CHAPTER-V

CARYOTA URENS
CHEMICAL CONSTITUENTS FROM THE BASE LEAVES OF CARYOTA URENS (PALMAE)

The genus Caryota comprises 15 species distributed in the tropical parts of India, Burma, Ceylon, Malaysia and Northern Australia. Out of these, three species are reported in India\(^1\) of which \textit{C. urens} is of economic importance.

\textit{Caryota} species have been reported for their medicinal properties such as internally nutritious and aphrodisiac and also laxative.\(^2\) Earlier investigations on this plant reported the isolation of Amino acids, sugars, ascorbic acid\(^3\), fatty acids, Kernel lipids\(^4\) and sugarsin.\(^5\)

Medicinal importance and scanty work on this plant accelerated our interest to carry out the comprehensive study of the plant \textit{Caryota urens}. The present discussion deals with the isolation and characterization of following compounds.

1. Triacontane
2. Lupeol
3. Myricadiol
4. \(\beta\)-sitosterol.
5. Tetracosonid
6. Ursolic acid
7. Sorbifolin 6-O-glucoside
8. 5,7-dihydroxy, 4'-O-methylflavone

The \textit{defatted} base leaves of \textit{Caryota urens} (2 Kg) procured from fort of A.M.U., Aligarh, India were extracted exhaustively with petroleum ether (60-80\(^0\)) and benzene. The base leaves were then extracted with methanol (3 liters x 3) at room temperature and finally on a steam bath.
The petroleum ether and benzene extracts of the base leaves were divided into neutral (I) and the acidic (ii) parts by treatment with alkali. Chromatographic resolution of the neutral part gave four products CY-1, CY-2, CY-3, CY-4. The chromatographic resolution of alkali soluble part (ii) over alummina column gave two compounds CY-5 and CY-6.

Both the hot and cold extracts of methanol showed almost same spots on TLC examination in different solvent systems and therefore were mixed together and concentrated under reduced pressure. The resultant mass was refluxed with petrol, benzene, chloroform, ethylacetate respectively and finally with acetone.

The ethylacetate and acetone concentrates on TLC examination in different solvent systems viz. TEF (5:4:1), BPF (36:9:5) and EtOAc: EtMeCO: AcOH: H₂O (2:3:1:1, 5:3:1) showed two compounds having same Rₖ values with varying concentrations. They were therefore, combined and subjected to column chromatography over silica gel column using benzene-ethylacetate mixture as eluting solvent in different ratios (9:1 to 1:1). The two compounds thus separated were purified by repeated column chromatography and marked as CY-7 and CY-8.
**Cn-1:**

Hexane yielded Cn-1 m.p. 62-67°C. It was found to be identical with triacontane on the basis of its elemental analysis (C30H62) and infrared spectrum, $\nu^\text{KBr}_{\text{max}}$ 2930 and 2860 cm$^{-1}$ (C-H, saturated), 1460 and 1380 cm$^{-1}$ (C-CH$_3$) and 720 cm$^{-1}$ (CH$_2$)$_n$. Finally, it was analysed by gas liquid chromatography which indicated Cn-1 to be a mixture of n-alkanes of series C24-C36, (Fig-I) containing mainly n-tri-triacontane (28.9%), n-nonacosane (21.2%), n-triacontane (2.9%) accompanied by hexacosane, pentacosane and pentatriacontane as minor constituents.

**Cn-2:**

Petroleum ether-benzene (1:4) solution from the neutral part afforded Cn-2, melting point 214-150°C, $[\alpha]^{20}_D + 23.64$ (CHCl$_3$). It gave positive Liebermann-Burchard and Noller's$^7$ tests and yellow colour with tetranitromethane. Elemental analysis agreed with the formula C$_{30}$H$_{50}$O. Infrared showed bands at 3360 and 1030 cm$^{-1}$ (OH), 1645 cm$^{-1}$ (C=C) and 1385 cm$^{-1}$ (geminal dimethyl), 885 cm$^{-1}$ (terminal methylene) (Fig-II). Mass spectrum of the triterpene alcohol gave M$^+$ at m/z 426 (11%) with other principal ions m/z 411 / (M-CH$_3$) (6%), 207 (34%), 189 (77%), and a base peak at m/z 95. It afforded an acetate m.p. 218-220°C. Infrared 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). $^1$H-NMR spectrum (Fig-III) gave the signals at $\delta$ 0.82, 0.87, 0.94, 1.04 (CH$_3$ protons), 1.27, 1.41, 1.46, 1.470 (CH$_2$ protons), $\delta$ 2.03 (OCOC$\text{H}_2$) and multiplets at $\delta$ 4.28 and 4.77 (>C CHOAc), $\delta$ 4.59, 4.67 (>C=CH$_2$). On the basis of the above physico-chemical data of the compound and its derivatives the Cn-2 was identified as lupeol$^8$ (I).
Elution of the column with benzene followed by crystallization from benzene-ethylacetate gave a colourless amorphous compound (CY-3), m.p. 259-60°C. It gave positive Liebermann-Burchard test showing it to be a triterpene which was confirmed by stannic chloride test. Its infrared spectrum (KBr) showed the bands at 3415 (OH), 2940 (unsaturation), 2880, 1470, 1450, 1390, 1380 (characteristic of triterpenic skeleton), 1080, 1030 (C-O stretching and O-H in plane deformation of secondary alcohol) and 815 cm⁻¹ (Fig-IV).

CY-3 on mass spectrometric analysis (Fig-V) showed a molecular ion peak at m/z 442 revealing its molecular formula to be C₃₀H₅₀O₂, further substantiated by elemental analysis. The other abundant ion peaks observed were 442 (M⁺-18 (H₂O)), 409 (M⁺-H₂O-CH₃), 339, 302, 287, 271, 245 (100), 220, 203, 202, 189.

The peaks at m/z 302 and 189 distinguished it to be having a Δ¹⁴ characteristic i.e. taraxerene skeleton. CY-3 on acetylation formed a diacetate, m.p. 245-47°C. Its mass spectrum (Fig-VI) showed the molecular ion peak at m/z 526 (C₃₄H₅₄O₂) confirming it to be a diacetate and hence in turn CY-3 to be a diol with one primary alcoholic group and one secondary alcoholic group (ir), other ion peaks observed at m/z 511 (M⁺-CH₃), 466 (M⁺-HOAc), 334(a), 329(b), 284 (a-HOAc), 269 (b-HOAc), 262 (c), 202 (c-HOAc) and 189 (100) (d); tallied with those of myricadiol diacetate⁹, can be explained as shown in the (scheme-I)
On the basis of m.p., acetate, ir and EIMS studies of C₃₋₃ and its diacetate,
the C₃₋₃ was identified as Myricadiol (II).
Cy-4:

Benzene and chloroform (1:1) eluate afforded another white crystalline CY-4 compound m.p. 159°C, [α]_D^{20} = 53.48° (CHCl_3). It gave positive Liebermann-Burchard test and responded to tetranitromethane colour test. IR spectrum (Fig-VII) showed the band at 3350 and 1050 cm^{-1} (OH) and at 1655 and 840 cm^{-1} (C=C). The ^1H-nmr spectrum (Fig-VIII) indicated signals at δ 0.7, 0.80, 0.88, 1.02 (CH_3 protons), 3.56 (3α-hydroxyl) and at δ 5.36 (1H, vinyl proton). Spectral data and elemental analysis (C_{29}H_{48}O) suggested it to be a β-sitosterol. Its acetate m.p. 126°C gave ir bands at 2990, 2880, 1740, 1680, 1470, 1390, 1262 and 970 cm^{-1}. Further derivatisation led to the preparation of benzoate, m.p. 144-45°C and 3,5-dintro benzoate m.p. 208-120°C.

For final confirmation gc-ms (Table-1) analysis were performed, using a 2.54 x 4 m. I.D. Glass column of 1% Dexil 300 G.C. on 100-120 Diatomic CQ at 260, flow rate 40 ml/min (helium carrier gas) connected through a silicone rubber membrane into an AEIMS-9 mass spectrometer (Fig-IX). This has been found to consist of the following four components.

Table -1

**GC data of sterols (TMS derivatives) on Dexsil 300 G.C.**

<table>
<thead>
<tr>
<th>Components</th>
<th>% sterol</th>
<th>RRT**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>0.4</td>
<td>0.60</td>
</tr>
<tr>
<td>Campesterol*</td>
<td>11.0</td>
<td>0.81</td>
</tr>
<tr>
<td>Stigmasterol*</td>
<td>41.5</td>
<td>0.85</td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>47.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Neither glc nor ms techniques are able to distinguish between sterol C_{24} epimers and these compounds may be either named compounds or its C_{24} epimers.

** Relative retention time (RRT) is expressed by the ratio of retention time for the substance under examination to the retention time of β-sitosterol.
The TMS ether of cholesterol, campesterol, stigmasterol and \( \beta \)-sitosterol gave molecular ions at m/z 458 (24\%), 472 (25\%), 484 (59\%) and at m/z 486 (30\%) respectively (Table-2). The characteristic peak at m/z 129 at \( \Delta^5 \) \( 3\beta \)-trimethyl silyloxy steroid for all sterols. The peak at 129 has been identified as the fragment originating from the cleavage of ring-A along with the TMS moiety.

Similarly, the other characteristic fragmentation from \( \Delta^5 \) \( 3\beta \)-trimethyl silyloxy steroid, as reported by Brook,\(^10\) was series of ions from M\(^+\)-129. These ions were also prominent at m/z 329 (100), 343 (100), 355 (36) and 357 (100) in the mass spectra of cholesterol, campesterol, stigmasterol and \( \beta \)-sitosterol trimethyl-silyloxy derivatives respectively.

The structural features which distinguish each of these sterols in the side chain of cholesterol contain C\(_8\)H\(_{17}\) chain, campesterol has a C\(_9\)H\(_{19}\) chain, stigmasterol has C\(_{10}\)H\(_{19}\) chain, due to the presence of double bond at carbon 22, \( \beta \)-sitosterol has a C\(_{10}\)H\(_{20}\) chain. The peaks at m/z 255, 275 were due to the loss of TMS and side chain moieties from the parent compounds of cholesterol and campesterol respectively.
Table 2

**Mass spectral data of sterols as their trimethylsilyl ether**

<table>
<thead>
<tr>
<th>Mole Formula</th>
<th>High mass species* m/z (A%)</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C27H45OsiMe3</td>
<td>459 (9.8), 458 (24) M^+; 443 (12) M'^-15, 369 (17), 368 (68)</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>M'^-90, 355 (9), 354 (11), 353 (36) M'^-90-15, 330 (20), 329</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(100) M'^-129, 328 (26), 274 (19), 255 (22) M'^-S.C., 247 (15).</td>
<td></td>
</tr>
<tr>
<td>C28H47OsiMe3</td>
<td>472 (25) M^+; 457 (12) M'^-15, 383 (18), 382 (64) M'^-90,</td>
<td>Campesterol</td>
</tr>
<tr>
<td></td>
<td>368 (11) 367 (34) M'^-90-15, 344 (26), 243 (100) M'^-129,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>342 (15), 389(5), 261(14), 255 (17) M'^-BC.S.C., 213 (13).</td>
<td>(or 24 epimer)</td>
</tr>
<tr>
<td>C29H47OsiMe3</td>
<td>485 (25), 484 (59) M^+; 469 (15) M'^-15, 395 (24) 394 (68)</td>
<td>Stigmasterol</td>
</tr>
<tr>
<td></td>
<td>351 (43), 255 (100) M'^-90-S.C., 215 (23), 213 (27).</td>
<td>(or 24 epimer)</td>
</tr>
<tr>
<td>C29H47OsiMe3</td>
<td>488 (11), 486 (30) M^+; 471 (13) M'^-15, 397 (22), 396 (73)</td>
<td>β-sitosterol</td>
</tr>
<tr>
<td></td>
<td>M'^-90, 382 (12) 381 (36) M'^-90-15, 358 (28), 357 (100),</td>
<td></td>
</tr>
</tbody>
</table>

*Only masses between m/z 650 and 205 are recorded as this is the most diagnostic region of the spectrum*
GC-MS  Fig.-IX
C\_\text{Y}-5:

The product \(C\_\text{Y}-5\), m.p. 87\(^{\circ}\)C showed \textit{ir} absorption \(u^\text{\textit{max}}\) at 2900, 1705, 1480, 1300, 940, 730 and 720 cm\(^{-1}\), thereby indicating it to be an aliphatic carboxylic acid. Elemental analysis showed the molecular formula to be \(C_{24}H_{48}O_{2}\), further confirmed by molecular ion peak at \(m/z\) 368. It gave methyl ester m.p. 58-59\(^{\circ}\)C. The compound was identified as \textit{tetracosnoic acid}\(^{11}(\text{III}).\)

\[C\_\text{Y}-6:\]

\(C\_\text{Y}-6\) on acetylation gave an acetate \((C\_\text{Y}-6\text{A})\) m.p. 263-64\(^{\circ}\)C. The \textit{ir} spectrum of the acetate showed absorptions at \(u^\text{\textit{nujol}}\) 1785, 1725, 1265 cm\(^{-1}\) characteristic of acetyl function.

The \(^1\text{H-nmr}\) spectrum of the compound revealed seven-methyl groups at \(\delta \ 0.8 \ (3\text{H}), \ \delta \ 0.92 \ (3\text{H}), \ \delta \ 1.0 \ (6\text{H})\) and \(\delta \ 1.1 \ (3\text{H})\) and one acetoxyl at \(\delta \ 2.1\). In addition there was a triplet centered at \(\delta \ 4.46\) for a proton \(\alpha\)- to the acetoxyl and a signal at \(\delta \ 5.2\) characteristic of the olefinic protons.

On methylation, \(C\_\text{Y}-6\text{A}\) gave an acetyl methyl ester m.p. 236-37\(^{\circ}\)C. Its \(^1\text{H-nmr}\) spectrum also showed methyl functions as signlets at \(\delta \ 0.78 \ (3\text{H}), \ 0.9 \ (6\text{H}), \ \delta \ 0.92 \ (3\text{H})\) and \(\delta \ 0.98 \ (3\text{H})\) and one acetoxyl function as a signlet at \(\delta \ 2.09\). A singlet at \(\delta \ 3.65\) showed the ester methoxyl function. The olefinic proton signal was at \(\delta \ 5.28\) and a triplet for the proton \(\alpha\)-to the acetoxyl at \(\delta \ 4.46\).

The acetate \((C\_\text{Y}-6\text{A})\) on deacetylation gave the \textit{genin} m.p. 268-70\(^{\circ}\)C. The above physical and spectral data of the genin and its derivatives showed that the compound is mono-hydroxy mono carboxylic acid. Its identify as \textit{Ursolic acid}\(^{(\text{III})}\) was established by comparing the \textit{ir} spectrum of its acetate with an authentic sample of ursolic acid acetate, which was super impossible.
C\textsubscript{Y}-7:

TLC examination of C\textsubscript{Y}-7 m.p. 261-63\textdegree{}C and its methyl ether m.p. 156-57\textdegree{}C indicated it to be acacetin.\textsuperscript{12} The \textsuperscript{1}H-nmr spectrum of C\textsubscript{Y}-7-acetate (Fig-X) m.p. 204\textdegree{}C showed two hydroxyl and one methoxyl groups. The uv spectrum of C\textsubscript{Y}-7 (Table-3) is comparable with the spectrum of acacetin and the \textsuperscript{1}H-nmr values of C\textsubscript{Y}-7A and acacetin diacetate are recorded in (Table-4).

**Table –3**

**UV Absorption spectra of C\textsubscript{Y}-7 and acacetin ($\lambda_{\text{max}}$, nm)**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>C\textsubscript{Y}-7</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>NaOMe</td>
<td>276, 295 sh, 383</td>
<td>275, 295 sh, 363</td>
</tr>
<tr>
<td>AlCl\textsubscript{3}</td>
<td>259 sh, 277, 292 sh,</td>
<td>260 sh, 277, 291 sh,</td>
</tr>
<tr>
<td></td>
<td>302, 344, 382</td>
<td>301, 344, 381</td>
</tr>
<tr>
<td>AlCl\textsubscript{3}/HCl</td>
<td>260 sh, 279, 294 sh,</td>
<td>260 sh, 280, 294 sh,</td>
</tr>
<tr>
<td></td>
<td>300, 338, 379</td>
<td>301, 337, 380</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>
</tbody>
</table>
Band II in methanol has a peak at 269 nm and a pronounced inflection at 327 nm. AlCl₃ produces a 55 nm shift of Band I showing thereby a free 5-hydroxyl group. Sodium acetate, shifts Band II 31 nm indicating the presence of a free 7-hydroxyl group. The sodium acetate-boric acid spectra of CY-7 showed a blue shift (9 nm) in Band I relative to apigenin (NaOAc/H₃BO₃) with a decrease in its relative intensity, showing thereby a protected 4'-hydroxy group.

UV, m.p., and ¹H-nmr spectra of CY-7A was found to be identical with that of acacetin diacetate (Table-4), CY-7 was, therefore, assigned the structure as 5,7-dihydroxy, 4'-O-methylflavone (IV).

![Chemical structure of CY-7](image)

(IV)

**Table-4**

<table>
<thead>
<tr>
<th>Assignment</th>
<th>CY-7A</th>
<th>Acacetin diacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-I-8</td>
<td>2.71 (1H, d, J=4 Hz)</td>
<td>3.45 (1H, d, J=3 Hz)</td>
</tr>
<tr>
<td>H-I-6</td>
<td>3.21 (1H, d, J=3 Hz)</td>
<td>3.80 (1H, d, J=2.5 Hz)</td>
</tr>
<tr>
<td>H-I-3</td>
<td>3.47 (IH, s)</td>
<td>3.63 (1H, s)</td>
</tr>
<tr>
<td>H-I-2', 6'</td>
<td>2.22 (2H, d, J=9 Hz)</td>
<td>2.20 (2H, d, J=9 Hz)</td>
</tr>
<tr>
<td>H-I-3', 5'</td>
<td>3.04 (2H, d, J=9 Hz)</td>
<td>3.05 (2H, d, J=9 Hz)</td>
</tr>
<tr>
<td>OMe/OAc:</td>
<td>3.04 (2H, d, J=9 Hz)</td>
<td>3.05 (2H, d, J=9 Hz)</td>
</tr>
<tr>
<td>-4'</td>
<td>(6.12) (3H, s)</td>
<td>(6.14) (3H, s)</td>
</tr>
<tr>
<td>-5</td>
<td>7.62 (3H, s)</td>
<td>7.57 (3H, s)</td>
</tr>
<tr>
<td>-7</td>
<td>7.68 (3H, s)</td>
<td>7.67 (3H, s)</td>
</tr>
</tbody>
</table>

s = singlet, d= doublet, spectrum run in CDCl₃ at 100 MHz, using TMS as internal standard = τ 10.00 Numbers in parentheses show chemical shifts of methoxy protons.
**Cγ-8:**

Cγ-8 was eluted from column with benzene-ethylacetate (2:8, 1:9) mixture. The glycosidic nature of the product (Cγ-8) was evidenced by the positive Molish test obtained after hydrolysis. The glycosidic nature was further supported by the $^1H$-nmr spectrum of the acetate of Cγ-8A (Table-5, Fig-XI) as it showed two aromatic acetoxyls at δ 2.46 (3H) and δ 2.27 (3H) and four alcoholic acetoxyls at δ 1.99 (9H, s, 3-OAc), δ 1.73 (3H, s, OAc) indicating it to be a glucoside or galactoside.

The glycoside gave pink colour with Zn/HCl and red colour on treatment with sodium amalgam followed by acidification indicating its flavone or flavanone nature. A yellow colour with Wilson boric acid reagent and λ_{max} at 269 and 333 nm in the uv spectrum indicated it to be a flavone glycoside. It gave a brownish green colour with FeCl₃ indicating the presence of hydroxyl group at C-5. The ir spectrum displayed strong bands at 3400 cm⁻¹ (OH) and 1700 cm⁻¹ (C=O). A red shift of 15 nm with AlCl₃ further confirmed the presence of a free 5-OH group. No shift with fused NaOAc, ruled out the possibility of a free hydroxyl at 7-position.

The $^1H$-nmr spectrum of Cγ-8A (Fig-XI), m.p. 100-11⁰C, showed a sharp singlet at δ 6.48 indicating the presence of a C-3 proton of γ-pyrone nucleus. The presence of one methoxyl group was indicated through a singlet at δ 3.99. The remaining singlet in the spectrum was at δ 6.78 and it integrates for one hydrogen and can be assigned to an aromatic proton shielded by two ortho and one para oxygen and was found to arise from the C-8 proton of 5,6,7-trioxygenated flavone. The aromatic region showed a pair of two ortho coupled doublets integrating for two protons each centered at δ 7.78 (J=9 Hz) and δ 7.15 (J=9 Hz), attributed to an
A$_2$B$_2$ pattern. The shifts were therefore assigned due to 2',6' and 3',5'-protons of ring-B respectively.

Cy-8 on hydrolysis with 10% HCl gave an aglycone m.p. 290-92°C (V-a). The sugar was identified as glucose by R$_f$-values, co-chromatography with an authentic sample and by the formation of osazone.

**Table -5**

<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>6.48 (s)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.78 (s)</td>
</tr>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.78 (d, J= 9Hz)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.15 (d, J=9Hz)</td>
</tr>
<tr>
<td>4 Aliphatic OAc of glucose moiety 2</td>
<td>12</td>
<td>1.73, 1.99 (s)</td>
</tr>
<tr>
<td>Aromatic OAc</td>
<td>3</td>
<td>2.27 (s)</td>
</tr>
<tr>
<td>Aromatic OAc</td>
<td>3</td>
<td>2.46 (s)</td>
</tr>
<tr>
<td>Aromatic OCH$_3$</td>
<td>3</td>
<td>3.99 (s)</td>
</tr>
</tbody>
</table>

s = singlet, d= doublet, spectrum run in CDCl$_3$, using TMS as internal standard (δ-scale).
Demethylation of the aglycone with hydroiodic acid gave a yellow product, m.p. >350\(^\circ\)C, elemental analysis indicated to the molecular formula C\(_{15}\)H\(_{10}\)O\(_6\). Acetylation of the compound with acetic anhydride and pyridine yielded a tetraacetate m.p. at 238-39\(^\circ\)C and showed no depression in melting point on admixture with an authentic sample of sceutellarein tetraacetate (V-b). The aglycone (V-a) was therefore characterized as sorbifolin by ferric reaction. \(R_f\) values, spectral and chromatographic comparison with an authentic sample.\(^{15}\)

\[
\begin{align*}
(V-a), & \quad R = H \\
(V-b), & \quad R = Ac
\end{align*}
\]

On the basis of the above colour reactions and examination of the products of hydrolysis, the glycoside was identified as flavone glucoside having sorbifolin as an aglycone.

The position of the sugar residue in the glucoside was confirmed by the hydrolysis of the methylated glucoside. The partial methyl ether thus obtained was characterized as 6-hydroxy 4',5,7-trimethoxyflavone (VI) (m.p. 221\(^\circ\)C) by m.p. m.m.p. with an authentic sample\(^{16}\) and \textbf{ultraviolet} spectral analysis with customary shift reagents.\(^{17}\)

\[
\begin{align*}
\text{(VI)}
\end{align*}
\]
The quantitative estimation of sugar by Somogyis Copper micro method\textsuperscript{18} showed the presence of one mole of glucose per mole of the aglycone.

\textbf{C\texttext{-8 was therefore characterized as Sorbifolin 6-glucoside (VII).}
EXPERIMENTAL
STUDY OF THE BASE LEAVES OF CARYOTA URENS

Well dried and crushed base leaves (2 kg) were extracted successively with petroleum ether (60-80°), benzene and methanol at room temperature and at their boiling points respectively. The petrol and benzene concentrates on TLC examination in petrol-ether as (4:1) solvent system showed at least six major spots, having the same Rf values. As the TLC behaviour of both the petroleum ether and benzene concentrates was the same, these two were combined (35 gm) together for further processing.

Separation into acidic and neutral part:

The dark green viscous mass (35 gm) was taken in ether, treated with aq. solution of potassium hydroxide (15%) and divided into alkali-soluble and alkali insoluble parts. The alkali insoluble part (25 gm) was refluxed with alcoholic potassium hydroxide (30 gm KOH dissolved in 600 ml of 80% ethanol) for half hour. Half of the solvent was then distilled off and the contents were diluted with water (2 liters) and extracted three times with ether. All the ether extracts were combined together and washed with water, till free of alkali. The ethereal layer was dried over anhydrous sodium sulphate. Sodium sulphate was filtered off and ether was recovered to give the neutral part. (≈ 15 gm).

The aqueous layer was acidified with hydrochloric acid and extracted with ether. The ether extract was washed with water and dried over anhydrous sodium sulphate. The ether was removed, a light yellow solid (≈ 5 gm) was obtained.

Both the hot and cold extracts of methanol showed almost same spots on TLC examination in different solvent systems and therefore were mixed together and concentrated under reduced pressure the resultant mass was refluxed with petrol, benzene, chloroform, ethylacetate respectively and finally with acetone.
The ethylacetate and acetone concentrates on TLC examination in solvent system viz. TEF (5:4:1), BPF (36:9:5) and EtOAc: EtMeCO: AcOH: H2O (2:3:1:1, 5:3:1) showed two compounds with varying concentration. They were combined and subjected to column chromatography over silica gel column using benzene-ethylacetate (9:1 to 1:1) yield two compounds CY-7 and CY-8 in different fractions. They were separated by repeated column chromatography.

Neutral part:

The neutral part (≈ 15 gm) was subjected to column chromatography over neutral alumina (1 Kg) and eluted with petrol, petrol-benzene and petrol-chloroform in different proportions and monitored by TLC. Following compounds CY-1, CY-2, CY-3 and CY-4 were isolated from different pools of identical fractions.

CY-1:

Elution of the column with petroleum ether (60-80°F) gave a dirty semi-solid mass. This was further purified by column chromatography over silica gel. On elution with hexane and repeated crystallization from carbon tetrachloride and acetone a colourless compound CY-1 m.p. 62-67°C was obtained. This showed an elongated spot on TLC (silica gel/AgNO₃, 5%) in petrol-benzene (4:1) solvent system. It was found to be a saturated hydrocarbon, \( \text{C}_{35} \) and the elemental analysis, compared with that of triacontane; \( \nu_{\text{max}} \) 2930, 2860 (C-H, saturated), 1460, 1380 (C-CH₃), 720 cm⁻¹ (CH₂)n.

Analysed for C₃₀H₆₂:

Calcd: C, 85.30; H, 14.69%

Found: C, 85.56; H, 14.58%
For final confirmation it was subjected to glc analysis which indicated this product to be a mixture of n-alkanes of the series C_{24}-C_{36} as given below (Table-6). Odd number homologues predominated as usual.

**Table-6**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>n-alkane</th>
<th>Composition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>n-Triacontane</td>
<td>36.3</td>
</tr>
<tr>
<td>2.</td>
<td>n-Tri-Triacontane</td>
<td>28.9</td>
</tr>
<tr>
<td>3.</td>
<td>n-Non-acosane</td>
<td>21.2</td>
</tr>
<tr>
<td>4.</td>
<td>n-Triacontane</td>
<td>2.9</td>
</tr>
<tr>
<td>5.</td>
<td>n-Heptacosane</td>
<td>2.0</td>
</tr>
<tr>
<td>6.</td>
<td>n-Hexatriacontane</td>
<td>2.0</td>
</tr>
<tr>
<td>7.</td>
<td>n-Octacosane</td>
<td>2.0</td>
</tr>
<tr>
<td>8.</td>
<td>n-Hexacosane</td>
<td>very small quantity</td>
</tr>
<tr>
<td>9.</td>
<td>n-Pentacosane</td>
<td>very small quantity</td>
</tr>
<tr>
<td>10.</td>
<td>n-Pentatriacontane</td>
<td>very small quantity</td>
</tr>
</tbody>
</table>
**C_Y-2:**

Further elution of the neutral part with petroleum ether-benzene (1:4) and purification by repeated crystallization from methanol-chloroform gave a crystalline solid (C_Y-2), m.p. 214-15°C, \([\alpha]_{D}^{20} + 23.64^0\) (CHCl_3). It gave positive Lieberman-Burchard and Noller's test and yellow colour with tetranitromethane.

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

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

C_Y-2 was confirmed as **Lupeol** by m.p. and mixed melting point with an authentic sample. Further confirmation of the identity of the compound was obtained by spectral studies and by its derivatisation.

**Acetylation of C_Y-2:**

The compound (50 mg) was treated with acetic anhydride (2 ml) and pyridine (0.2 ml), allowed to stand overnight at room temperature and then heated on a water bath for 6 hours. The solid product obtained, after usual work up, was crystallized from methanol-chloroform mixture as colourless flakes (60 mg), m.p. 218-20°C.

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

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

**^1H-NMR (CDCl_3) on \delta scale:**

0.82, 0.87, 0.94, 1.04, 1.27, 1.41, 1.46, 1.70, 2.03, (OCOCH_3), 2.28, 4.77 (CHOAc).
Analysed for C_{32}H_{52}O_{2}:

Calcd.: C, 82.05; H, 11.11%

Found: C, 82.14; H, 11.17%

\textbf{C_{y}-3:}

Elution of the column with benzene, followed by crystallization from benzene-ethylacetate gave a colourless amorphous powder, m.p. 259-60^\circ C. It gave a positive Stannic chloride test showing it to be a triterpene.

\textbf{IR, }\nu^{bBr}_{\text{max}} \text{ cm}^{-1}:

3415 (OH), 3060, 2940 (unsaturation), 2880, 1470, 1450, 1390, 1380 (characteristic of triterpenic skeleton), 1080, 1030 (C-O stretching and O-H in plane deformation of secondary alcohol), 815.

\textbf{Mass, }m/z:


\textbf{Acetylation of C_{y}-3:}

The compound (C_{y}-3), (100 mg) was treated with acetic anhydride (2.0 ml) and pyridine (1.0 ml) and left overnight at room temperature. After usual work up followed by crystallisation from ethanol colourless needles m.p. 245-47^\circ C, were obtained.

\textbf{Mass, }m/z:

M^+ 526, 511, (M^+-Me), 466 (M^+-HOAc), 344, 329, 284, 269, 262, 202, 189 (100%).
**CY-4:**

Elution of the column with benzene-chloroform (1:1) and purification of the product obtained from the column by repeated crystallization from methanol-chloroform afforded white crystalline solid (CY-4) m.p. 159-60°C, \([\alpha]^D_{20} = 53.48^\circ\). It gave positive Libermann-Burchard test and yellow colour with tetranitromethane.

Analysed for C_{29}H_{48}O:

Calcd:  C, 84.40; H, 11.72%

Found:  C, 84.46; H, 11.91%

\[ \text{IR, } \nu_{\text{max}}^{\text{cm}^{-1}}: \]

3350, 1050 (OH), 1655 (C=C), 840 (terminal methylene).

\[ \text{\textsuperscript{1}H-NMR, (CDCl}_3 \text{) on } \delta \text{ scale:} \]

0.70, 0.80, 0.88, 1.02 (CH\textsubscript{3} protons), 3.56 (3\alpha-hydroxyl), 5.36 (1H, vinyl proton).

**Acetylation of CY-4:**

The above product (100 mg) was acetylated by the usual method, using acetic anhydride (2 ml) and pyridine (0.4). The acetate was crystallized from methanol-chloroform mixture as colourless flakes m.p. 126°C.

\[ \text{IR, } \nu_{\text{max}}^{\text{cm}^{-1}}: \]

1740 (C=O), 1680 (C=C), 1262 (acetate), 970 (terminal).
**Benzoate:**

C\textsubscript{Y}-4, (50 mg) was dissolved in minimum amount of pyridine and benzoyl chloride (1 ml) was added to it. The mixture was heated over a boiling water bath for 8 hours, cooled to room temperature and poured into crushed ice with stirring. The solid obtained was washed with aqueous solution of potassium hydroxide (2%) followed by excess of water. The solid was crystallized from methanol, m.p. 144-45\textdegree{}C (35 mg).

**3,5-Dinitrobenzoate:**

C\textsubscript{Y}-4, (50 mg) was treated with freshly prepared 3,5-dinitrobenzoyl chloride (60 mg) and pyridine (0.5 ml) and heated over a water bath for 45 mints. After usual work up the crude derivative was crystallized from acetone and methanol m.p. 208-12\textdegree{}C.

Finally gc-ms analysis of the sterol (TMS derivative) indicated it to be a mixture of cholestrol (M\textsuperscript{+} 458), campesterol (M\textsuperscript{+} 472, m/z 457, 383, 382, 368, 344, 255, 213 etc.), stigmasterol (M\textsuperscript{+} 484, m/z 469, 395, 394, 379, 355, 354, 255, 215, 213 etc) and β-sitosterol (M\textsuperscript{+} 486, m/z 471, 397, 396, 382, 357, 275, 255, 213 etc.).

**Alkali soluble part:**

The yellow acidic part (≈ 5 gm) was dissolved in benzene-ether (8:1, v/v) and chromatographed over silica gel (300 gm). Mainly two products were obtained marked as C\textsubscript{Y}-5 & C\textsubscript{Y}-6.
**C_{5}-5:**

On elution with petroleum ether (60-80\(^{0}\)) \(\text{C}_{5}-5\), m.p. 87\(^{0}\)C was obtained. This appeared to be a saturated (negative tetranitromethane test) aliphatic acid \(\nu_{\text{max}}\) 2900, 1300, 940 (OH), 1705, 1480 and 730, 720 cm\(^{-1}\) (CH\(_2\)\(_n\)).

The methyl ester of \(\text{C}_{5}-5\) was prepared by treatment with absolute methanol. On crystallization with acetone, low melting (58-59\(^{0}\)C) crystals were obtained.

**C_{5}-6:**

Further elution of the column with ethylacetate, a colourless product was obtained. It was crystallized from chloroform-methanol as colourless shining needles, m.p. 284-88\(^{0}\)C. It appeared to be ursolic acid on the basis of its m.p., m.m.p. and co-TLC with an authentic sample. Its identity as **Ursolic acid** was further supported by derivitisation of the \(\text{C}_{5}-6\).

**Acetylation of \(\text{C}_{5}-6\):**

Acetate was prepared by usual method, m.p. 263-64\(^{0}\)C. It showed no depression in melting point when mixed with an authentic sample of ursolic acid acetate.

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

1785, 1725, 1265.

**\(^{1}\text{H-NMR (CDCl}_{3}\), on \(\delta\) scale:**

0.8 (3H, s), 0.9 (s), 0.92 (3H, s), 1.0 (6H, s), 1.1 (3H, s), 2.1 (s), 4.46 (t), 5.26 (m).
**Mass, m/z:**

\[ 498 (M^+), 438, 249, 203, 189. \]

**Acetyl methyl ester:**

The above compound was treated with diazomethane. After usual work up followed by crystallization from methanol gave colourless needles m.p. 236-37°C.

**\(^1\)H-NMR (CDCl\textsubscript{3}) on \( \delta \) scale:**

\[
0.78 (3 \text{ H, s}), 0.9 (6\text{ H, s}), 0.92 (3\text{ H, s}), 0.98 (3\text{ H, s}), 0.98 (s), 2.09 (s), 3.65 (s), 4.46 (s), 5.28 (m).
\]

**Mass, m/z:**

\[ 512 (M^+), 452, 262, 249, 203, 184. \]

**Cy-7:**

Cy-7 (60 mg) was refluxed with dimethyl sulphate (0.7 ml), anhydrous potassium carbonate (1 gm) in dry acetone (100 ml). After usual work up a methyl ether corresponding to trimethyl ether of apigenin (55 mg) m.p. 156-57°C was obtained.

**5,7-Diacetoxy, 4'-O-methylflavone (Cy-7A):**

Cy-8 (100 mg) was acetylated with pyridine and acetic anhydride. On crystallization from CHCl\textsubscript{3}-MeOH colourless needles m.p. 204°C (80 mg) were obtained.

**\(^1\)H-NMR (CDCl\textsubscript{3}) on \( \delta \) scale:**

\[
2.71 (1\text{ H, d, H-I-8}), 3.21 (1\text{ H, d, H-I-6}), 3.47 (1\text{ H, s, H-I-3}), 2.22 (2\text{ H, d, H-I-3',6'}), 3.04 (2\text{ H, d, H-I-3',5'}), 6.12 (3\text{ H, s, OMe-I-4'}), 7.62, 7.68 (s, 3\text{ H, each, OAc-5,7 respectively}).
\]
UV with shift reagents, \( \lambda_{max} \) nm:

- MeOH: 269, 303 sh, 327
- NaOMe: 276, 295 sh, 383
- AlCl\(_3\): 259 sh, 277, 292 sh, 302, 344, 382
- AlCl\(_3\)/HCl: 260 sh, 279, 294 sh, 300, 338, 379
- NaOAc: 276, 297 sh, 358
- NaOAc/H\(_3\)BO\(_3\): 269, 309 sh, 331

\( \text{C}_{22} \text{H}_{22} \text{O}_{11} \):

\( \text{C}_{22} \text{H}_{22} \text{O}_{11} \) was crystallized from ethylacetate-methanol as yellow needles, m.p. >310\(^\circ\)C.

Analysed for \( \text{C}_{22} \text{H}_{22} \text{O}_{11} \):

- Calcd.: C, 57.14; H, 4.76%
- Found: C, 57.20; H, 4.80%.

UV with shift reagents, \( \lambda_{max} \) nm:

- MeOH: 269, 333
- AlCl\(_3\): 280, 290, 348, 375 sh
- AlCl\(_3\)/HCl: 280, 291, 348, 375 sh
- NaOAc: 269, 375
- NaOAc/H\(_3\)BO\(_3\): 271, 334
- NaOMe: 370, 388
Acetylation of C_y-8:

A crystalline glycoside (35 mg), was heated with acetic anhydride (3 ml) and dry pyridine (1.5 ml) at 100°C for 3 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The separated solid was filtered, washed well with water and dried. On crystallization from dilute ethanol it gave colourless crystals (25 mg), m.p. 110-111°C.

$^1$H-NMR (CDCl$_3$) on δ scale:

$\delta$ 6.48 (1H, s, H-3), 6.78 (1H, s, H-8), 7.15 (2H, d, J=9 Hz, H-3',5'), 7.78 (2H, d, J=9 Hz, H-2',6'), 3.99 (3H, s, OCH$_3$-7), 2.27 (3H, OAc-4'), 2.46 (3H, OAc-5'), 1.99 (9H, 3 x OAc), 1.73 (3H, s, OAc).

Acid hydrolysis of C_y-8:

The glycoside (100 mg) was dissolved in 25 ml of 10% aqueous HCl-MeOH (1:1) and heated on a water bath. The hydrolysis appeared to be completed within 30 minutes. The heating was continued for two hours to ensure complete hydrolysis. After leaving overnight, the yellow aglycone thus separated out was filtered, washed well with water and dried. The crude product on crystallization from methanol gave yellow needles (70 mg), m.p. 290-92°C showed no depression on admixture with an authentic sample of sorbifolin.

Analysed for C$_{16}$H$_{12}$O$_6$:

Calcd.: C, 64.00; H, 4.00%

Found: C, 63.96; H, 3.98%.
UV with shift reagents, $\lambda_{\text{max}}$ nm:

- MeOH 253, 308
- AlCl$_3$ 263, 323
- NaOAc 254, 309

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

3260, 1655

Acetylation of Sorbifolin:

Sorbifolin (25 mg) was heated under reflux with acetic anhydride (2.5 ml) and fused sodium acetate (100 mg) on a water bath for two hours. After cooling, the mixture was poured over crushed ice and left overnight. The solid was collected, washed with water and dried, on crystallization from ethanol it gave colorless needles (18 mg), m.p. 238-39$^\circ$C.

Analysed for C$_{22}$H$_{18}$O$_9$:

Calcd.: C, 61.95; H, 4.26%

Found: C, 62.03; H, 4.30%.

$^1$H-NMR (CDCl$_3$) on $\delta$ scale:

7.90 (2H, d, J=9 Hz, H-2',6'), 7.28 (2H, d, J=9 Hz, H-3',5'), 6.60 (1H, s, H-8), 6.58 (1H, s, H-3), 3.80 (3H, s, OCH$_3$-7). 2.42 (3H, s, OAc-5), 2.35 (6H, s, OAc-4',6).

Methylation of Sorbifolin:

Sorbifolin (20 mg), dimethyl sulphate (0.5 ml), anhydrous potassium carbonate (1.0 gm) and acetone (100 ml) were refluxed for 24 hours. The reaction
mixture was filtered and the residue washed several times with hot acetone. On distilling off the solvent, a brown viscous semisolid mass was left behind. It was washed with hot petroleum ether to remove the excess of dimethyl sulphate. The solid residue on crystallization from ethylacetate-methanol gave colourless needles (10 mg), mp. 188-89°C.

Analysed for C_{19}H_{18}O_{6}:

Calcd.: C, 66.66; H, 5.26%

Found: C, 66.61; H, 5.23%.

**Chromatographic identification of sugar:**

The acidic filtrate, left after filtering the aglycone was extracted with ether and then with ethylacetate to ensure the complete removal of the aglycone. The solution was concentrated to a syrup in vacuum over NaOH pellets. The concentration was continued till the syrup was neutral to litmus paper. The syrup was chromatographed on Whatman No. 1 filter papers using butanol-acetic acid-water (4:1:5) and butanol-water-ethanol (60:28.5:16.5) as solvent systems, employing descending techniques. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying at room temperature were sprayed with aniline pthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05°C showed the presence of only glucose.

**Estimation of sugar:**

The anhydrous glycoside (20.5 mg) was hydrolysed by refluxing for two hours with 2% H_{2}SO_{4}. After cooling overnight, the aglycone was filtered, washed, dried and weighed (13.2 mg). Thus the ratio to the glycoside is 64.3% and this ratio indicates the presence of one mole of sugar per mole of the aglycone.
The quantitative estimation of sugar by Somogyis copper micro method gave the value (0.44 ml) which corresponds to 1 mole of sugar / mole of aglycone.

6-Hydroxy-4',5,7-trimethoxyflavone:

Glycoside (30 mg) was dissolved in dry acetone and refluxed with an excess of dimethyl sulphate (1.2 ml) and fused potassium carbonate (3 gm) for 36 hours on a water bath. The mixture was filtered and the residue was washed with hot acetone. After distilling off the solvent from the filtrate brown residue was left behind. The excess of dimethyl sulphate was removed by washing the methylated product several times with hot petroleum ether. It was hydrolysed by heating with 7% H$_2$SO$_4$ for two hours. The reaction mixture was left overnight, a faintly yellowish powder separated out. It was filtered washed with water and dried. On several crystallization from methanol it gave straw needles (16 mg), m.p. 221$^\circ$C.

Analysed for C$_{18}$H$_{16}$O$_6$:

Calcd.: C, 65.85; H, 4.87%

Found: C, 65.81; H, 4.82%.
REFERENCE

