms which showed the presence of one sugar. The R_{f}-value of sugar was identical with that of glucose.

**Acetylation of FL-1**

Crystalline FL-1 (40 mg) was acetylated by heating it with acetic anhydride (2 ml) and dry pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on water bath for two hours. The reaction mixture was cooled at room temperature and poured on crushed ice. The solid separated was collected, washed well with water and dried. On crystallization from methanol-chloroform gave colourless crystals (30 mg), m.p. 166°C.

_{1}H-nmr data (300 MHz, CDCl_{3}), values on δ-scale

0.71-1.04 (18H, s, 6 x CH_{3}), 1.95 (3H, s, OCOCH_{3}), 1.99 (3H, s, OCOCH_{3}), 2.02 (3H, s, OCOCH_{3}), 2.04 (3H, s, OCOCH_{3}), 4.2-5.3 (7H, m, protons α- to acetoxyl), 5.4 (1H, m, H-6).

**Mass m/z (rel.intensity)**

FL-2
FL-2 was obtained from the column with benzene-chloroform (1:1) mixture and crystallized from chloroform-methanol as light cream coloured crystals (165 mg), m.p. 59-60 °C.

Analysed for C\textsubscript{16}H\textsubscript{14}O\textsubscript{2}

Calcd.: C, 80.67; H, 5.88 %
Found : C, 80.63; H, 5.85 %

IR\textsubscript{KBr} \nu\text{max} \text{cm}\textsuperscript{-1}

1661 (C=O), 1475 (C=C).

UV data with shift reagent \lambda\text{max} \text{nm}

<table>
<thead>
<tr>
<th>Reagent</th>
<th>245</th>
<th>345</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOAc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOAc/H\textsubscript{3}BO\textsubscript{3}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlCl\textsubscript{3}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlCl\textsubscript{3}/HCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOMe</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}H-nmr data (300 MHz, CDCl\textsubscript{3}), values on \delta-scale

3.87 (3H, s, OCH\textsubscript{3}), 6.92 (2H, d, J=8.7 Hz, H-3, 5), 7.59 (2H, d, J=8.7 Hz, H-2, 6), 7.39 (1H, d, J=15.0 Hz, H-\alpha), 7.65 (1H, d, J=15.0 Hz, H-\beta), 7.90 (2H, d, J=8.4 Hz, H-2',6'), 7.52-7.63 (3H, m, H-3', 4', 5').

Mass m/z (rel.int.)

238 [M\textsuperscript{+}] (100), 239 [M\textsuperscript{+}+1] (25.75), 223 [M\textsuperscript{+}-CH\textsubscript{3}] (10.60), 210[M\textsuperscript{+}-CO] (18.18), RDA fragments, 161 (49.8), 134 (15.15), 133 [134-H\textsuperscript{+}] (1.5), 105 (22.7), 78 (40.90), 77 [78-H\textsuperscript{+}] (1.5).
**FL-3**

The compound FL-3 was eluted from the column by benzene-ethylacetate (8:2) mixture. It was crystallized from chloroform-methanol as white crystals (180 mg), m.p. 185-87°C. It gave greenish-brown colour with ferric chloride solution.

Analysed for C₁₇H₁₄O₅

Calcd.: C, 68.45; H, 4.69%

Found: C, 68.41; H, 4.65%

**UV data with shift reagent λ_max nm**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>λ_max nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>244, 369.5</td>
</tr>
<tr>
<td>NaOAc</td>
<td>249, 367.5</td>
</tr>
<tr>
<td>NaOAc/H₃BO₃</td>
<td>251.5, 366.5</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>276.5, 300.5, 329.5, 360</td>
</tr>
<tr>
<td>AlCl₃/HCl</td>
<td>277, 354, 300.5, 330, 372.5</td>
</tr>
<tr>
<td>NaOMe</td>
<td>269, 298</td>
</tr>
</tbody>
</table>

**IR ν_max cm⁻¹**

KBr: 3429 19 (OH), 2980 87, 2850 18, 1669 38 (C=O), 1507 94, 1464 34, 1383 70, 1270 73, 1162 82, 833 55, 818 35.

**¹H-nmr data (300 MHz, CDCl₃), values on δ-scale**

3.88 (6H, d, J=3.0 Hz, 2xOCH₃), 6.36 (1H, d, J=3.0 Hz, H-6), 6.48 (1H, d, J=3.0 Hz, H-8), 6.58 (1H, s, H-3), 7.00 (2H, d, J=9.0 Hz, H-3', 5'), 7.83 (2H, d, J=9.0 Hz, H-2', 6'), 12.81 (1H, s, OH-5).

**Mass m/z (rel.intensity)**

FL-4

It was obtained by eluting the column with benzene-ethylacetate (7:3) mixture and crystallized with chloroform-methanol as pale yellow plates (180 mg), m.p. 198-200°C. It gave a dark green colour with ferric chloride.

Analysed for C₁₈H₁₆O₈
Calcd: C, 60.00; H, 4.40%.
Found: C, 60.58; H, 4.37%.

IR

<table>
<thead>
<tr>
<th>ν_max cm⁻¹</th>
<th>KBr</th>
</tr>
</thead>
<tbody>
<tr>
<td>3433.14 (OH), 1662.82 (C=O), 1622.55 (aromatic).</td>
<td></td>
</tr>
</tbody>
</table>

UV data

<table>
<thead>
<tr>
<th>λ_max nm</th>
<th>MeOH</th>
<th>262, 329 sh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl₃</td>
<td>274, 328 sh, 372.</td>
<td></td>
</tr>
<tr>
<td>NaOAc</td>
<td>272, 326.</td>
<td></td>
</tr>
<tr>
<td>NaOMe</td>
<td>268, 272, 332 sh.</td>
<td></td>
</tr>
</tbody>
</table>

¹H-nmr (300 MHz, DMSO-d₆), values on δ-scale

3.67 (3H, s, OMe), 3.73 (3H, s, OMe), 3.76 (3H, s, OMe), 6.63 (1H, d, J=3.0 Hz, H-6), 6.68 (1H, d, J=3.0 Hz, H-8), 8.36 (1H, s, H-2), 6.48 (1H, s, H-5'), 9.33 (1H, s, OH-4'), 10.32 (1H, s, OH-7), 13.01 (1H, s, OH-5).

Mass m/z

Methylation of FL-4

Crystalline FL-4 (50 mg), dry acetone (50 ml), dimethylsulphate (1 ml) and anhydrous potassium carbonate (0.3 g) were refluxed for 24 hours. The reaction mixture was filtered and the inorganic residue washed several times with hot acetone. On distilling off the solvent, a brown semi solid mass was left behind. It was washed with hot petroleum ether to remove the excess of dimethyl sulphate. The solid residue on crystallization from ethyl acetate gave colourless shining plates (28 mg), m.p. 156 °C.

Analysed for C_{21}H_{22}O_{8}
Calcd.: C, 62.69; H, 5.47%.
Found: C, 62.65; H, 5.45%.

\[^1\text{H-nmr (300 MHz, CDCl}_3\text{), values on }\delta\text{-scale}\]

3.85-3.96 (18H, s, 6x OMe), 6.66 (1H, d, J=2.5 Hz, H-6), 6.75 (1H, d, J=2.5 Hz, H-8), 7.23 (1H, s, H-3'), 7.85 (1H, s, H-2).

\text{Mass m/z}

402 [M^+], 387 [M-15], 372 [M-CH}_2\text{O or M-2Me}], 357 [M-3Me], 371 [M-OMe], RDA fragments, 167 [A_1-Me+2H^+], 179 [B_1-Me-CO].

Oxidative degradation of the methyl ether with alkaline H\textsubscript{2}O\textsubscript{2}

A solution of the methyl ether of FL-4 (20 mg) in acetone (50 ml) was treated with 5% alc. KOH aqueous (10 ml) followed by 30% H\textsubscript{2}O\textsubscript{2} (1 ml), the mixture was kept at 45 °C for two hours. It was cooled, poured into ice-cold water (10 ml), acidified with cold conc. HCl and extracted with ether. The ether solution was washed with water and shaken with saturated NaHCO\textsubscript{3} solution, the bicarbonate fraction was acidified and extracted with ether. Evaporation of ether followed by micro vaccum sublimation gave a colourless crystalline solid, m.p. 185-86 °C. It was identified as 2, 3, 4, 6-tetramethoxybenzoic acid, m.p.; m.m.p. and Co-chromatography with an authentic sample.
**FL-5**

The compound FL-5 was eluted from the column by benzene-ethylacetate (6:4) mixture. It was crystallized from chloroform-methanol as cream coloured crystals (150 mg), m.p. 255°C. It gave greenish colour with ferric chloride solution.

Analysed for C$_{22}$H$_{22}$O$_{10}$

Calcd.: C, 59.19; H, 4.93%

Found: C, 59.16; H, 4.89%

**UV data with shift reagent $\lambda_{\text{max}}, \text{nm}$**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>$\lambda_{\text{max}}, \text{nm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>268, 321</td>
</tr>
<tr>
<td>NaOAc</td>
<td>268, 318</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>268, 324</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>277, 300, 331</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>277, 300, 331</td>
</tr>
<tr>
<td>NaOMe</td>
<td>253, 295, 382 sh</td>
</tr>
</tbody>
</table>

**IR $\nu_{\text{max}}, \text{cm}^{-1}$**

1656 (C=O), 3399 (OH), 1615, 1498, 1304, 1173, 1081 and 832

**$^1$H-nmr (300 MHz, DMSO-d$_6$) values on $\delta$-scale**

3.87 (3H, s, OMe), 5.06(1H, d, J=9.0 Hz, H-1' (glu)), 5.06-5.41(7H, m), 6.45 (1H, d, J=3.0 Hz, H-6), 6.85 (1H, d, J=3.0 Hz, H-8), 6.96 (1H, s, H-3), 7.12 (2H, d, J=9.0 Hz, H-3', 5'), 8.05 (2H, d, J=9.0 Hz, H-2', 6'), 12.91(1H, s, 5-OH).

**Hydrolysis of FL-5**

The glycoside FL-5 (80 mg) was dissolved in water and acidified with 6% hydrochloric acid. The reaction mixture was refluxed over water bath for four hours and left over night at room temperature. The separated aglycone was filtered, washed well with water and dried. It was crystallized from chloroform-methanol as light yellow crystals (60 mg), m.p. 261-63 °C.
Identification of sugar

The acidic filtrate left after removing the aglycone, was extracted with ethylacetate to ensure the complete removal of any residuale aglycone. After usual work-up as described earlier one coloured spot was developed on the chromatograms which showed the presence of one sugar. The R_t-value of sugar was identical with that of glucose.

UV data of FL-5(Agl)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>269, 303 sh, 327</td>
</tr>
<tr>
<td>NaOAc</td>
<td>276, 297 sh, 358</td>
</tr>
<tr>
<td>NaOAc/H_3BO_3</td>
<td>269, 309 sh, 331</td>
</tr>
<tr>
<td>AlCl_3</td>
<td>259 sh, 277, 292 sh, 302, 344, 382</td>
</tr>
<tr>
<td>AlCl_3/HCl</td>
<td>260 sh, 279, 294 sh, 300, 338, 379</td>
</tr>
<tr>
<td>NaOMe</td>
<td>276, 295 sh, 383</td>
</tr>
</tbody>
</table>

Acetylation of FL-5(Agl)

Crystalline FL-5(Agl) (40 mg) was acetylated by heating it with acetic anhydride (2ml) and pyridine (1ml) and allowed to stand overnight at room temperature and then heated on water bath for two hours. As described earlier the separated solid was crystallized from chloroform-methanol as colourless needles (30 mg), m.p. 204°C.

^1H-nmr data (300 MHz, CDCl_3), values on δ-scale

7.83 (1H, d, J=4.0 Hz, H-8), 6.79 (1H, d, J=3.0 Hz, H-6), 6.53 (1H, s, H-3), 7.78 (2H, d, J=9.0 Hz, H-2', 6'), 6.96 (2H, d, J=9.0 Hz, H-3', 5'), 3.88 (3H, s, OMe-4'), 2.38 (3H, s, OAc-5), 2.32 (3H, s, OAc-7)

FL-6

The compound FL-6 was eluted from the column by benzene–ethylacetate (1:1) mixture. It was crystallized as white crystals from chloroform-methanol (270 mg), m.p.266-68°C. It gave greenish-brown colour with ferric chloride solution.
Analysed for C_{28}H_{32}O_{14}

Calcd: C, 55.86; H, 5.51%

Found: C, 55.75; H, 5.61%

**UV data with shift reagent** $\lambda_{\text{max}}$ nm

- MeOH: 269, 325
- NaOAc: 269, 326
- NaOAc/H_3BO_3: 270, 328
- AlCl_3: 278, 301, 344, 384
- AlCl_3/HCl: 279, 302, 340, 380
- NaOMe: 245 sh, 289, 360

**IR** $\nu_{\text{max}}$ cm$^{-1}$

3438.93 (OH), 3369 (OH), 1660.81 (C=O), 1605.26, 1578.88, 1496.47, 1300.50, 1245.15, 1184.79, 1070.72, 1033.70, 835.45, 771.02.

$^1$H-nmr data (300 MHz, DMSO-d$_6$), values on $\delta$-scale

1.07 (3H, d, J=6.0 Hz, CH$_3$-rhm), 3.86 (3H, s, OCH$_3$), 4.45-5.43 (12 H, m, sugar protons-H-1', H-2', H-3', H-4', H-5', H-6', H-1'', H-2'', H-3'', H-4'', H-5''), 4.55 (1H, d, J=2.0 Hz, H-1''' (rhm)), 5.09 (1H, d, J=11.0 Hz, H-1''' (glu)), 6.45 (1H, s, H-3), 6.79 (1H, s, H-6), 6.95 (1H, s, H-8), 7.14 (2H, d, J=9.0 Hz, H-3',5''), 8.04 (2H, d, J=9.0 Hz, H-2', 6'), 12.91 (1H, s, 5-OH).

**Hydrolysis of FL-6**

The glycoside FL-6 (40 mg) was dissolved in water and acidified with 6% HCl. The mixture was refluxed over water bath for four hours and then left over night at room temperature. After usual work-up the aglycone separated was filtered, washed well with water and dried. The aglycone was crystallized from chloroform-methanol as light yellow crystals. Yield (25 mg) m.p. 230°C.
Anlysed for C_{16}H_{12}O_{5}

Calcd.: C, 67.60; H, 4.22%

Found: C, 67.59; H, 4.31%

**UV data with shift reagent $\lambda_{\text{max}}$ nm**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>268, 304 sh, 328</td>
</tr>
<tr>
<td>NaOAc</td>
<td>277, 298 sh, 360</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>270, 310 sh, 333</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>258 sh, 279, 292 sh, 301, 344, 385</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>216 sh, 280, 295 sh, 303, 339, 380</td>
</tr>
<tr>
<td>NaOMe</td>
<td>277, 293 sh, 366</td>
</tr>
</tbody>
</table>

**Identification of sugar**

The acidic filtrate left after removing the aglycone, was extracted with ethylacetate to ensure the complete removal of any residual aglycone. The filtrate was concentrated to a syrup in vacuum dessicator over KOH. The syrup was co-chromatographed on Whatmann No. 1 paper using BuOH-AcOH-H$_2$O (5:4:1) as developing solvents along with authentic sugars. After usual work-up, as described earlier, the sugars were identified as glucose and rhamnose.

**Partial hydrolysis:**

FL-6 (40 mg) was hydrolysed by heating it with 1% H$_2$SO$_4$. Aliquots were taken at different intervals and examined by paper chromatography (EPW, 2:1:2). After one hour, it gave a partial glycoside labelled as FL-4 (pg) and a sugar. The sugar was identified by usual method as rhamnose.

**Acetylation of FL-6**

Crystalline FL-6 (40 mg) was acetylated by heating it with acetic anhydride (2ml) and pyridine (1ml) and allowed to stand overnight at room temperature. After usual work-up the acetylated FL-6 (Ac) was obtained and crystallized from methanol as light cream crystals (25 mg), m.p. 216-20°C.
**Periodate Oxidation**

The glycoside methyl ether (15 mg) was dissolved in methanol (10 ml) and aq. NaIO₄ (0.47 N, 15 ml) was added to it. The mixture was allowed to stand at 20 °C in dark for 24 hours. Solid NaHCO₃ (2 mg) was then added followed by the addition of NaHSO₃ (0.05 N, 25 ml). The resultant mixture was titrated against I₂ using starch as indicator. One mole of methyl ether consumed 3.14 mole of periodate with liberation of 1.12 mole of formic acid.

**¹H-nmr data (300 MHz, CDCl₃), values on δ-scale**

1.15 (3H, d, J=6.0 Hz, CH₃-rhm), 3.90 (3H, s, OCH₃-4'), 1.60-2.02 (18H, m, 6-aliphatic acetoxyls), 2.45 (3H, s, 5-aromatic acetoxyls), 4.98 (1H, d, J=2.0 Hz, rhm-1''''), 5.25 (1H, d, J=11.0 Hz, glu. H-1'''), 3.35-5.26 (12H, m, sugar protons H-1'', 2'', 3'', 4'', 5'', 6'', H-1''', 2''', 3''', 4''', 5'''), 6.25 (1H, s, H-3), 6.60 (1H, d, J=2.5 Hz, H-6), 6.72 (1H, d, J=2.5 Hz, H-8), 7.09 (2H, d, J=9.0 Hz, H-3’, 5’), 7.81 (2H, d, J=9.0 Hz, H-2’, 6’).

**Mass m/z (rel. intensity)**

[M⁺] absent, 289 [glu(Ac)₃] (33.2), 273 [rhm (Ac)₃] (32.5), 284 [aglycone] (95.7), RDA fragments, 152 [A₁⁺] (18.0), 132 [B⁺₁] (36.1), 135 [B⁺₂] (5.3).
REFERENCES


   a) 32
   b) 23 - 24
   c) 19 - 131

   a) 227-230
   b) 44-52
   c) Chapter VIII, The $^1$H-nmr spectra of flavonoids, P. 254-273
   d) Spectrum No. 29 and 30.
   e) 269-271


CHAPTER 5
DISCUSSION
Chemical constituents from the roots of **Bergenia ligulata** (**Saxifragaceae**)

*Bergenia ligulata*, is a medicinal plant distributed in South and East Asia\(^1\). In India it grows at high altitudes in the Himalayas usually in rocky areas and cliffs. It is popularly known in the Indian system of medicine as Paashaanbheed. It is extensively used in Indian medicine as cure of kidney stones\(^2\). The rhizomes have been used for centuries in the Ayurvedic formulations of ailments\(^1\). The roots are used in fever, diarrhea and cough\(^3\).

Alcoholic extract of the plant has exhibited significant analgesic, anti-inflammatory and diuretic properties\(^4\). Previous chemical investigations of the plant have indicated the presence of \(\beta\)-sitosterol, \(\beta\)-sitosterol-D-glucoside, bergenin\(^2\) and afzelechin\(^5\). Medicinal importance and scanty work on this plant accelerates our interest to carry out the comprehensive investigations of the plant *Bergenia ligulata*. The present discussion deals with the isolation and characterization of the following five compounds, in addition to bergenin from the roots of *Bergenia ligulata*.

1. Lanost-7(8)-en-3\(\beta\)-formyl-6\(\beta\)-acetoxy-11\(\beta\),15\(\beta\)-dil-26-heptanoxy-27-oic acid (BL-1).
2. Urs-12(13)-en-3\(\beta\)-22\(\alpha\)-diol-15\(\beta\)-formyl-21\(\beta\)-glucoside (BL-2).
3. 9,11-Seco-lanost-20(22)-en-3\(\beta\)-formyl-18-phenoxyoate-12\(\beta\)-D-glucose (BL-3).
4. Sumatrol (BL-4).
5. 5\(^\prime\)-Methyleriodictyol-3\(^\prime\)-O-\(\beta\)-D-galactopyranosyl(1\(\rightarrow\)4)-\(\alpha\)-L-rhamnopyranose (BL-5).
The dried and powdered roots (2.0 kg) of *Bergenia ligulata*, procured from Saudi Arabia, were consecutively extracted with the following solvents and yielded the indicated fractions.

Two extractions (24 hours, periods) with light petroleum (60-80°C) (2x4 l) yielded fraction ‘A’ (60 + 15 gm). Two continuous extractions (24 hours, periods) with hot chloroform yielded fraction ‘B’ (80+10 gm). Finally, the roots were extracted with hot acetone, yielded fraction 'C' followed by methanol, fraction ‘D’.

Fractions ‘A’ and ‘B’ on TLC examinations in different solvent systems showed a number of compounds with the same Rf-values, in varying concentrations. The two fractions were therefore mixed together. The combined material was chromatographed over silica gel column using successively petroleum ether, petroleum ether-chloroform mixtures, chloroform, chloroform-methanol mixtures and methanol as eluting solvents. Appropriate fractions (IR spectra and TLC) were combined. Repeated column chromatography of the fractions followed by fractional crystallizations afforded four crystalline TLC homogenous substances, labeled as BL-1, BL-2, BL-3 and BL-4.

The acetone extract gave a positive test for flavonoids. TLC examination of the extract in different solvent systems, showed the presence of only one major compound along with some minor impurities. Repeated column chromatography over silica gel and fractional crystallization failed to purify the compound, therefore, a recourse was taken to the preparative TLC using EtOAc-EtMeCO-AcOH-H₂O (5:3:1:1) as a solvent system. A TLC homogenous, light yellow substance was obtained, labeled as BL-5.

The methanol extract, on evaporation in vacuum, gave a dark red gummy residue. The tannins and the non-tannins materials present in the residue were separated by the conventional 'lead acetate' method. The alcoholic filtrate containing the non-tannins portion was concentrated on a water bath. The concentrate was left overnight in a refrigerator, when a white semi-solid mass was separated. It was filtered and crystallized from methanol and labeled as BL-6.
**BL-1**

BL-1 was obtained as colourless amorphous powder from petroleum ether-chloroform (9:1) mixture. It was crystallized by chloroform-methanol (200 mg), m.p.161-62°C. It gave effervescence with sodium bicarbonate solution and responded positively to Liebermann-Burchard test. Its ir spectrum showed the characteristic absorptions for hydroxyls (3425 cm\(^{-1}\)), ester group (1734 cm\(^{-1}\)) and unsaturation (1595 cm\(^{-1}\)). Its molecular weight was established as 704 [M]+ relating to C\(_{40}H_{64}O_{10}\) on the basis of positive ion FAB mass spectrum.

The \(^1\)H-nmr spectrum of BL-1 (Fig.1) displayed a one-proton downfield singlet at δ 8.55 assigned to the formyl proton. A one-proton multiplet at δ 5.31 was ascribed to H-7. Two one-proton each doublets with coupling interaction of 6.5 Hz at δ 4.13 and 4.08 were associated with the C-26 ester substituted methylene group. The H-6, H-3α and H-11α carbinolic protons appeared at δ 4.05 (d, J = 5.5 Hz), 3.65 (m) and 3.57 (m), respectively. The methyl signals resonated as broad singlets at δ 1.28 (Me-30), 1.18 (Me-28), 1.15 (Me-29), 0.96 (Me-19), 0.83 (Me-18) and as a doublet at δ0.88 (J = 6.0 Hz, Me-21). The signals in the range δ1.23-2.80 were due to the remaining methine and methylene groups.

The mass spectrum (Fig.2) exhibited typical ion fragments at m/z 661 [M-Ac]+, 390 [661-C\(_{13}H_{27}O_{4}\), SC, 271]+, 591 [M-C\(_{6}H_{13}CO\)]+, 320 [591-SC]+, 686 [M-H\(_2\)O]+, 675 [M-CHO]+, 128 [ion a]+, 576 [ion b]+, 558 [ion b-H\(_2\)O]+, 445 [558-C\(_{6}H_{13}CO\)]+, 532 [ion b-CO\(_2\)]+, 305 [ion b-SC]+, 287 [305-H\(_2\)O]+, 269 [287-H\(_2\)O], 168 [ion c]+, 139 [ion c-CHO]+, and 153 [ion c-Me]+, suggesting the lanostenic type nature of the molecule possessing a formyl group in ring-A, which was placed at C-3 on the basis of biogenetic ground. It also possessed an acetoxy and two hydroxyl groups and a C\(_8\)-saturated side chain with a carboxylic and a heptanoyl groups. The existence of acetoxy group at C-6 was deduced from the ion peaks of m/z 240 [ion d]+, 211 [ion d-CHO]+, 197 [ion d-CO\(_2\)CH\(_3\)]+, 308 [ion g]+, 279 [ion g-CHO]+, 265 [ion g-Ac]+ and 278 [ion g-C\(_2\)OH]+. The ion peaks at m/z 463 [ion e]+, 445 [ion e-H\(_2\)O]+, 426 [ion f]+, 355 [ion h]+, 325 [ion h-C\(_2\)OH]+, 340 [ion h-Me]+, 311 [ion h-CO\(_2\)]+, 242 [ion h-C\(_{6}H_{13}CO\)]+, 393 [ion i]+, 379 [ion i-CH\(_2\)]+.
and 349 [379-CHOH]$^+$ reflected the presence of the hydroxyl groups at C-11 and C-15. (Scheme-I)

The BL-1 on methylation with diazomethane yielded a monomethyl ester BL-1 (Me). Acetylation of BL-1 with acetic anhydride-pyridine formed a diacetyl product, BL-1 (Ac).

On the basis of above spectral studies BL-1 was identified as Lanost-7(8)-en-3β-formyloxy-6β-acetoxy-11β,15β-diol-26-heptanoxy-27-oic acid (I).

BL-2

BL-2 was obtained as a crystalline solid from petroleum ether-chloroform (7:3) eluants. It was crystallized by chloroform-methanol mixture as white needles (230 mg), m.p. 190-191°C. It gave a positive Liebermann-Burchard test and exhibited strong IR bands at 3425 (hydroxyl-group), 1735 (ester-group) and 1605 cm$^{-1}$ (unsaturation). It had a molecular ion peak in its positive ion FAB mass spectrum at m/z 664 corresponding to a pentacyclic triterpenic glycoside, C$_{37}$H$_{60}$O$_{10}$. It indicated seven degrees of double bonds.

The $^1$H-nmr spectrum of BL-2 (Fig.3) displayed a one-proton downfield singlet at δ 8.50 assigned to formate group, a one-proton triplet at δ 5.30 (J=5.5 Hz) due to H-12, a one-proton doublet at δ 3.85 (J=5.5 Hz) for H-22, two one-proton each broad singlets at δ4.83 and 3.80 associated with H-1′ anomeric and H-21 methine protons, a one-proton broad multiplet at δ 4.30 due to H-15 methine proton and a one double doublet at δ 3.53 for carbinol placed at C-3 on the basis of biogenetic analogy.
Scheme - 1
and its coupling interactions of 4.5 and 8.5 Hz indicated its α-orientation. The glucose protons appeared as broad singlets at δ4.13 (1H, H-6'a), 4.06 (1H, H-6'b) and 3.60 (4H, H-2', H-3', H-4', H-5'). The eight methyl signals, all located on saturated carbons, resonated at δ1.20 (Me-24, Me-25), 1.16 (Me-27), 1.13 (Me-23), 1.08 (d, J=6.6 Hz, Me-29), 0.86 (d, J=6.5 Hz, Me-30), 0.80 (Me-28), and 0.76 (Me-26).

The presence of a doublet at δ 2.30 (J= 9.0 Hz), attributed to H-18, indicated the compound to be related to the ursane series. The remaining methine and methylene groups appeared in the range of δ 1.56-2.80.


More compelling evidence for the structure of BL-2 was provided by chemical reactions. Treatment of BL-2 with acetic anhydride and pyridine afforded a peracetylated product BL-2 (Ac). Acid hydrolysis of BL-2 yielded the aglycone, BL-2 (Agl), and glucose which was identified by co-chromatography with an authentic sample of glucose.
On the basis of above spectral studies the BL-2 was identified as Urs-12(13)-en-3β, 22α-diol-15β-formyl-21β-glucoside (II).

BL-3

BL-3 was eluted from the column by petroleum ether-chloroform (3:1) mixture as amorphous mass. It was crystallized from methanol (180 mg) m.p. 210-11°C. It gave positive tests of triterpenoids and glycosides. Its ir spectrum demonstrated the presence of hydroxyl groups (3435 cm⁻¹), ester (1735, 1725 cm⁻¹) and unsaturation (1600 cm⁻¹). It had molecular ion peak in its positive ion FAB mass spectrum at m/z 742 [M]⁺, corresponding to C₄₃H₆₆O₁₀.

The ¹H-nmr spectrum of BL-3 (Fig.5) displayed a one-proton downfield singlet at δ 8.43 assignable to OCHO and three aromatic protons as multiplets at δ 7.07 (2H, H-2', H-6''), 6.95 (2H, H-3'', H-5'') and 6.70 (1H, H-4''). A one-proton broad singlet at δ 5.26 was attributed to H-22 vinylic proton. The carbinolic protons appearing as a double doublet at δ 4.36 (J= 4.5, 8.5 Hz), and as a multiplet at δ 4.15 were ascribed to H-3β and H-12. The protons of glucose moiety resonated at δ 4.87 (d, J= 6.0 Hz, H-1'), 4.05 (d, J= 6.0 Hz, H-6'a), 4.00 (d, J= 6.0 Hz, H-6'b), 3.96 (m, H-5'), 3.53 (m, H-4') and 3.40 (m, H-2', H-3'). A three-protons broad singlet at δ 1.80 was attributed to C-21 methyl group attached to C-20 olefinic carbon. The signals for three-protons each at δ 1.08, 1.02, 0.80 and 0.76 were associated with the tertiary Me-29, Me-19, Me-28 and Me-30 respectively. The secondary Me-26 and Me-27 appeared as doublets with coupling interactions of 6.5 Hz at δ 0.97 and 0.87. The signals in the range δ 1.15-2.71 were accounted to the remaining methylene and methine functionalities.
In its mass spectrum (Fig. 6), the ion fragments at m/z 727 [M-Me]⁺, 631 [M-C₆H₁₅, SC]⁺, 579 [M-C₆H₁₁O₃]⁺, 562 [M-C₆H₁₂O₃]⁺, 111 [C₈H₁₅, SC]⁺, 163 [C₆H₁₁O₃]⁺, 180 [C₆H₁₂O₆]⁺, 93 [C₆H₅O]⁺ and 410 [562-C₁₁H₂₀]⁺ suggested that the compound belonged to lanostene-type triterpene which possessed C₈H₁₅ an unsaturated side chain, a glucose moiety, a formyl group and a phenolic ester. The ion peaks at m/z 128 [ion a]⁺, 113 [ion a-Me]⁺, 99 [ion a-CHO]⁺, 168 [ion b]⁺, 139 [ion b-CHO]⁺, 223 [ion c]⁺, 194 [ion c-CHO]⁺ and 177 [ion c-HCOOH]⁺, indicated the presence of formyl group in ring-A which was placed at C-3 on biogenetic grounds. The ion fragments at m/z 519 [ion d]⁺, 408 [ion d-SC]⁺, 426 [ion d-C₆H₅O]⁺, 356 [ion d-C₆H₁₁O₃]⁺, 339 [ion d-C₆H₁₂O₆]⁺, 207 [CH(CH₃)OC₆H₁₁O₃]⁺, 312 [ion d-207]⁺, 219 [312-C₆H₅O]⁺ and 297 [312-Me]⁺ supported the C₉-C₁₁ seco-nature of ring-C and the existence of phenoxy group at C-18 and glucosyl moiety at C-12. (Scheme-III)

Acid hydrolysis of BL-3 yielded an aglycone, BL-3 (Agl), and a sugar moiety which was identified as D-glucose by comparing it with an authentic sample of glucose on TLC.

On the basis of above spectral studies the BL-3 was identified as 9,11-Secolanost-20(22)-en-3β-formyl-18-phenoxyoate-12β-D-glucose (III).

BL-4 was eluted with chloroform-methanol (8:2) mixture. Recovery of the solvent left a residu, which was crystallized from acetone as colourless needles (750 mg), m.p. 190-92 °C. It was sparingly soluble in methyl alcohol, acetic acid and dilute aq. sodium hydroxide, but readily dissolved in chloroform. With ferric chloride it gave a deep brown colouration tinged with green. The analytical results obtained for BL-4 gave the molecular formula as C₂₃H₂₀O₅(OMe)₂.
FIG. 5
Scheme - III
results obtained for BL-4 gave the molecular formula as C_{23}H_{20}O_{5}(OMe)_2. A purple colouration with Durham test\(^{10}\) and a green one in the Rogers and Calamari test\(^{11}\) indicated that the compound might be rotenoid.

The formation of an oxime, m.p. 245-46 °C established the presence of an active carbonyl group. This fact, in conjunction with the strong ferric reaction indicated the presence of a phenolic group in the ortho position to the carbonyl group.

Treatment of BL-4 with iodine and sodium acetate and elimination of iodine from the product by means of zinc dust and acetic acid according to the standard procedure yielded a colourless compound, m.p. 190-92°C, which like the parent compound was optically active, almost insoluble in dilute aq. NaOH and gave a strong ferric reaction. It was identified as dehydrosumatrol\(^{12}\) (IV) by melting and mixed melting point with an authentic sample.

The above observations showed that the BL-4 is sumatrol (V). Further confirmation to its identity was furnished by spectral studies, as the authentic sample of sumatrol could not be obtained.
The \(^1\)H-nmr spectrum (Fig.7) revealed a vinyl methyl at \(\delta\) 1.75, two methoxyls at \(\delta\) 3.79 and 3.82, three aromatic protons at \(\delta\) 6.0, 6.46 and 6.86, all of which appeared as singlets besides signals for nine other protons between \(\delta\) 2.5-5.2. A careful study of the last mentioned region showed that three of these formed a ABX system centred at \(\delta\) 2.8 (dd), 2.3 (dd) and 5.2 (t) attributed to the dihydrofuran ring protons. The two broad singlets at \(\delta\) 4.95 and 5.08 could be assigned to olefinic protons of \((\text{CH}_3\text{C}=\text{CH}_2)\) unit consistent with the presence of a vinyl methyl. Four other signals at \(\delta\) 3.8 (d, \(J= 4.0\)Hz), 4.18 (d, \(J= 12.0\)Hz), 4.6 (dd, \(J= 12.0\)Hz, 4.0Hz) and 4.9 (t, \(J= 4.0\)Hz) were reminiscent of rotenoid skeleton. The presence of a chelated hydroxyl was indicated by a low field signals at \(\delta\) 12.5 the data suggested the structure (V).

The \(^{13}\)C-nmr spectrum (Fig. 8) further confirmed the above structure. The assignments of individual carbon atoms are given in Table-1.

The lower field position of the carbonyl at 194 is suggestive of a reduced pyrone ring system. The two aliphatic carbons of this ring appearing at \(\delta\) 44 and 72 (both doublets in off resonance) is indicative of an elaboration of isoflavanone chromophore to rotenoid type skeleton. The presence of isopropenyl dihydrofuran ring system is also substantiated by signals at \(\delta\) 17 (\text{CH}_3), 30 (\text{CH}_2), 88 (oxygenated carbon), 112 (terminal methylene) and 143 (quarternary olefinic carbon). There were three protonated aromatic carbon resonances at \(\delta\) 92, 101 and 110 of which the first can be assigned to phloroglucinol ring methine carbon. There were two methoxyl carbon resonances near \(\delta\) 56. The remaining signals were other aromatic quaternary carbons falling in two classes, three nonoxygenated 101.4, 104.3 and 104.8 and six oxygenated 144, 147, 149.9, 156, 166 and 169. Thus these data also lead to the same conclusion derived from \(^1\)H-nmr and confirmed that BL-4 is sumatrol (V).
Table-I

$^{13}$C-nmr spectral data of BL-4

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>110</td>
</tr>
<tr>
<td>C-2</td>
<td>144</td>
</tr>
<tr>
<td>C-3</td>
<td>149.9</td>
</tr>
<tr>
<td>C-4</td>
<td>101</td>
</tr>
<tr>
<td>C-4a</td>
<td>147</td>
</tr>
<tr>
<td>C-6</td>
<td>66</td>
</tr>
<tr>
<td>C-6a</td>
<td>72</td>
</tr>
<tr>
<td>C-7a</td>
<td>156</td>
</tr>
<tr>
<td>C-8</td>
<td>101.4</td>
</tr>
<tr>
<td>C-9</td>
<td>166</td>
</tr>
<tr>
<td>C-10</td>
<td>92</td>
</tr>
<tr>
<td>C-11</td>
<td>169</td>
</tr>
<tr>
<td>C-11a</td>
<td>104.3</td>
</tr>
<tr>
<td>C-12</td>
<td>194</td>
</tr>
<tr>
<td>C-12a</td>
<td>44</td>
</tr>
<tr>
<td>C-4'</td>
<td>30</td>
</tr>
<tr>
<td>C-5'</td>
<td>88</td>
</tr>
<tr>
<td>C-6'</td>
<td>143</td>
</tr>
<tr>
<td>C-7'</td>
<td>112</td>
</tr>
<tr>
<td>C-8'</td>
<td>17</td>
</tr>
<tr>
<td>2 x OCH$_3$</td>
<td>56</td>
</tr>
</tbody>
</table>

**BL-5**

BL-5 was obtained as light yellow solid from chloroform-methanol (3:1) mixture. The solid on TLC examination showed a major spot with some minor impurities. It was therefore, further purified by preparative TLC (ethyl acetate-ethyl methyl ketone-acetic acid-water (5:3:1:1)). A light yellow substance was obtained, labelled as BL-5 and crystallized from ethanol as pale yellow needles.
(350 mg), m.p. >250°C. It responded positively to Shinoda’s test\(^6\), Molisch test\(^9\) and the uv spectrum showed \(\lambda_{\text{max}}\) in methanol at 275 nm (Band II). Analysis of functional groups revealed the presence of phenolic OH (3450 cm\(^{-1}\)), \(\alpha, \beta\)-unsaturated ketonic C=O (1680 cm\(^{-1}\)) and a complex aromatic substitution pattern (1500, 1365, 1140, 800 cm\(^{-1}\)) besides a strong band at 2950 cm\(^{-1}\). The colour reaction, ir, uv spectral data with diagnostic shift reagents\(^{13}\) coupled with appearance of a double doublet (C\(_2\)-1H, \(\delta\) 8.5.2, \(J=5.0\) and 10.0 Hz) and a multiplet (C\(_3\)-2H, \(\delta\) 2.95-3.20) in the \(^1\)H-nmr spectrum confirmed the presence of flavanone skeleton bearing hydroxyl groups at 4', 5, 7-positions.

Acid hydrolysis with 0.2 N hydrochloric acid gave an aglycone and equimolar quantity of galactose and rhamnose, identified by paper chromatography. The aglycone showed characteristic features of 3', 4'-dihydroxyflavanone as it rapidly decomposed in the presence of NaOAc, in uv spectrum (absent in glycoside), thus suggesting that sugars may be attached to 3'-position of the aglycone\(^{14}\). The formation of tetraacetate derivative and the presence of one C-methyl group (\(^1\)H-nmr signal at \(\delta\) 2.40 corresponding to 3H of C-Me) was in agreement with the structure of the aglycone as 3',4',5,7-tetrahydroxy-5'-methylflavanone (5'-methyleriodictyol) (VI) corresponding to molecular formula C\(_{16}\)H\(_{14}\)O\(_6\).

On acetylation with acetic anhydride and pyridine, BL-5 formed a crystalline nonaacetate BL-5(AC), m.p. 85-86 °C. The \(^1\)H-nmr spectrum (Fig.9) of BL-5 (Ac) (Table-II) showed multiplets at \(\delta\) 2.01-2.48 integrating for 30 protons due to one C-methyl group and nine acetoxyls (3 aromatic and 6 aliphatic). A quartet centred at \(\delta\) 2.95-3.20 is assigned to C-3 methylene and double doublet at \(\delta\) 5.20 (\(J=5.0\) and 10.0 Hz) identified the C-2 proton. The 5,7-disubstitution was demonstrated by the presence of two meta coupled doublets at \(\delta\) 6.82 and 7.28 assigned to C-6 and C-8 protons which have shifted considerably downfield due to derivatisation. A doublet at \(\delta\) 7.75 is ascribed to 2', 6'-protons of the ring-B. The mass spectrum of BL-5 (Ac) (Fig.10) fully supported the structure of BL-5 as it clearly exhibited M\(^+\) at m/z 428 (M-Gly) in accordance with the flavanone containing three acetoxyl and one C-methyl substituents. The subsequent
removal of three acetoxyls gave fragment ions at m/z 386, 344 and 302. The fragment at m/z 302 was observed as the base peak as it corresponds to the aglycone. Other major fragments were observed at m/z 286, 283, 273, 245, 236, 153, 137 and 111 (Scheme-IV).

Table-II

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.75 (d, J=9.0Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.28 (d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.82 (d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-2</td>
<td>1</td>
<td>5.20 (dd, J1=10.0Hz, J2=5.0Hz)</td>
</tr>
<tr>
<td>H-3, 3</td>
<td>2</td>
<td>2.95-3.20 (q, J1=12.0Hz, J2=4.0Hz, J3=17.0Hz)</td>
</tr>
<tr>
<td>6 Aliphatic acetoxyls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Aromatic acetoxyls</td>
<td>30</td>
<td>2.01-2.48 (m)</td>
</tr>
<tr>
<td>C-methyl group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

s = singlet, d=doublet, q=quartet, m=multiplet. Spectrum run in CDCl₃ at 300 MHz, using TMS as internal standard.

Kuhn methylation¹⁵ of the glycoside followed by acid hydrolysis gave 2,3-di-O-methyl-rhamnose¹⁶, 2,3,4,6-tetra-O-methyl-D-galactose¹⁷ indicating the disaccharide to be galactosyl (1→4) rhamnose and an aglycone characterized as 5'-methyl-4',5,7-tri-O-methyleriodctylo(VII) by its spectral studies. The sugar moieties were found to be attached to position-3' by the formation of this partial methyl ether as well as by the comparison of uv spectrum of the aglycone and the glycoside in the presence of NaOMe. Further evidence for the vicinal dihydroxyl system in the aglycone was obtained by the conversion to diphenylmethylenedioxy derivative with diphenyldichloromethane¹⁸. The glycoside however
failed to form this derivative thus finally confirming the sugar linkage at 3' position. Emulsion hydrolysis of the glycoside gave only galactose in aqueous hydrolysate showing it to be terminal and β-linked. Quantitative estimation of sugars and periodate oxidation showed two moles of sugars per mole of the aglycone and both the sugars to be in pyranose form.

On the basis of these results, the flavanone glycoside has been identified as 5'-methyleriodictyol-3'-O-β-D-galactopyranosyl(1→4)-α-L-rhamnopyranose (VIII).
FIG-10
Scheme-IV
The solid obtained from the methanol extract of the roots was crystallised from methanol as fine, colourless needles (5 gm), m.p. 237-38° C (after drying in high vacuum at 80°C for several hour). The uv spectrum in ethanol gave a $\lambda_{\text{max}}$ at 272 nm, showing a red shift to 309 nm, on being made alkaline with aqueous NaOH. The ir spectrum of BL-6 showed absorption at $\nu_{\text{nujol}}$ 3460 (bonded OH), 1710 (C=O, open chain or 6-membered ring), 1613, 1520, 1460 cm$^{-1}$ (aromatic ring). It readily reduced Fehling and Tollens reagents but did not respond to Molisch test$^9$. On treatment with acetic anhydride and pyridine it gave an acetate, crystallized from methanol, m.p. 207-08° C.

Analytical and other data on the substance BL-6 and its derivatives, showed it to be Bergenine (IX) a C-glycoside compound that had already been reported from the same plant$^2$. 

![Chemical structure of Bergenine (IX)](attachment:chemical_structure.png)
EXPERIMENTAL
**BL-1**

The compound BL-1 was eluted from the column by petroleum ether-chloroform (9:1). It was crystallized by chloroform-methanol as colourless amorphous powder (200 mg), m.p. 161-62°C. Purified by preparative TLC, (n-hexane-petroleum ether, 3:1).

**UV** \( \lambda_{\text{max}} \) (MeOH) nm

208

**IR** \( \nu_{\text{max}} \) (cm\(^{-1}\)):

3425, 2925, 2860, 1734, 1595, 1561, 1441, 1367, 1254, 1240, 1180, 1112, 1066, 1044, 965, 815, 745.

**\(^1\)H-nmr** (300 MHz, CDCl\(_3\) + MeOD), values on \( \delta \)-scale:

\( \delta \) 8.55 (1H, br s, OCHO), 5.31 (1H, m, H-7), 4.13 (1H, d, J=6.5, Hz, H-26'a), 4.08 (1H, d, J=6.5 Hz, H-26'b), 4.05 (1H, d, J=5.5 Hz, H-6), 3.65 (1H, m, H-3\( \alpha \)), 3.57 (1H, m, H-11\( \alpha \)), 3.50 (1H, m, H-15\( \alpha \)), 2.80 (2H, m, H-2'-1), 2.31 (5H, m, H-2'-2', H-16, H-5), 2.17 (1H, m, H-17), 2.10 (4H, m, H-16, H-24), 1.93 (3H, m, COCH\(_3\)), 1.77 (1H, d, J= 5.5, H-9), 1.65 (5H, m, H-22, H-23, H-20), 1.28 (3H, br s, Me-30), 1.23 (10H, br s, 5x CH\(_2\)), 1.18 (3H, br s, Me-28), 1.15 (3H, br s, Me-29), 0.96 (3H, br s, Me-19), 0.88 (3H, d, J= 6.0 Hz, Me-21), 0.83 (3H, br s, Me-18).

**FABMS** m/z (rel. int.)

704 [M]\(^+\) (3.3), 686 (3.2), 675 (2.0), 663 (11.9), 661 (3.8), 647 (8.7), 591 (2.5), 576 (1.7), 558 (1.7), 532 (4.7), 463 (1.7), 445 (2.6), 427 (2.9), 426 (2.1), 408 (3.6), 396 (3.9), 393 (3.1), 379 (3.5), 364 (2.3), 355 (4.5), 350 (3.2), 349 (2.3), 340 (4.9), 338 (4.4), 325 (5.7), 323 (4.9), 320 (4.8), 311 (8.2), 308 (7.9), 305 (6.2), 287 (3.3), 279 (7.8), 278 (3.9), 271 (4.2), 269 (4.0), 265 (8.8), 253 (5.8), 242 (5.6), 240 (8.2), 225 (5.7), 211 (6.8), 210 (2.8), 207 (16.3), 197 (6.1), 192 (11.1), 173 (9.3), 168 (13.2), 155 (13.9), 153 (11.0), 139 (8.3), 128 (21.7), 119 (30.8), 117 (25.9), 109 (41.5), 107 (39.1), 105 (59.0), 99 (10.3), 97 (46.2), 95 (94.3), 93 (100).
**Methylation of BL-1**

The compound BL-1 (50 mg) was dissolved in ether (100 ml). Diazomethane was passed throughout the solution till it's saturated. The flask was corked and kept over night at room temperature. The ether was evaporated and the residue was washed well with sodium bicarbonate solution and then with water. It was dried and purified on silica gel column using the chloroform as the eluants.

The methylated product was crystallized from chloroform-methanol mixture as colourless needles (35 mg), m.p. 114-15 °C.

Analysed for \( \text{C}_{41}\text{H}_{66}\text{O}_{10} \)

Calcd.: C, 68.52; H, 9.19

Found : C, 68.49; H, 9.16

**Acetylation of BL-1**

Crystalline BL-1 (25 mg) was treated with a acetic anhydride (1 ml) and dry pyridine (0.5 ml). The mixture was allowed to stand overnight at room temperature (22-27°C) and then heated on water bath for two hours. The reaction mixture was cooled at room temperature and poured on crushed ice. The solid was separated, filtered, washed with water and dried over sodium sulphate. It was crystallized from chloroform-methanol mixture as white-needles, m.p. 110 °C.

Analysed for \( \text{C}_{44}\text{H}_{69}\text{O}_{12} \)

Calcd.: C, 66.92; H, 8.74

Found : C, 66.89; H, 8.79

**BL-2**

Elution of the column with petroleum ether-chloroform (7:3) gave BL-2. It was crystallized from chloroform-methanol as white needles (230 mg), m.p. 190-91°C.
UV $\lambda_{\text{max}}$ (MeOH) nm

210

KBr

IR $\nu_{\text{max}}$ cm$^{-1}$

3425, 2925, 2820, 1735, 1605, 1562, 1449, 1375, 1255, 1100, 820.

$^1$H-nmr (300 MHz, CDCl$_3$ + MeOD) values of $\delta$-scale

$\delta$ 8.50 (1H, s, OCHO), 5.30 (1H, t, J= 5.5 Hz, H-12), 4.83 (1H, br s, H-1'), 4.30 (1H, br m, H-15), 4.13 (1H, br s, H-6'a), 4.06 (1H, br s, H-6'b), 3.85 (1H, d, J= 5.5, H-22), 3.80 (1H, br s, H-21), 3.60 (4H, br s, H-2', H-3', H-4', H-5'), 3.53 (1H, dd, J= 4.5, 8.5 Hz, H-3a), 2.80 (2H, m, CH$_2$-1), 2.53 (2H, m, CH$_2$-16), 2.30 (1H, d, J= 9.0 Hz, H-18), 2.16 (3H, m, CH$_2$, CH), 2.03 (5H, m, 2x CH$_2$, CH), 1.90 (3H, br s, CH, CH$_2$), 1.80 (1H, d, J= 5.5 Hz, CH-5), 1.56 (3H, m, CH$_2$, CH), 1.20 (6H, br s, Me-24, Me-25), 1.16 (3H, br s, Me-27), 1.13 (3H, br s, Me-23), 1.08 (3H, d, J= 6.6 Hz, Me-29), 0.86 (3H, d, J= 6.5 Hz, Me-30), 0.80 (3H, br s, Me-28), 0.76 (3H, br s, Me-26).

FABMS m/z (rel. int.)

664 [M]$^+$ (1.4), 646 (1.0), 618 (1.8), 533 (1.4), 503 (2.1), 460 (23.9), 458 (3.7), 444 (1.2), 426 (1.5), 416 (1.4), 415 (3.6), 413 (3.6), 398 (6.2), 398 (1.1), 397 (2.3), 390 (1.3), 386 (3.0), 368 (3.8), 355 (14.0), 346 (1.2), 340 (11.2), 334 (1.3), 332 (1.3), 328 (2.8), 324 (1.9), 318 (1.7), 314 (1.4), 281 (33.8), 278 (8.7), 274 (2.0), 266 (14.7), 264 (10.5), 263 (4.0), 259 (2.4), 256 (3.6), 250 (5.1), 246 (3.0), 235 (3.9), 220 (43.1), 210 (3.4), 207 (43.9), 192 (12.3), 191 (14.6), 189 (10.0), 176 (11.0), 163 (5.8), 152 (5.6), 146 (100), 145 (13.4), 138 (3.9), 133 (23.3), 131 (17.4), 123 (12.4), 117 (16.3), 111 (14.7), 107 (20.3), 105 (28.0), 101 (14.3), 98 (42.4), 93 (30.0).

Acetylation of BL-2

Crystalline BL-2 (50 mg) was treated with acetic anhydride (1 ml) and dry pyridine (0.5 ml). The mixture was allowed to stand at room temperature (22-27°C) and then heated on water bath for 2 hours. The solid product obtained,
after usual work-up, was crystallized from chloroform-methanol as white needle-shaped crystals. BL-2(Ac), m.p. 125 °C.

\[
KBr \quad \text{IR } v_{\text{max}} \quad \text{cm}^{-1} \\
1735, 1725, 1720.
\]

**Acid hydrolysis of BL-2**

The glycoside BL-2 (100 mg) was dissolved in 80% methyl alcohol and 20 ml of 0.6N hydrochloric acid was added to it. The mixture was refluxed over a water bath for three hours. After leaving over night, the aglycone thus separated out was filtered, washed well with water and dried. The crude product was crystallized from methanol as white needles, m.p. 230-31 °C

Analysed for C_{31}H_{50}O_{5}

Calcd.: C, 74.10; H, 9.96

Found: C, 73.97; H, 9.94

**Chromatographic identification of sugar**

The acidic filtrate left after filtering the aglycone was extracted with ether to ensure the complete removal of any residual aglycone. The clear filtrate thus obtained was concentrated to a syrup in vacuum over KOH pellets. The concentration was continued till the syrup was neutral to litmus paper. The syrup was chromatographed on a Whatmann No.1 filter paper using butanol: acetic acid: water (4:1:5) and n-butanol: water: ethanol (60:25.8:16.5) as solvent mixtures, using descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05°C showed the presence of glucose only.

**BL-3**

The compound BL-3 was eluted from the column by petroleum ether-chloroform (3:1) mixture. It was crystallized from methanol (180 mg), m.p. 210-
1°C. Purified by preparative silica-gel TLC, Rf = 0.3 (n-hexan-petroleum ether, 3:1).

**UV λ<sub>max</sub> (MeOH) nm**

222, 275.

**KBr IR ν<sub>max</sub> cm<sup>-1</sup>**

3435, 2900, 2855, 1735, 1725, 1600, 1563, 1441, 1412, 1377, 1265, 1250, 1155, 935.

**<sup>1</sup>H-nmr (300 MHz, CDCl<sub>3</sub> + MeOD), values on δ-scale**

δ 8.43 (1H, br s, OCHO), 7.07 (2H, m, H-2'), 6.95 (2H, m, H-3', H-5'), 6.70 (1H, m, H-4'), 5.26 (1H, br s, H-22), 4.87 (1H, d, J=6.0 Hz, H-1'), 4.36 (1H, dd, J=4.5, 8.5 Hz, H-3β), 4.15 (1H, m, H-12), 4.05 (1H, d, J=6.0 Hz, H-6'a), 4.00 (1H, d, J=6.0 Hz, H-6'b), 3.96 (1H, m, H-5'), 3.53 (1H, m, H-4'), 3.40 (2H, m, H-2', H-3'), 2.71 (2H, m, H2-1), 2.30 (1H, m, H-5), 2.17 (1H, m, H-8), 2.13 (1H, m, H-22), 2.07 (2H, m, H2-7), 1.87 (2H, m, H2-15), 1.80 (3H, br s, Me-21), 1.67 (1H, m, H-17), 1.60 (1H, m, H-25), 1.50 (4H, m, H2-2, H2-16), 1.30 (3H, d, J=6.0 Hz, Me-11), 1.15 (8H, br s, H2-6, H2-9, H2-23, H2-24), 1.08 (3H, br s, Me-29), 1.02 (3H, br s, Me-19), 0.97 (3H, d, J=6.5 Hz, Me-26), 0.87 (3H, d, J=6.5 Hz, Me-27), 0.80 (3H, br s, Me-28), 0.76 (3H, br s, Me-30).

**FABMS m/z (rel. int.)**

742 [M]+ (2.8), 727 (3.7), 705 (11.0), 663 (16.3), 647 (13.1), 631 (3.1), 594 (15.4), 579 (2.2), 562 (2.2), 519 (2.9), 445 (100), 426 (3.3), 414 (3.9), 410 (2.2), 408 (2.6), 356 (4.5), 339 (5.0), 312 (8.2), 297 (9.3), 282 (10.7), 266 (12.0), 223 (9.8), 219 (5.6), 208 (12.3), 207 (12.2), 194 (105), 193 (9.3), 180 (14.4), 179 (18.2), 177 (17.1), 168 (10.0), 163 (28.2), 153 (12.5), 146 (29.7), 141 (11.4), 139 (20.4), 128 (36.3), 115 (62.1), 113 (3.7), 111 (3.7), 105 (65.9), 99 (5.4), 93 (21.1), 91 (99.1).

**Hydrolysis of BL-3**

The glycoside, BL-3 (80 mg) was dissolved in 80% methyl alcohol and 30 ml of 0.6N hydrochloric acid was added to it. After usual work-up the aglycone (30 mg) obtained was crystallized by petrol-chloroform mixture, m.p. 250-51 °C.
The sugar was identified as glucose by co-chromatography with an authentic sample of glucose.

Analysed for C$_{37}$H$_{56}$O$_5$

Calcd: C, 76.55; H, 9.65
Found: C, 76.52; H, 9.62

**BL-4**

Elution of the column with chloroform-methanol (8:2) gave BL-4 (750 mg). The compound was sparingly soluble in methyl alcohol and in dilute aq. Sodium hydroxide. It gave colourless needles m.p. 190-92 °C from acetone. With alcoholic ferric chloride it gave a deep brown colour.

Analysed for C$_{23}$H$_{20}$O$_5$(OMe)$_2$:

Calcd: C, 67.31; H, 5.36; OMe, 15.12.
Found: C, 67.19; H, 5.4; OMe, 16.1.

**UV $\lambda_{max}$ (EtOH) nm**

237, 275, 305, 335.

**mull**

**IR $v_{max}$ (cm$^{-1}$)**

1686, 1631, 1587, 1546, 1504 and 937.

**$^1$H-nmr (CDCl$_3$), values on $\delta$ scale**

1.75 (vinyl methyls), 3.79, 3.82 (s, 2 x OCH$_3$), 6.0, 6.46 and 6.86 (singlet for three aromatic protons), 2.8 (dd), 2.3 (dd) and 5.2 (t), 4.95 and 5.08 (H$_3$C-C=CH$_2$); 3.8 (d, J= 4.0 Hz), 4.18 (d, J= 12.0 Hz), 4.6 (dd, J= 12.0 and 4.0 Hz) and 4.9 (t, J= 4.0 Hz), 12.0 (s, OH).

**$^{13}$C-nmr data of BL-4, values on $\delta$ scale**

17 (C-8'), 30 (C-4'), 44 (C-12a), 56 (2 x OCH$_3$), 66 (C-6), 76 (C-5a), 88 (C-5"'), 92 (C-10), 101 (C-4), 101.4 (C-8), 104.3 (C-11a), 110 (C-1), 112(C-7'), 143 (C-6'), 144 (C-2), 147 (C-4a), 149.9 (C-3), 156(C-7a), 166 (C-9), 169(C-11), 194 (C12).
**Oxime**

A mixture of BL-4 (250 mg), hydroxyl amine hydrochloride (250 mg) and dry pyridine (2.5 ml) was heated on the water bath for twenty hours, cooled and poured on ice. After being kept for one hour, the resulting oxime was collected, washed, dried and crystallised from alcohol as colourless needles m.p. 245-46°C.

Analysed for $C_{23}H_{23}O_7N$

Calcd.: C, 64.94; H, 5.41; N, 3.29.

Found: C, 65.17; H, 5.46; N, 3.45.

**Dehydrosumatrol**

Iodine (400 mg), dissolved in a little alcohol, was added in the course of 5-10 minutes to a solution of sumatrol (BL-4) (300 mg) in boiling alcohol (50 ml), containing sodium acetate (1 gm), the mixture then boiled for two hours, after the addition of greater part of the iodine (about 360 mg) the solution retained a permanent brown colour. Next day the resulting crystalline iodo-derivative (150 mg) was collected, washed and dried, a further quantity of (300 mg) of crude iodo-compound was obtained when the alcoholic filtrate was concentrated and treated with water. The crystalline product gave a deep green ferric reaction and on being heated decomposed at about 200°C.

A mixture of iodo-derivative (150 mg), acetic acid (3 ml) and zinc dust (270 mg) was refluxed for two hours, after one and half hours more zinc (125 mg) was added. The hot solution was filtered and the zinc washed with boiling acetic acid (2 ml). On cooling the combined solution deposited the dehydro-compound (100 mg) which separated from chloroform-methyl alcohol in tiny, pale yellow prisms, m.p. 190-92°C. With alcoholic ferric chloride it gave a deep green colour.

Analysed for $C_{23}H_{20}O_7$

Calcd: C, 67.64; H, 4.90.

Found: C, 67.45; H, 4.88.
BL-5

Elution of the column with chloroform-methanol (3:1) mixture gave BL-5. It was crystallized from ethanol as pale yellow needles m.p. > 250°C.

Analysed for C_{28}H_{34}O_{15}
Calcd.: C, 55.08; H, 5.57
Found: C, 54.68; H, 5.49

UV data $\lambda_{\text{max}} \text{nm}$:

<table>
<thead>
<tr>
<th></th>
<th>MeOH</th>
<th>AlCl$_3$/HCl</th>
<th>NaOAc</th>
<th>NaOMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$</td>
<td>275, 326 sh</td>
<td>299, 370</td>
<td>272 sh, 309</td>
<td>274, 310</td>
</tr>
</tbody>
</table>

IR $v_{\text{max}} \text{ cm}^{-1}$

3450 (OH), 1680(C=O), 2950, 1500, 1365, 1205, 1140, 800.

Acetylation of BL-5

BL-5 (40 mg) was heated with pyridine (1.5 ml) and acetic anhydride (3 ml) on a water bath for 2 hours. After usual work-up the crude product was crystallized from chloroform-methanol as colourless needles, m.p. 85-86 °C.

$^1$H-nmr (CDCl$_3$) on δ-scale:

7.75 (2H, d, J= 9.0 Hz, H-2', 6'), 7.28 (1H, d, J = 2.5 Hz, H-8), 6.82 (1H, d, J=2.5 Hz, H-6), 5.20 (1H, q, J=5.0 Hz, 10.0 Hz, H-2), 3.20(2H, m, H-3,3), 2.01-2.48(30H, m, 9xOAc, C-Me), 2.95-3.20 (2H, q, J=12.0 Hz, 4.0 Hz, 17.0 Hz, H-3).

Mass m/z (rel. int.)

**Acid hydrolysis on BL-5**

BL-5 (100 mg) was hydrolysed by heating it with 0.2 N HCl over a water bath for 45 minutes. The solid obtained was crystallized with methanol as yellow silky needles, m. p. 245-246°C.

Analysed for C_{16}H_{14}O_{6}:

Calcd.: C, 63.57; H, 4.63

Found: C, 63.06, H, 4.90

**Acetylation of BL-5 (AgI)**

The aglycone (30mg) was acetylated by the method described earlier. The crude product on crystallization from ethanol gave white shining needles m.p. 135-36 °C.

Analysed for C_{24}H_{22}O_{10}:

Calcd.: C, 51.06; H, 4.68

Found: C, 51.26, H, 4.78

**Emulsin hydrolysis**

BL-5 (5 mg) was hydrolysed with emulsin prepared from almond at 30-40 °C for 70 hours. Liberation of galactose in the hydrolysate was confirmed by paper chromatography.

**Identification of sugars**

The neutral aq. hydrolysate on paper chromatographic examination as described earlier showed the presence of galactose and rhamnose.

**GLC of sugars**

The neutral aqueous layer obtained after the hydrolysis of the glycoside (BL-5) was extracted with ethyl acetate and evaporated to dryness. The aqueous residue (4mg) was dissolved in dry pyridine and trimethylsilyl ether, prepared by addition of hexamethyldisilazane (1 ml) and trimethyl chlorosilazane (0.5 ml). The mixture was separated on a column of 3% OV-I on silanized chromosorb W operated at 180 °C, helium flow rate at 35 ml/minute. The R₆(minute) observed
for investigated TMSi derivatives correspond to α- and β-rahmbose 0.21 and 0.28, α- and β-galactose 0.57 and 0.69. The observed Rt-values are in agreement with those of authentic samples of rhamnose and galactose.

**Methylation of the glycoside followed by acid hydrolysis**

The glycoside BL-5 (40 mg) in dimethyl formamide (1 ml) was treated with CH$_3$I (2 ml) and Ag$_2$O (30 mg) and kept for sixty hours at room temperature. The mixture was filtered and the residue washed with little dimethyl formamide. The filtrate was evaporated to dryness and the residue was treated with ethyl alcohol (25 ml). The syrup obtained after removal of ethyl alcohol was hydrolysed with 0.3 N HCl (4 hours). Work-up in the usual manner afforded 5'- (methyl-4', 5, 7-tri-O-methyleryodictoyl, 2, 3-di-O-methyl-L-rhamnose and 2, 3, 4, 6-tetra-O-methyl-D-galactose.

**Diphenyl methylenedioxy derivatives**

5'-methyleriodictoy (25 mg), dichlorodiphenyl methane (0.25 ml) were heated on a metal bath at 185°C for 5 minutes. The mixture in benzene was passed through small column of silica gel and the product crystallized from ethanol as straw coloured needles (15 mg), m.p. 254 °C.

Analysed for C$_{29}$H$_{24}$O$_6$:

Calcd.: C, 74.35; H, 5.12

Found: C, 74.48, H, 5.21

**Estimation of sugars**

The glycoside (BL-5) (30 mg) was hydrolysed by refluxing it with 2% H$_2$SO$_4$ for two hours. After cooling overnight, the aglycone was filtered and dried. The ratio of the aglycone to the glycoside was found to be 44.6% indicating the presence of 2 moles of sugar per mole of the aglycone. Somogyis copper-micro method gave the value (1.64 cc) which also corresponds to two moles of sugar per mole of the aglycone.
BL-6

It was obtained from methanol extract and crystallized from methanol as fine, colourless needles (5 gm), m.p. 237-38 °C.

**UV data** $\lambda_{\text{max}}$ nm:
- Ethanol 272
- aq. NaOH 309

**IR** $v_{\text{max}}$ cm$^{-1}$
- 3460 (bonded-OH), 1710 (C=O, open chain or 6-membered ring), 1613, 1520, 1460 (aromatic ring).

**Acetylation of BL-6**

Crystalline BL-6 (150 mg) was treated with acetic anhydride (2.0 ml) and dry pyridine (1.0 ml). The mixture was allowed to stand overnight at room temperature (22-27 °C) and then heated over water bath for two hours. The solid obtained, after usual work-up, was crystallized from methanol as needle shaped crystals BL-6(Ac) (125 mg), m.p. 207-08 °C.

**UV data** $\lambda_{\text{max}}$ nm:
- Ethanol 252

**IR** $v_{\text{max}}$ cm$^{-1}$
- 3500 (very weak), 1785, 1750, 1613, 1574, 1472.
3 Council of Scientific and Industrial Research, Delhi, ‘*The Wealth of India*’, Raw material, Vol I, P 179 (1948)
10 Jones and S Smith, *Ind. Eng. Chem. Analyt.*, **5**, 75 (1933)
CHAPTER 6
DISCUSSION
Chemical constituents of *Cassia siamea* (Fabaceae)

The family Fabaceae consists of about 482 genera and 1200 species of evergreen, trees, herbs, water plants and shrubs\(^1\). The genus *Cassia* comprises about 580 species distributed mostly in the tropical region of the World\(^2\). About 20 species found in India, find extensive use in folk medicines for the treatment of cheloid tumors, ringworms, insectbites and rheumatism\(^3\).

The earlier works lead to the isolation of 2', 3', 6-trihydroxy-4'-methoxy-7-O-neohesperidoside\(^4\). 2', 4', 5, 7-tetrahydroxy-8-C-glucosylisoflavone (2'-hydroxygenistin-8-C-glucoside\(^5\)).

The present discussion deals with the isolation and characterisation of the following compounds:

1) Bergapten.
2) Seselin.
3) 5-Methoxy-4'-hydroxy-6, 7-methylenedioxyisoflavone.
4) 5, 4'-Dihydroxy-6, 7-methylenedioxyisoflavone.
5) 5, 7, 4'-Trihydroxy-3', 6-dimethoxyisoflavone.

The leaves of *Cassia siamea* (2.0 kg) procured from A.M.U. campus were dried under shade. After defatting with petroleum-ether (40-60° C) they were refluxed exhaustively with benzene, ethylacetate, acetone and methanol respectively. The benzene and ethylacetate extracts responded positively with Shinoda's test\(^6\) for flavonoids and showed the same pattern of complex spots on TLC examination in benzene-ethylacetate (9:1, 7:3) solvent systems, hence they were mixed together. After removal of the solvents, a gummy greenish mass was obtained. The gummy mass was chromatographed over silica gel column, using different solvent systems, namely petrol, petrol-benzene (9:1-1:1), benzene, benzene-ethylacetate (9:1-1:1) respectively. The fractions obtained from petrol-benzene (1:1) and benzene showed two fluorescent spots on TLC in benzene-ethylacetate (9:1) system along with some impurities. Repeated column chromatography followed by fractional crystallization with chloroform-methanol gave two TLC homogenous substances labeled as CS-1 and CS-2. Benzene-ethylacetate eluates (9:1-7:3) were found to be a mixture of two closely related compounds, which were separated.
by repeated fractional crystallization with chloroform-methanol mixture and labeled as CS-3 and CS-4. The benzene-ethylacetate (1:1) eluate on repeated column chromatography followed by fractional crystallization gave one homogenous compound, labelled as CS-5.

**CS-1**

It was crystallized with chloroform-methanol as light yellow crystals (115 mg), m.p. 190-91°C. Elemental analysis along with the molecular ion peak at m/z 216 agreed with the molecular formula C_{12}H_{8}O_{4}. Its ir spectrum (Fig.1) showed strong absorption at 1733 cm^{-1} supporting the pyrone-carbonyl stretching frequency of a coumarin nucleus. It gave no colour with ferric chloride indicating the absence of phenolic hydroxyl group. It showed a yellow-green fluorescence under uv light which intensified by spraying the plate with 10% KOH. The fluorescence remains unchanged on treatment of the chromatogram with NH_{3} vapours. This supporting the presence of furano-coumarin which is further confirmed by a strong sharp band at 1623-1646 cm^{-1} for C=C stretching frequency of furan ring in addition to the aromatic bands at 1507-1607 cm^{-1} in its ir spectrum. The uv light showed four zones of absorption at 203-222, 240-250, 260-268 and 310-320 nm indicating the presence of furanocoumarin.

The {^1}H-nmr spectrum (Table-1, Fig-2) showed a pair of ortho-coupled doublet of one proton each at δ 6.26 (J=9.6 Hz) and δ 8.14 (J=9.6 Hz) ascribed to H-3 and H-4 protons of pyrone ring. Another pair of doublets of one proton each which arise from H-2 and H-3 protons of furan ring appeared at δ 7.59 (J=2.4 Hz) and 7.02 (J=2.4 Hz) respectively. The methoxyl group resonated as three protons singlet at δ 4.27. The remaining one proton singlet at δ 7.14 is attributed to H-5 or H-8 proton. On the basis of the above results the following two structures 1 and 2 are proposed.

![Diagram](image)

(1) Bergaptin  
(2) Xanthotoxin  
(8-alkoxypsoralen)
Xanthotoxine (2) was ruled out as CS-1 gave a yellow-green fluorescence under uv light and the same coloured fluorescence under uv light on exposing the chromatogram with NH₃ vapours while Bergapten (1) gives yellow coloured fluorescence under uv light which remains unchanged with NH₃. Furthermore, the upfield shift of H-8 at δ 7.14 as compared to H-5 at δ 7.3-7.4⁸ and characteristic maximum absorption at 268 and 310 nm in its uv spectrum⁹, suggesting it to be Bergapten (1).

The assigned structure was also supported by the mass spectrum (Fig-3) (Scheme-1) which showed the molecular ion peak at m/z 216 and the major fragment ions at m/z 201 (M-CH₃) and m/z 173 (201-CO) respectively.

In the light of above results, CS-1 was characterized as Bergapten (I)⁹.

\[ \text{OMe} \]
\[ \text{I} \]

Table-1

\[ ^1\text{H-nmr data of CS-1 (δ-scale)} \]

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-4</td>
<td>1</td>
<td>8.14 (d, J=9.6 Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.26 (d, J=9.6 Hz)</td>
</tr>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.59 (d, J=2.4 Hz)</td>
</tr>
<tr>
<td>H-3'</td>
<td>1</td>
<td>7.02 (d, J=2.4 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.14 (s)</td>
</tr>
<tr>
<td>OCH₃</td>
<td>3</td>
<td>4.27 (s)</td>
</tr>
</tbody>
</table>

s= singlet, d= doublet, spectrum run in CDCl₃ at 300 MHz using TMS as internal standard
Scheme I

\[
\begin{align*}
\text{C}_{12}\text{H}_{8}\text{O}_{4}^{+}, \text{m/z 216 (100\%)} & \quad \text{C}_{12}\text{H}_{10}\text{O}_{3}^{+}, \text{m/z 201 (34.1\%)} \\
& \quad \text{Scheme I}
\end{align*}
\]
CS-2

It was crystallized with chloroform-methanol as light yellow coloured crystals (130 mg), m.p. 119-20°C. It showed a blue coloured fluorescence under uv light responded negatively to ferric chloride and specific colour tests for flavonoids and alkaloids. Its elemental analysis and molecular ion peak at m/z 228 agreed with molecular formula C_{14}H_{12}O_{3}. The ir spectrum (Fig-4) showed the characteristic bands at 1720 cm\(^{-1}\) (C=O group), 3051-3416 cm\(^{-1}\) (C-H str.), 1633-1595 (C=C) and the uv spectrum showed the maximum absorptions at 280, 295 and 330nm. The ir spectrum along with uv spectrum supporting the presence of pyranocoumarin nucleus\(^8c\).

Its \(^1\)H-nmr spectrum (Fig-5) (Table-2) displayed the presence of three pairs of ortho-coupled doublets. One pair of ortho-coupled doublets of one proton each centered at \(\delta\) 6.25 (J=9.3 Hz) and 7.91 (J=9.3 Hz) were attributed to H-3 and H-4 protons while the other pair of ortho-coupled doublets consisting of one proton each at \(\delta\) 7.43 (J=8.4 Hz) and \(\delta\) 6.75 (J=8.4 Hz) were ascribed to H-5 and H-6 protons. The remaining pair of ortho-coupled doublets of one proton each at \(\delta\) 5.87 (J=10.0 Hz) and 6.68 (J=10.0 Hz) corresponded to H-3\(^{\prime}\) and H-4\(^{\prime}\) protons of pyran ring. A six protons singlet at \(\delta\) 1.14 was assigned to geminal dimethyl groups of pyran ring. The \(^1\)H-nmr spectrum suggesting the presence of angular pyran ring.

The assigned structure was further supported by the mass spectrum (Fig-6) (Scheme-2) which showed molecular ion peak at m/z 228 and the characteristic fragment ions at m/z 213 (M-CH\(_3\)), 184 (M-CO\(_2\)) and 202 (M-C\(_2\)H\(_2\)).

On the basis of above results, CS-2 was characterized as Seselin\(^{10a,b}\) (II).
Table-2

\(^1\text{H-nmr data of CS-2 (d-scale)}\)

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.25 (d, J=9.3 Hz)</td>
</tr>
<tr>
<td>H-4</td>
<td>1</td>
<td>7.91 (d, J=9.3 Hz)</td>
</tr>
<tr>
<td>H-5</td>
<td>1</td>
<td>7.43 (d, J=8.4 Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.75 (d, J=8.4 Hz)</td>
</tr>
<tr>
<td>H-3'</td>
<td>1</td>
<td>5.87 (d, J=10.0 Hz)</td>
</tr>
<tr>
<td>H-4'</td>
<td>1</td>
<td>6.68 (d, J=10.0 Hz)</td>
</tr>
<tr>
<td>Geminal methyl groups</td>
<td>6</td>
<td>1.41 (s)</td>
</tr>
</tbody>
</table>

s= singlet, d= doublet, spectrum run in DMSO-d\(_6\) at 300 MHz using TMS as internal standard.

**CS-3**

CS-3 was eluted from the column by benzene-ethylacetate (9:1) mixture. It was crystallized with chloroform-methanol as white crystals (225 mg), m.p. 258-60\(^\circ\)C. Elemental analysis along with the molecular ion peak at m/z 312 agreed with the molecular formula C\(_{17}\)H\(_{12}\)O\(_6\). It gave light green colour with FeCl\(_3\). On treatment with sodium amalgam followed by acidification it gave a pink colour pointing out the presence of isoflavone nucleus\(^{11}\) which was further confirmed by the uv spectrum which showed the maximum absorption at 264 nm and an inflection at 330 nm. Analysis with shift reagents indicated the absence of free 5-OH and 7-OH groups.

Its \(^1\text{H-nmr spectrum (Table-3) (Fig-7), showed a sharp singlet of one proton at }\delta 7.87\) for C-2 proton of \(\gamma\)-pyrone ring which further suggesting the isoflavone nucleus.

A singlet integrating for three protons at \(\delta 4.02\) was assigned to methoxyl group. B-ring protons displayed a typical A\(_2\)B\(_2\) pattern as it showed a pair of ortho-coupled doublets of two protons each at \(\delta 6.82\) (J=8.4 Hz) and \(\delta 7.31\) (J=8.4 Hz) due to 3',5'-protons and 2',6'-protons respectively. An independent singlet for two protons at \(\delta 6.11\) could be assigned to CH\(_2\)-protons of methylenedioxy group. A sharp singlet in the offset region at \(\delta 9.19\) was ascribed to hydroxyl group at 4'-position which was confirmed by
the fragment ion at \( m/z \) 118 and monoacetate (3b). The remaining one proton singlet at \( \delta 6.7 \) could be assigned to H-8 proton. Therefore, the methoxyl group is placed at 5-position.

The above assigned structure was further supported by the mass spectrum (Scheme-3) (Fig-8) which showed the molecular ion peak at \( m/z \) 312 (100%) and the RDA fragments were exhibited by the fragment ions at \( m/z \) 194 and \( m/z \) 118 respectively.

On the basis of above data, CS-3 was characterized as 5-Methoxy-4'-hydroxy-6, 7-methylenedioxyisoflavone\(^{12}\) (IIIa).

![Diagram of 5-Methoxy-4'-hydroxy-6, 7-methylenedioxyisoflavone](image)

\( a) \ R= H \\
\( b) \ R= Ac \)

Table-3

\(^1\)H-nmr data of CS-3 (\( \delta \)-scale)

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMe</td>
<td>3</td>
<td>4.02 (s)</td>
</tr>
<tr>
<td>CH(_2)</td>
<td>2</td>
<td>6.11 (s)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.70 (s)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>6.82 (d, ( J=8.4 ) Hz)</td>
</tr>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.31 (d, ( J=8.4 ) Hz)</td>
</tr>
<tr>
<td>H-2</td>
<td>1</td>
<td>7.87 (s)</td>
</tr>
<tr>
<td>4'-OH</td>
<td>1</td>
<td>9.19 (s)</td>
</tr>
</tbody>
</table>

s= singlet, \( d= \) doublet, spectrum run in CDCl\(_3\) at 300 MHz using TMS as internal standard.
Scan: 3  P1: 1: 3  M ions: 195  Base: 76.3%F  TIC=402974

[Chemical structure diagram]

MeO

O

HO

[Mass spectrum graph]
C_{17}H_{12}O_{6}  
[M^{+}], m/z 312 (100%)  
[M^{++}], m/z 156 (14.4%)  

\[ \text{Scheme - III} \]
CS-4

It was eluted from the column by benzene-ethylacetate (7:3) and crystallized from chloroform-methanol as cream coloured crystals (180 mg), m.p. 236-37°C. Elemental analysis and the molecular ion peak at m/z 298 (100%) agreed with the molecular formula C_{16}H_{16}O_{6}. It gave a negative Durham test and no colour with Mg/HCl\textsuperscript{6} which ruled out the possibility of flavanone or flavone nucleus for CS-4. On the other hand, treatment with sodium amalgam followed by acidification resulted in a pink colouration suggesting an isoflavone nucleus\textsuperscript{11}. Its uv spectrum showed a maximum absorption at 272 nm and an inflection at 340 nm. The colour reaction, uv spectral studies and a sharp singlet of one proton at δ 8.41 in its \textsuperscript{1}H-nmr spectrum suggesting it to be an isoflavone.

CS-4 gave a dark green colour with ferric chloride pointing out the presence of chelated hydroxyl group which was further supported by the presence of a band at 3300 cm\textsuperscript{-1} in its ir spectrum. A bathochromic shift of 12 nm with anhydrous AlCl\textsubscript{3} in its uv spectrum\textsuperscript{13a} and the presence of a sharp singlet at δ 12.91 in \textsuperscript{1}H-nmr spectrum further confirmed the presence of chelated 5-hydroxyl group.

The \textsuperscript{1}H-nmr spectrum (Fig-9, Table-4) revealed a sharp singlet at δ 8.41 indicating the presence of C-2 proton of γ-pyrone ring nucleus.

B-ring protons showed a typical A\textsubscript{2}B\textsubscript{2} pattern consisting of a pair of ortho-coupled doublet at δ 6.78 (J=8.4 Hz) and 7.35 (J=8.4 Hz) integrating for two protons each were assigned to 3', 5' and 2',6'-protons respectively. A sharp one proton singlet at δ 6.87 was ascribed to H-8. The remaining two proton singlet at δ 6.16 could only be attributed to CH\textsubscript{2} protons of methylenedioxy group\textsuperscript{13b}, while the hydroxyl groups appeared as two singlet at δ 12.91 (5-OH) and δ 9.63 (4'-OH). The above assigned structure was further confirmed by the mass spectrum (Fig-10, Scheme-4) which showed the molecular ion peak at m/z 298 (100%). The RDA fragments at m/z 180 (due to A-ring) and 118 (due to B-ring), further confirmed the presence of methylenedioxy group and the hydroxyl group at 4'-position. Acetylation with Ac\textsubscript{2}O/py afforded diacetate (IVb).

On the basis of above results, CS-4 was characterized as 5, 4'-dihydroxy-6, 7-methylenedioxyisoflavone (IVa)\textsuperscript{14}. 
a) $R=H$

b) $R=Ac$

Table 4

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-CH$_2$-O</td>
<td>2</td>
<td>6.16 (s)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>6.78 (d, J=8.4 Hz)</td>
</tr>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.35 (d, J=8.4 Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.87 (s)</td>
</tr>
<tr>
<td>H-2</td>
<td>1</td>
<td>8.41 (s)</td>
</tr>
<tr>
<td>OH-4'</td>
<td>1</td>
<td>9.63 (s)</td>
</tr>
<tr>
<td>OH-5</td>
<td>1</td>
<td>12.91 (s)</td>
</tr>
</tbody>
</table>

s= singlet, d= doublet, spectrum run in DMSO-d$_6$ at 300 MHz using TMS as internal standard.

CS-5

It was obtained by elution of the column with benzene-ethylacetate (1:1) mixture and crystallized with chloroform-methanol as cream coloured crystals (160 mg), m.p. 255-56°C. Elemental analysis agreed with the molecular formula C$_{17}$H$_{14}$O$_7$. The mass spectrum showed the molecular ion peak at m/z 330 in agreement with the molecular formula assigned to it. The isoflavone nucleus of CS-5 was indicated by the appearance of a pink colour on treatment with sodium amalgam/HCl, further supported by the UV absorption at 266 nm and an inflection at 330 nm. A red shift of 10 nm with AlCl$_3$ indicated the presence of chelated 5-hydroxyl group which was further confirmed by a band at 3400 cm$^{-1}$ in its IR spectrum (Fig. 11) and a signal at $\delta$ 13.05 in the $^1$H-nmr spectrum. Furthermore,
FIG-10
a bathochromic shift of 10 nm with NaOAc and $^1$H-nmr signal at $\delta$ 10.52 indicated the hydroxyl group at 7-position. Active hydrogen estimation showed the presence of three hydroxyl groups. This was confirmed by the formation of triacetate (Vb). Micro-Ziesel determination showed the presence of two methoxyl groups.

In $^1$H-nmr spectrum (Fig-12, Table-5), the isoflavone nucleus was evidenced by the presence of a sharp singlet at $\delta$ 8.31 for C-2 proton. The presence of two methoxyl groups was indicated by two independent singlets at $\delta$ 3.72 and 3.77. The remaining signals, excluding the three in the offset region for phenolic hydroxyl, at $\delta$ 6.47 could be assigned to either C-6 or C-8 proton. The methoxyl group was placed at 6-position on the evidence of mass spectrum discussed later, thus, assigning the singlet at $\delta$ 6.47 to C-8 proton. The B-ring protons showed a typical complex system consisting of an ortho-coupled doublet at $\delta$ 6.78 ($J=8.1$ Hz) for one proton, assigned to 5'-proton, a double doublet of one proton at $\delta$ 6.94 ($J_1=8.1$ Hz and $J_2=2.0$ Hz) ascribed to H-6' and a meta-coupled doublet at $\delta$ 7.10 ($J=2$ Hz) was attributed to 2'-proton. The oxygenation pattern for B-ring was further confirmed by alkaline degradation of CS-5 which furnished 4-hydroxy-3-methoxy benzoic acid, identified by m.p., m.m.p. and Co-chromatography with an authentic sample. Thus the fourth OH group is placed at C-4', further confirmed by the mass fragmentation.

The mass spectrum (scheme-5, Fig-13) showed the molecular ion peak at m/z 330 (100%) and the fragment ion corresponding the loss of methyl group at m/z 315 and is about 56.4%. This is extremely significant and provides the justification for putting the OMe at C-6 position. For 8-OMe, 5-OH flavonoids, the order are reversed and the predominant peak is that resulted from the loss of CH$_3$. The RDA fragments appeared at m/z 182 and m/z 148. Other fragments are shown in the Scheme-5.

On the basis of the above results, CS-5 has been characterized as 5, 7, 4'-trihydroxy-3', 6-dimethoxyisoflavone (Va).
Table 5

$^1$H-nmr data of CS-5 (δ-scale)

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMe</td>
<td>3</td>
<td>3.72 (s)</td>
</tr>
<tr>
<td>OMe</td>
<td>3</td>
<td>3.77 (s)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.47 (s)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>6.78 (d, J=8.1 Hz)</td>
</tr>
<tr>
<td>H-6'</td>
<td>1</td>
<td>6.94 (dd, J1=8.1 Hz and J2=2.0 Hz)</td>
</tr>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.10 (d, J=2.0 Hz)</td>
</tr>
<tr>
<td>H-2</td>
<td>1</td>
<td>8.31 (s)</td>
</tr>
<tr>
<td>4'-OH</td>
<td>1</td>
<td>9.30 (s)</td>
</tr>
<tr>
<td>7-OH</td>
<td>1</td>
<td>10.52 (s)</td>
</tr>
<tr>
<td>5-OH</td>
<td>1</td>
<td>13.05 (s)</td>
</tr>
</tbody>
</table>

$s=$ singlet, $d=$ doublet, $dd=$ double doublet, spectrum run in DMSO-d$_6$ at 300 MHz, using TMS as internal standard.
Scan: 5  File: 02-01-02  13B  Base= 40.3%F  TIC= 86712

FIG-13
EXPERIMENTAL
\textbf{CS-1}

It was crystallized with chloroform-methanol as light yellow crystals (115 mg) m.p. 190-91\degree C. It gave yellow-green fluorescence under uv light, which remain unchanged on exposing the TLC plate with vapours of NH\textsubscript{3} but the yellow-green florescence intensified when the chromatogram was sprayed with 10\% KOH.

Analysed for C\textsubscript{12}H\textsubscript{8}O\textsubscript{4}
Calcd:  C, 66.66; H, 3.70\%.
Found:  C, 66.64; H, 3.68\%.

\textbf{IR} \textit{v}\textsubscript{max} cm\textsuperscript{-1}

1733 (C=O), 1623-646, 1507-1607, 1471, 1360.

\textbf{UV data} \lambda\textsubscript{max} (MeOH) nm


\textbf{\textsuperscript{1}H-nmr} (300 MHz, CDCl\textsubscript{3}), values on \delta-scale

4.27 (3H, s, OCH\textsubscript{3}), 7.14 (1H, s, H-8), 7.02 (1H, d, J=2.4 Hz, H-3'), 7.59 (1H, d, J=2.4 Hz, H-2'), 6.26 (1H,d, J=9.6 Hz, H-3), 8.14 (1H, d, J=9.6 Hz, H-4).

\textbf{Mass m/z (rel. int.)}

216 (M\textsuperscript{+}, 100\%), 201 (M-CH\textsubscript{3}, 34.1), 173 (201-CO, 50.8).

\textbf{CS-2}

It was crystallized with chloroform-methanol as light yellow shining crystals (130 mg) m.p. 119-20\degree C. It gave blue fluorescence under uv light and no colour with FeCl\textsubscript{3}.

Analysed for C\textsubscript{14}H\textsubscript{12}O\textsubscript{3}
Calcd.:  C, 73.68; H, 5.26\%.
Found:  C, 73.65; H, 5.24\%.

\textbf{IR} \textit{v}\textsubscript{max} cm\textsuperscript{-1}

3416-3051 (C-H, str.), 1720 (C=O), 1595-1633 (C=C).

\textbf{UV data} \lambda\textsubscript{max} (MeOH) nm

280, 295, 330.
$^1$H-nmr (300 MHz, DMSO-d$_6$), values on δ-scale

6.25 (1H, d, J=9.3 Hz, H-3), 7.91 (1H, d, J=9.3, H-4), 7.43 (1H, d, J=8.4 Hz, H-5), 6.75 (1H, d, J=8.4 Hz, H-6), 5.87 (1H, d, J= 10.0 Hz, H-3'), 6.68 (1H, d, J=10.0 Hz, H-4'), 1.41 (6H, s, CH$_3$-C-CH$_3$).

Mass m/z (rel. int.)


CS-3

It was obtained by eluting the column with benzene-ethylacetate (9:1) and crystallized as white crystals with chloroform-methanol (225 mg), m.p. 258-60°C.

Analysed for C$_{17}$H$_{12}$O$_6$
Calcd.: C, 65.38; H, 3.84%.
Found: C, 65.36; H, 3.83%.

$^{KBr}$ IR $v_{max}$ cm$^{-1}$

3400 (OH), 1685 (C=O), 1450, 1250.

UV data $\lambda_{max}$ nm

MeOH 264,330 sh.
AlCl$_3$ 265, 329 sh.
AlCl$_3$/HCl 266, 329 sh.
NaOAc 264, 328 sh.
NaOAc/H$_3$BO$_3$ 265, 328 sh.
NaOMe 263, 338 sh.

$^1$H-nmr (300 MHz, CDCl$_3$ + DMSO-d$_6$), values on δ-scale

4.02 (3H, s, OMe), 6.11 (2H, s, O-CH$_2$-O), 6.70 (1H, s, H-8), 6.82 (2H, d, J=8.4 Hz, H-3', 5'), 7.31 (2H, d, J=8.4 Hz, H-2', 6'), 7.87 (1H, s, H-2), 9.91 (1H, s, 4'-OH).
Mass m/z


Acetylation of CS-3

Crystalline CS-3 (60 mg), acetic anhydride (1ml) and dry pyridine (0.5 ml) were heated over a water bath for three hours. After cooling, the mixture was poured on crushed ice and left over night. The solid obtained was collected, washed with water and dried. On several crystallization from aq. ethanol, it gave colourless needles, m.p. 187 °C.

Analysed for C₁₉H₁₄O₇
Calcd.: C, 64.40; H, 3.95
Found: C, 64.37; H, 3.91

^H-nmr (300 MHz, CDCl₃), values on δ–scale
2.33 (3H, s, OAc), 4.10 (3H, s, OMe), 6.15 (2H, s, O-CH₂-O), 6.74 (1H, s, H-8), 6.86 (2H, d, J=8.4 Hz, H-3', 5'), 7.35 (2H, d, J=8.4 Hz, H-2', 6'), 8.01 (1H, s, H-2).

CS-4

It was obtained by the elution of the column with benzene-ethylacetate (7:3) and crystallized with chloroform-methanol as cream coloured crystals (180 mg), m.p. 236-37 °C. CS-4 gave pink colour with Na/Hg-HCl, but no change in colour was observed when its ethanolic solution was treated with Mg/HCl. It gave dark greenish colour with FeCl₃.

Analysed for C₁₆H₁₀O₆
Calcd.: C, 64.42; H, 3.35%.
Found: C, 64.40; H, 3.33%.

IR  v_max  cm⁻¹
3300 (OH), 1680 (C=O), 1620, 1570, 1460, 1250, 1090.
**UV data** $\lambda_{\text{max}} \text{nm}$

<table>
<thead>
<tr>
<th>Substance</th>
<th>Wavelengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>261, 330 sh.</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>273, 308 sh, 375.</td>
</tr>
<tr>
<td>AlCl₃/HCl</td>
<td>273, 307 sh, 374.</td>
</tr>
<tr>
<td>NaOAc</td>
<td>261, 331 sh.</td>
</tr>
<tr>
<td>NaOAc/H₂BO₃</td>
<td>361, 329 sh.</td>
</tr>
<tr>
<td>NaOMe</td>
<td>267, 356 sh.</td>
</tr>
</tbody>
</table>

**$^1$H-nmr (300 MHz, DMSO-d₆), values on $\delta$–scale**

6.16 (2H, s, O-CH₂-O), 6.78 (2H, d, $J=8.4 \text{ Hz, H-3', 5'}$), 6.87 (1H, s, H-8), 7.35 (2H, d, $J=8.4 \text{ Hz, H-2', 6'}$), 8.41 (1H, s, H-2), 9.63 (1H, s, 4'-OH), 12.91 (1H, s, 5-OH).

**Mass m/z**


**Acetylation of CS-4**

Crystalline CS-4 (50 mg), acetic anhydride (1ml) and dry pyridine (0.5 ml) were heated over a water bath for three hours. After cooling, the mixture was poured on crushed ice and left over night. The solid was collected, washed with water and dried. On several crystallization from aq. ethanol, it gave colourless needles, m.p. 187 °C.

Analysed for C₂₀H₁₄O₈

Calcd.: C, 62.82; H, 3.66%.

Found: C, 64.78; H, 3.63%.

**$^1$H-nmr (300 MHz, CDCl₃) values on $\delta$–scale**

2.45 (3H, s, 5-OAc), 2.34 (3H, s, 4'-OAc), 6.44 (2H, s, O-CH₂-O), 6.80 (2H, d, $J=8.4 \text{ Hz, H-3', 5'}$), 6.90 (1H, s, H-8), 7.40 (2H, d, $J=8.4 \text{ Hz, H-2', 6'}$), 8.44 (1H, s, H-2).

**CS-5**

The compound CS-5 was obtained from the column by eluting it with benzene-ethylacetate (1:1) mixture and crystallized with chloroform-methanol as light cream crystals (160 mg), m.p. 255°C. It gave greenish-brown colour with FeCl₃.
Analysed for C\textsubscript{17}H\textsubscript{14}O\textsubscript{7}

Calcd.: C, 61.81; H, 4.24%.
Found: C, 61.86; H, 4.26%.

\textbf{IR} \textit{\nu}_{\text{max}} \text{ cm}^{-1}

3400 (OH), 1660 (C=O), 1620, 1510, 1460, 1370, 1190.

\textbf{UV data} \lambda_{\text{max}} \text{ nm}

MeOH 266, 330 sh.
AlCl\textsubscript{3} 276, 310 sh, 378.
AlCl\textsubscript{3}/HCl 277, 309 sh, 368.
NaOAc 276, 333.
NaOAc/H\textsubscript{3}BO\textsubscript{3} 269, 335 sh.
NaOMe 277, 329 sh.

\textbf{\textsuperscript{1}H-nmr} (300 MHz, DMSO-\textit{d}_6), \textit{values on} \delta–\textit{scale}

3.72 (3H, s, OMe), 3.77 (3H, s, OMe), 6.47 (1H, s, H-8), 6.78 (1H, d, J=8.1 Hz, H-5’), 6.94 (1H, dd, J\textsubscript{1}=8.1 Hz, J\textsubscript{2}=2.0 Hz, H-6’), 7.10 (1H, d, J=2.0 Hz, H-2’), 8.31 (1H, s, H-2), 9.30 (1H, s, 4’-OH), 10.52 (1H, s, 7-OH), 13.05 (1H, s, 5-OH).

\textbf{Mass} m/z (rel. int.)

330 [M\textsuperscript{+}] (100%), 331 [M+1] (22.00), 312 [M\textsuperscript{+}-H\textsubscript{2}O] (52.80), 315 [M\textsuperscript{+}-Me] (56.40), 300 [315-Me] (3.10), RDA fragments, 182 [A\textsubscript{1}\textsuperscript{+}] (1.06), 136 [A\textsubscript{1}-CO- H\textsubscript{2}O] (6.00), 148 [B\textsubscript{1}\textsuperscript{+}] (4.80), 133 [B\textsubscript{1}-Me] (7.60), 132 [133-H\textsuperscript{+}] (7.60).

\textbf{Acetylation of CS-5}

Crystalline CS-5 (50 mg), acetic anhydride (1ml) and dry pyridine (0.5 ml) were heated over a water bath for three hours. After usual work-up the solid obtained on several crystallization from ethanol, gave colourless needles, m.p. 135°C.

Analysed for C\textsubscript{23}H\textsubscript{20}O\textsubscript{10}

Calcd.: C, 60.52; H, 4.38%.
Found: C, 60.48; H, 4.35%.
**H-nmr (300 MHz, CDCl₃) values on δ-scale**

2.44 (3H, s, 5-OAc), 2.39 (3H, s, 7-OAc), 2.34 (3H, s, 4'-OAc), 3.74 (3H, s, OMe), 3.80 (3H, s, OMe), 6.50 (1H, s, H-8'), 6.81 (1H, d, J=8.1 Hz, H-5'), 6.99 (1H, dd, J₁=8.1 Hz, J₂=2.0 Hz, H-6'), 7.15 (1H, d, J=2.0 Hz, H-2'), 8.35 (1H, s, H-2).

**Oxidative degradation of the methyl ether with alkaline H₂O₂**

A solution of the methyl ether of CS-5 (25 mg) in acetone (50 ml) was treated with 5% alc. KOH aqueous (10 ml) followed by 30 % H₂O₂ (1 ml), the mixture was kept at 45 °C for two hours. Then it was cooled, poured into ice cold water (10 ml), acidified with cold HCl and extracted with ether. The ether solution was washed with water and then shaken with saturated NaHCO₃ solution, the bicarbonate fraction was acidified and extracted with ether. Evaporation of ether followed by micro-vacuum sublimation gave a colourless crystalline solid, m.p. 180-82 °C. It was identified as 3, 4-dimethoxybenzoic acid by m.p., m.m.p. and co-chromatography with an authentic sample.
REFERENCES


flavonoids. p. 165-171. b) Part-III. The structure analysis of flavonoids by $^1$H-nmr spectroscopy. Spectrum no. 82 & 83. c) 165-171


