CHAPTER 7

CHEMICAL EXAMINATION OF THE HEARTWOOD OF SAPIUM EUGNIFOLIUM
**Sapium eugnifolium** Ham. is called 'Korha' in 'Hindi' and belongs to the family 'Euphorbiaceae'. It is a middle sized tree attaining a height of 50 feet and 60 inches in girth. The bark is greyish in colour, fairly smooth with horizontal wrinkles, 0.25 inches thick, inside light brown, fibrous and mottled. The wood is white in colour. The leaves are 2-6.5 inches by 1-2.5 inches, ovate or elliptic, acute or subacute rather subcoriaceous, entire glabrous and often glaucous beneath. The lateral neves are 6-10 on either half and the base is rounded. Petiole is 1-4 inches long and is biglandular at the apex. The flowers are recemes dense and 3-4 inches long. The capsules are subglobose, 0.4-0.5 inches, across and woody. The seeds are globose.

It is distributed in the Himalayas from Kumaun to Sikkim and in Assam and Manipur, ascending to an altitude of 4000 feet.

A survey of the available literature on the heartwood of **S. eugnifolium** showed that no work is reported so far. Although, the bark of this plant showed the presence of taraxerone, taraxerol, moretenone, β-sitosterol, 3,3'-di-O-methyl ellagic acid\(^{107,108}\) and 16,22-dihydroxy-methyl-hopanoate\(^{109}\).

The author has examined the heartwood of **S. eugnifolium** and he has been able to isolate and characterize three known compounds. One of the known compounds is reported for the first time in this plant.
ISOLATION OF THE CONSTITUENTS FROM THE HEARTWOOD OF SAPIUM EUGNIFOLIUM

The air dried and powdered heartwood of *S. eugnifolium* (3 kg) was exhaustively extracted with ethanol under reflux for 30 days. The total ethanolic extract (20 litres) was filtered while hot and the filtrate was concentrated under reduced pressure to get a gummy mass which was extracted with petroleum ether and benzene respectively.

The petroleum ether extract (1.5 litres) on thin layer chromatography examination showed the presence of single entity which was concentrated by distilling off the excess of solvent under reduced pressure on a water bath. The product was passed through a column of neutral alumina and eluted with hexane:petroleum ether (8:2 v/v) mixture. The eluate (1.2 litres) was concentrated under reduced pressure on a water bath to afford a colourless substance which was crystallized as colourless needles from chloroform (yield 350 mg), m.p. 134-136° and designated as compound-A.

The benzene extract (2 litres) on thin layer chromatography examination showed the presence of a mixture of two substances. The benzene extract was concentrated by distilling off the solvent under reduced pressure on a water bath and chromatographed over the column of neutral alumina. The column was eluted successively with petroleum ether : benzene (2:8 v/v) mixture (Fraction-I) and benzene:chloroform (5:5 v/v) mixture (Fraction-II).

The fraction-I (800 ml) on thin layer chromatography examination showed the presence of single entity. This fraction was concentrated on a water bath under reduced pressure to a small volume
and kept in a refrigerator for few days which gave a colourless amorphous substance. It was crystallized as colourless needle shaped crystals from methanol, m.p. 198-199° (yield 300 mg) and designated as compound-B.

The fraction-II on thin layer chromatography examination showed the presence of single entity. This fraction was concentrated on a water bath under reduced pressure to a small volume and kept in a refrigerator for few days which gave a colourless substance. It was crystallized as colourless needles from ether : methanol (5:5 v/v) mixture, m.p. 201-202° (yield 520 mg) and designated as compound-C.

The structural studies of the compounds-A, B and C have been described in the Sections - I, II and III respectively of Chapter-7.
STRUCTURAL STUDY OF THE COMPOUND-A

The compound-A, \( \text{C}_{29}\text{H}_{50}^0 \) \((\text{M}^+ \text{ at m/e 414}), \text{m.p. } 134-136^0, \quad (\alpha)_D^{25} - 32^0 \) (in chloroform) gave all positive colour reactions \((1-9)^{61-69}\) as described on page 156 characteristic for a sterol.

From the molecular formula and colour reactions, it is evident that the compound-A is a steroid. It also gave a positive test with tetranitromethane\(^{67}\) showing the presence of an olefinic bond in the compound-A.

Since the compound-A formed a monoacetate, \( \text{C}_{31}\text{H}_{52}^0\text{O}_2 \), m.p. 126-127\(^0\), \((\alpha)_D^{25} - 42^0 \) (in chloroform) and a monobenzoate, \( \text{C}_{36}\text{H}_{54}^0\text{O}_2 \), m.p. 142-143\(^0\), \((\alpha)_D^{25} - 15^0 \) (in chloroform), the oxygen function of the compound-A was present as an alcohol.

The infra-red spectrum (KBr) of the compound-A was found to be similar to the compound-A as given in the Section-I of Chapter-6, which were indicative of \(\beta\)-sitosterol.

The compound-A was proved to be \(\beta\)-sitosterol by its co-chromatographic behaviour and mixed melting point with an authentic sample of \(\beta\)-sitosterol\(^{70}\). It was further confirmed by completely superimposable infra-red spectra of the compound-A with an authentic sample of \(\beta\)-sitosterol. Thus the compound-A can be represented as follows.
COMPOUND - A

The above proposed structure for the compound-A as  
β-sitosterol was further supported by its mass spectrum. The mass  
spectrum of the compound-A showed the fragments at m/e 414 (M⁺), 399,  
396, 381, 329, 303, 275, 273 and 255.
EXPERIMENTAL

The isolation, purification and crystallization of the compound-A has already been described on page 184. The compound-A was found to be soluble in benzene, chloroform, methanol, ethanol, pyridine and dioxan but insoluble in water.

COLOUR REACTIONS OF THE COMPOUND-A

The compound-A gave all the colour reactions (1-9)\textsuperscript{67-69} as described on page 156 characteristic for a sterol.

THIN LAYER CHROMATOGRAPHY EXAMINATION OF THE COMPOUND-A

Thin layer chromatography of the compound-A was done on silica gel 'G' plates in the following solvent systems as described on page 159. It showed the presence of single spot in each case.

<table>
<thead>
<tr>
<th>Solvent systems</th>
<th>( R_f ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether:benzene; 7:3 v/v</td>
<td>0.78</td>
</tr>
<tr>
<td>Benzene : chloroform; 8:2 v/v</td>
<td>0.95</td>
</tr>
</tbody>
</table>

ANALYTICAL AND SPECTRAL DATA OF THE COMPOUND-A

Found : C, 84.02; H, 12.02; \( \text{C}_{29}\text{H}_{50}\text{O} \) required: C, 84.05; H, 12.07%.

IR : \( \text{KBr} \quad \text{max} \quad 3400 \text{ (OH)}, 2952, 2875, 1660, 1465, 1390, 1300, 1265, 1066, 1020, 950, 832 \text{ and } 800 \text{ cm}^{-1} \) respectively.

MS at m/e : 474 (M\(^+\)), 399, 396, 381, 329, 303, 275, 273 and 255.

ACETYLATION OF THE COMPOUND-A

The compound-A (100 mg) was acetylated with acetic anhydride (5 ml) and pyridine (5 ml) by the method as described on page 160. The acetylated product was crystallized from benzene : chloroform (5:5
v/v) mixture, m.p. 126-127° (lit. m.p. 127°)\(^7\) .

Found : C, 81.46; H, 11.35; \(\text{C}_3\text{H}_6\text{O}_2\) required: C, 81.58; H, 11.40%.

**BENZOYLATION OF THE COMPOUND-A**

The compound-A (100 mg) was benzoylated with benzoyl chloride (6 ml) and pyridine (5 ml) in the usual manner as described on page 160. The benzoylated product was crystallized from ethanol, m.p. 142-143° (lit. m.p. 143-144°)\(^7\) .

Found : C, 83.40, H, 10.35; \(\text{C}_{36}\text{H}_{54}\text{O}_2\) required: C, 83.39; H, 10.42%.
SECTION - II

STRUCTURAL STUDY OF THE COMPOUND-B

The compound-B, C_{30}H_{50}O (M^+ at m/e 426), m.p. 198-199°,
(α)_{D}^{25} + 16° (in chloroform) gave positive colour reactions (1-7)\textsuperscript{61-67}
as described on page 156 characteristic for a triterpene. In addition
to these tests, the compound-B gave a reddish-violet colouration in
Brieskorne reaction\textsuperscript{68} as described on page 157 and no precipitate
with digitonin\textsuperscript{69}. From the above tests it was clear that compound-B
is a triterpenoid.

The compound-B formed a monoacetate, C_{32}H_{52}O_{2}, m.p. 161-162°,
(α)_{D}^{25} - 3.2° (in chloroform) and a monobenzoate, C_{37}H_{54}O_{2},
m.p. 224-225°, (α)_{D}^{25} + 70° (in chloroform) indicating the presence of
one hydroxyl group in it. The compound-B on oxidation gave a positive
Zimmerman test\textsuperscript{72,73} for 3-keto group. Thus the hydroxyl group was
present at position C-3 in the compound-B.

The compound-B has been characterized as epi-lupeol by its
cocromatography and mixed melting point with an authentic sample of
epi-lupeol. The identity of the compound-B was further confirmed by
superimposable IR spectra of the compound-B with an authentic sample\textsuperscript{110}. This known compound-B was reported first time from this plant.

Thus the compound-B can be assigned the following structure.

\[
\text{COMPOUND - B}
\]
EXPERIMENTAL

The isolation, purification and crystallization of the compound-B has already been described on page 184. The compound-B was found to be soluble in benzene, chloroform, ethyl acetate, acetone, methanol and dioxan but insoluble in water.

THIN LAYER CHROMATOGRAPHY EXAMINATION OF THE COMPOUND-B

Thin layer chromatography was done on silica gel 'G' plates using the following solvent systems as described on page 159. The iodine was used as a developer to detect the spot. A single spot was observed in each case.

Solvent systems
1. Petroleum ether:benzene; 5:5 v/v
2. Benzene : chloroform; 7:3 v/v

Rf values
0.52
0.64

ANALYTICAL AND SPECTRAL DATA OF THE COMPOUND-B

Found: C, 84.46; H, 11.70; C_{30}H_{50}O required: C, 84.50; H, 11.73%.

IR: $\nu_{\text{KBr}}^{\text{max}}$ 3450 (br, OH), 2965, 1630, 1450, 1380, 1100, 1045, 1020, 950 and 885 cm$^{-1}$.

MS at m/e: 426 (M$^+$), 411, 408, 393, 383, 220, 218, 207, 191, 189 and 187.

ACETYLATION OF THE COMPOUND-B

The compound-B (100 mg) was acetylated with acetic anhydride (6 ml) and pyridine (6 ml) in a 100 ml round bottomed jointed flask fitted with a water condenser by the method as described on page 160. The product was crystallized with methanol as colourless needles, m.p. 161-162$^0$ (lit. m.p. 161$^0$)\textsuperscript{111}, $\alpha$\textsubscript{D}$^{25}$ - 3.2$^0$ (in chloroform).

Found: C, 82.00; H, 11.10; C_{32}H_{52}O\textsubscript{2} required: C, 82.05; H, 11.11%.
BENZOYLATION OF THE COMPOUND-B

The compound-B (100 mg) was benzoylated with benzoyl chloride (5 ml) and pyridine (5 ml) in a 100 ml round bottomed jointed flask fitted with a water condenser and refluxed on a water bath using the same method as described on page 160. The benzoylated product was crystallized with ether as colourless crystals, m.p. 224-225° (lit. m.p. 225-226°) \textsuperscript{112}, \((\alpha)_D^{25} + 70^\circ\) (in chloroform).

Found : C, 83.72; H, 10.16; \(\text{C}_3\text{H}_5\text{O}_2\) required: C, 83.77; H, 10.18%. 
SECTION - III

STRUCTURAL STUDY OF THE COMPOUND-C

The compound-C, C_{30}H_{48}O (M^+ at m/e 424), m.p. 201-202°, \((\alpha)_{D}^{25} + 50^0\) (in chloroform) gave positive colour reactions \((1-7)^{61-67}\) as described on page 156 characteristic for a triterpene. In addition to these tests the compound-C gave a positive Brieskorne reaction\(^68\) and no precipitate with digitonin\(^69\) showing the triterpenoidal nature of the compound-C.

From the molecular formula and colour reactions it is evident that the compound-C is a triterpene. It also gave a positive Zimmesman test\(^72,73\) for 3-keto group in the ring-A of the compound-C.

The infra-red spectrum (KBr) of the compound-C showed the absorptions at 2925, 2852, 1702 (carbonyl), 1653, 1452, 1382 and 1370 cm\(^{-1}\).

The compound-C on treatment with lithium aluminium hydride yielded a known product, moretenol, m.p. 228-229° (lit. m.p. 228-230°, mixed melting point and co-thin layer chromatography)\(^113-115\). Moretenol on further catalytic hydrogenation afforded hopan-3β-ol, m.p. 236-237°, \((\alpha)_{D}^{25} + 36^0\) (in chloroform) [lit. m.p. 236-238°, lit. \((\alpha)_{D}^{25} + 37.4^0\) (in chloroform)]\(^113\) which clearly indicated that the compound-C was moretenone (lit. m.p. 200-202°)\(^109\).

The compound-C was proved to be moretenone by its co-chromatographic behaviour and mixed melting point with an authentic sample of moretenone\(^109\).
Thus the compound-C can be represented as follows:

![Compound-C](image)

**COMPOUND - C**

The above proposed structure for the compound-C as moretenone was further supported by its mass spectrum. The mass spectrum of the compound-C showed the fragment ions at 424 (M⁺), 409, 406, 391, 222, 205 and 189.
EXPERIMENTAL

The isolation, purification and crystallization of the compound-C has already been described on page 184. The compound-C was found to be soluble in benzene, chloroform, ethyl acetate, methanol, ethanol, pyridine and dioxan but insoluble in water.

COLOUR REACTIONS OF THE COMPOUND-C

The compound-C gave all the positive colour reactions (1-7)61-67 as described on page 156 characteristic for a triterpene.

THIN LAYER CHROMATOGRAPHY EXAMINATION OF THE COMPOUND-C

Thin layer chromatography of the compound-C was done on silica gel 'G' plates in the following solvent systems as described on page 159 which showed single spot in each case.

<table>
<thead>
<tr>
<th>Solvent Systems</th>
<th>Rf Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Petroleum ether:benzene; 5:5 v/v</td>
<td>0.62</td>
</tr>
<tr>
<td>2. Benzene : chloroform; 4:6 v/v</td>
<td>0.92</td>
</tr>
</tbody>
</table>

ANALYTICAL AND SPECTRAL DATA OF THE COMPOUND-C

Found : C, 84.74; H, 11.28; C_{30}H_{48}O required: C, 84.90; H, 11.32%.

IR : $\nu_{\text{KBr}}$ max : 2925, 2852, 1702, 1653, 1452, 1382 and 1370 cm$^{-1}$.

MS at m/e : 424, 409, 406, 391, 222, 205 and 189.

LITHIUM ALUMINIUM HYDRIDE REDUCTION OF THE COMPOUND-C

A solution of the compound-C (400 mg) in ether (20 ml) was added dropwise with stirring to a solution of lithium aluminium hydride (3.5 g) in ether (20 ml) and the reaction mixture was stirred
for 3 hours which afforded moretenol (mixed melting point and co-thin layer chromatography).

**CATALYTIC HYDROGENATION OF MORETENOL**

A solution of moretenol (100 mg) in ethanol (40 ml) was shaken with hydrogen in the presence of PtO₂ (300 mg) for 4 hours at room temperature. The solution was filtered and evaporated. The residue on crystallization from acetone gave colourless needles of hopan-3β-ol (mixed melting point and co-thin layer chromatography).

Found : C, 84.09; H, 12.10; C₃₀H₅₂O required: C, 84.11; H, 12.14%.