CHAPTER - 4

CHEMICAL INVESTIGATION OF THE STEM BARK OF

AEGLE MARMELOS CORR.
The description, distribution, medicinal importance and the compounds already reported in different parts of *Aegle marmelos* has already been described on pages 76, 77 of the Chapter - III.

**ISOLATION OF THE CONSTITUENTS FROM THE STEM BARK OF AEGLE MARMELOS CORR.**

The air dried and powdered stem bark of *A. marmelos* (5 kg) was extracted with ethanol in a 10 litres round bottomed joined flask fitted with a water condenser under reflux condition on a water bath for 30 days (three times, using fresh EtOH). The ethanolic extract (20 litres) was filtered. The filtrate was concentrated under reduced pressure to get a small volume (1 litre) and left overnight which gave a white deposit. It was then filtered. The excess of the solvent (EtOH) from the filtrate (1 litre) was further removed under reduced pressure to get a solid mass. The solid mass was then transferred into a 500 ml round bottomed jointed flask fitted with a water condenser and successively extracted with petroleum ether and benzene on a water bath.

The white deposit was purified over the column of neutral alumina. The column was eluted with petroleum ether.
The eluate showed a single spot on TLC examination. The excess of solvent from the petroleum ether eluate (800 ml) was distilled under reduced pressure on a water bath to give a colourless substance which was then crystallised from chloroform as colourless crystalline substance (yield : 1.3 g), m.p. 197-198°. This compound is designated as compound - A.

The petroleum ether fraction could not be worked out due to the paucity of the material.

The benzene fraction was found to be a mixture of two substances on TLC examination. The excess of the solvent was removed by distillation under reduced pressure on a water bath to yield a yellowish coloured mass. It was then passed through a column of neutral alumina. The column was carefully eluted with petroleum ether-benzene (2:8 v/v) and benzene-chloroform, (8:2 v/v) respectively. Both these eluates on TLC examinations showed the presence of single entity.

The excess of the solvent from petroleum ether-benzene eluate (1 litre) was distilled under reduced pressure on a water bath to get a yellow substance which was then crystallized as pale yellow coloured crystalline substance from benzene-chloroform mixture (yield : 2 g), m.p. 122-123°. It is designated as compound-B.

The excess of the solvent from benzene-chloroform eluate (1.2 litres) was distilled under reduced pressure on a water bath to get a white substance which was then crystallised as
colourless amorphous substance from chloroform (yield: 1.2 g), m.p. 192-193°. It is designated as compound - C.

The benzene insoluble fraction could not be worked out due to the paucity of the material.

The isolation of various constituents from the stem bark of *A. marmelos* has been briefly shown in (Chart - VII). The structural study of the compounds - A, B, and C has been described in sections - I, II and III respectively.
**CHART - VII**

A BRIEF DESCRIPTION OF THE CONSTITUENTS ISOLATED FROM THE STEM BARK OF AEGLE MARMELOS CORR.

Air dried and powdered stem bark of *A. marmelos* (5 kg) was extracted with ethanol under reflux for 30 days (three times, using fresh EtOH) filtered and the filtrate (20 litres) was concentrated under reduced pressure to get a small volume (1 litre) which gave a white deposit. The deposit was filtered out and the filtrate (1 litre) was further distilled to get a solid mass which was successively extracted with petroleum ether and benzene.

<table>
<thead>
<tr>
<th>White deposit</th>
<th>Petroleum ether fraction</th>
<th>Benzene fraction</th>
<th>Benzene insoluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>The white deposit was purified over the column of neutral alumina, eluted with petroleum ether and then crystallised from chloroform as colourless crystalline substance.</td>
<td>This fraction could not be worked out due to the paucity of the material.</td>
<td>Concentrated and passed through a column of neutral alumina and eluted successively with petroleum ether-benzene (2:8 v/v) and benzene chloroform (8:2 v/v).</td>
<td>This fraction could not be worked out due to the paucity of the material.</td>
</tr>
</tbody>
</table>

**M.P.** : 197-198°

Molecular formula: **C_{36}H_{50}O**

Designated as **Compound - A**

(Described in Section - I)

**epi-Lupeol**

**Petroleum ether-benzene (2:8 v/v) eluate**

Concentrated and crystallised as pale yellow coloured crystalline substance from benzene-chloroform mixture.

**M.P.** : 122-123°

Molecular formula : **C_{19}H_{24}O_{5}**

Designated as **Compound - B**

(Described in Section - II)

**Benzene-chloroform (8:2 v/v) eluate**

Concentrated and crystallised as colourless amorphous substance from chloroform.

**M.P.** : 192-193°

Molecular formula : **C_{14}H_{14}O_{4}**

Designated as **Compound - C**

(Described in Section - III)

**Marmin**

**Marmesin**
SECTION - I

STRUCTURAL STUDY OF THE COMPOUND - A

The compound - A, molecular formula C_{30}H_{50}O (M^+ 426), m.p. 197-198°, (α)_{D}^{25} + 16° (in chloroform) responded all the colour reactions (1-7)_{85-91} as described on page 27 characteristic for a triterpene. In addition to these tests, the compound - A gave a reddish-violet colouration in Brieskorne reaction_{92} as described on page 28 and no precipitate with digitonin_{93}. From the above tests it was clear that compound - A was a triterpenoid.

The compound - A formed a monoacetate, C_{32}H_{52}O_{2}, m.p. 160-161°, (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 - A on oxidation gave a positive Zimmerman test_{140,141} for 3-ketogroup. Thus the hydroxyl group was present at position C-3 in the compound - A.

The IR spectrum (KBr) of the compound - A showed absorptions at 3450, 2965, 1630, 1450, 1380, 1100, 1045, 1020, 950 and 885 cm^{-1} which were indicative of epi-lupeol_{147}.

The compound - A was found to be lupeol by its co-TLC examination and m.m.p. with an authentic sample of epi-lupeol_{147}. Thus the compound - A can be represented as follows:
The above structure of the compound - A as epi-lupeol was further supported by its mass spectrum which showed the fragment ions at m/z 426 \((M^+)\), 411, 408, 393, 383, 220, 218, 207, 191, 189 and 187 respectively.
EXPERIMENTAL

The isolation, purification and crystallisation of the compound - A has already been described on page 110.

The compound - A was found to be soluble in benzene, chloroform, ethyl acetate, acetone, methanol and dioxan but insoluble in water.

COLOUR REACTIONS OF THE COMPOUND - A

The compound - A gave all the colour reactions (1-7)85-91 and also gave a positive Brieskorne reaction92 and did not give precipitate with digitonin93 as described on page 27 characteristic for a triterpenoid.

THIN LAYER CHROMATOGRAPHY EXAMINATION OF THE COMPOUND - A

TLC was done on silica gel 'G' plates using the following solvent systems as described on page 30. A single spot was observed in each case.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>systems</th>
<th>$R_f$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Benzene :</td>
<td>chloroform; 6:4 v/v</td>
<td>0.54</td>
</tr>
<tr>
<td>2. Benzene :</td>
<td>chloroform; 5:5 v/v</td>
<td>0.71</td>
</tr>
</tbody>
</table>

ANALYTICAL AND SPECTRAL DATA OF THE COMPOUND - A

Found: C, 84.44; H, 11.68; calculated for $C_{30}H_{50}O$;

C, 84.50; H, 11.73%.
IR : $\nu_{\text{max}}^{\text{KBr}}$ 3450, 2965, 1630, 1450, 1380, 1100, 1045, 1020, 950 and 885 cm$^{-1}$.

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

**ACETYLATION OF THE COMPOUND - A**

The compound - A (100 mg) was acetylated with acetic anhydride (6 ml) and pyridine (6 ml) and worked up as usual (on page 31). The product was crystallised from methanol as colourless needles, m.p. 160-161$^\circ$ (Lit. m.p. 161$^\circ$)$^{148}$, ($\alpha$)$_D^{25}$ = 3.0$^\circ$ (in chloroform).

Found : C, 82.00; H, 11.08; calculated for C$_{32}$H$_{52}$O$_2$ :

C, 82.05; H, 11.11%.

**BENZOYLATION OF THE COMPOUND - A**

The compound - A (100 mg) was benzyolated with benzoyl chloride (5 ml) and pyridine (5 ml) and worked up as usual (on page 31). The benzyolated product was crystallised with ether as colourless crystals, m.p. 224-225$^\circ$ (Lit. m.p. 225-226$^\circ$)$^{149}$, ($\alpha$)$_D^{25}$ + 70$^\circ$ (in chloroform).

Found : C, 83.70; H, 10.15; calculated for C$_{37}$H$_{54}$O$_2$ :

C, 83.77; H, 10.18%.
SECTION - II

STRUCTURAL STUDY OF THE COMPOUND - B

The compound - B, molecular formula C₁₉H₂₄O₅ (M⁺ 332), m.p. 122-123° and (α)²⁵ + 25° (in ethanol). It gave a negative Molisch's test showing the absence of glycosidic nature of the compound - B.

The compound - B responded the positive reactions (1-3)¹³⁰ as described on page 60 indicative of a coumarin nature of the compound - B which was also supported by the following facts:

1. The compound - B did not consume perphthalic acid and gave a negative Angeli-Rimini test showing that the compound - B did not contain any methoxy, methylenedioxy, ketone and aldehyde functions and was shown to contain a coumarin nucleus by its behaviour towards the alkali. The compound did not produce an α-glycol with oxalic acid and did not react with diethylamine and potassium chloride, the presence of an epoxide group was also excluded.

2. The compound - B on thermal decomposition yielded umbelliferone, m.p. 231-232° (Lit. m.p. 232°)¹⁵⁰.

3. The compound - B on catalytic hydrogenation afforded dihydroumbelliferone, m.p. 132-133° (Lit. m.p. 133°, m.m.p. and co-TLC)¹⁵⁰.
4. The compound - B on treatment with glacial acetic acid gave umbelliferone and a terpenaceous oil having odour of geraniol\textsuperscript{150}.

The above reactions (1-4) were indicative for the presence of umbelliferone ether type structure for the compound - B.

Thus the compound - B can be represented as follows:

$$\text{C}_{10}\text{H}_{19}\text{O}_{2}$$

The compound - B showed its ultra-violet spectrum at 324 nm characteristic for marmin as reported in literature (Lit. UV : 324 nm)\textsuperscript{151}.

**INFRA-RED SPECTRUM OF THE COMPOUND - B**

The significant peaks obtained in the IR spectrum (KBr, cm\textsuperscript{-1}) (FIGURE - XX) of the compound - B along with their structural assignments with the help of available literature\textsuperscript{108-110} are recorded in table - 16.
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peaks (KBr, cm(^{-1}))</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3455-3350</td>
<td>Hydroxyl group</td>
</tr>
<tr>
<td>2.</td>
<td>1725</td>
<td>Coumarin lactone</td>
</tr>
<tr>
<td>3.</td>
<td>840</td>
<td>Trisubstituted double bond</td>
</tr>
<tr>
<td>4.</td>
<td>2975, 2890, 1615, 1515, 1465, 1400, 1390, 1350, 1285, 1230, 1180, 1160, 1120, 1085, 1010, 990, 915, 825, 775, 640</td>
<td>Methyl, methylene, methine and a complex aromatic substitution pattern in the coumarin lactone ring</td>
</tr>
</tbody>
</table>

From the above discussion it is clear that the compound - B was marmin. The compound - B as marmin was further confirmed by the following sets of reactions:

1. The compound - B on dehydration with phosphorus oxychloride in benzene afforded umbelliferone as one of the products (m.m.p. and co-TLC\(^1\)).

2. The chromic acid oxidation of the compound - B gave acetone and levulinic acid which clearly indicated the presence of:
FIGURE - XX : INFRA-RED SPECTRUM OF THE COMPOUND - B.
(i) a terminal hydroxy-isopropyl group.

(ii) an α-glycol linkage and

(iii) a double bond between 2' and 3' carbon atom of ether side chain.

All the above results for the compound - B were in agreement with the structure of marmin. Thus the structure of the compound - B can be represented as follows:

![Chemical structure of Compound - B]

**Compound - B**

The above proposed structure of the compound - B was further supported by its $^1$H-NMR spectrum and also by its mass spectrum data.

$^1$H-NMR SPECTRUM OF THE COMPOUND - B

The $^1$H-NMR spectrum (CDCl$_3$, 220 MHz, TMS, δ) of the compound - B (FIGURE - XXI) was found to be in complete conformity with the proposed structure of the compound - B. The significant signals along with their structural assignments are given in table - 17.
TABLE - 17

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value (δ)</th>
<th>Nature</th>
<th>J (Hz)</th>
<th>Number of protons</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.60</td>
<td>d</td>
<td>9.70</td>
<td>1</td>
<td>C-4</td>
</tr>
<tr>
<td>2.</td>
<td>7.35</td>
<td>d</td>
<td>9.70</td>
<td>1</td>
<td>C-5</td>
</tr>
<tr>
<td>3.</td>
<td>6.80</td>
<td>fused, dd</td>
<td>-</td>
<td>2</td>
<td>C-6 and C-8</td>
</tr>
<tr>
<td>4.</td>
<td>6.20</td>
<td>d</td>
<td>9.70</td>
<td>1</td>
<td>C-3</td>
</tr>
<tr>
<td>5.</td>
<td>5.50</td>
<td>t</td>
<td>5.00</td>
<td>1</td>
<td>C-2'</td>
</tr>
<tr>
<td>6.</td>
<td>4.62</td>
<td>d</td>
<td>6.60</td>
<td>2</td>
<td>C-1'</td>
</tr>
<tr>
<td>7.</td>
<td>3.35</td>
<td>dd</td>
<td>9.60 each</td>
<td>1</td>
<td>C-6'</td>
</tr>
<tr>
<td>8.</td>
<td>2.40</td>
<td>s</td>
<td>-</td>
<td>2</td>
<td>2 x OH</td>
</tr>
<tr>
<td>9.</td>
<td>1.80</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>1 x CH3</td>
</tr>
<tr>
<td>10.</td>
<td>1.20</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>1 x CH3</td>
</tr>
<tr>
<td>11.</td>
<td>1.16</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>1 x CH3</td>
</tr>
</tbody>
</table>

MASS SPECTRUM OF THE COMPOUND - B

The mass spectrum (FIGURE - XXII) of the compound - B (CHART - VIII) showed the fragment ions at m/z 332 (M⁺), 273, 272, 171, 162, 153, 134, 81, 71 and 59 respectively which supports the above proposed structure of the compound - B.

On reviewing the literature it has been found that the compound - B has already been reported by other workers in nature which was finally confirmed by m.m.p. and co-TLC with an authentic sample 138, 139.
FIGURE - XXI: $^1$H-NMR SPECTRUM OF THE COMPOUND-B
SCALE EXPANSION OF PEAK AT δ 5.50 AND δ 3.35 IN THE $^1$H-NMR SPECTRUM OF THE COMPOUND-B
FIGURE - XXII: MASS SPECTRAL FRAGMENTATION PATTERN OF THE COMPOUND-B.
CHART - VIII

Mass Spectral Fragmentation Pattern of the Compound - B
EXPERIMENTAL

The isolation, purification and crystallisation of the compound - B has already been described on page 110. The compound - B was found to be soluble in benzene, chloroform, ethyl acetate, acetone, methanol, ethanol, pyridine and dioxan.

COLOUR REACTIONS OF THE COMPOUND - B

The compound - B gave a negative reaction to Molisch's test and responded all the positive tests (1-3)\textsuperscript{130} as described on page 60 which are characteristic for a coumarin lactone.

THIN LAYER CHROMATOGRAPHY EXAMINATION OF THE COMPOUND - B

TLC was done on silica gel 'G' plates using the following solvent systems as described on page 30. A single spot was observed in each case by developing the plates in iodine vapours.

<table>
<thead>
<tr>
<th>Solvent systems</th>
<th>$R_f$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Benzene : chloroform; 6:4 v/v</td>
<td>0.49</td>
</tr>
<tr>
<td>2. Chloroform : methanol; 9:1 v/v</td>
<td>0.68</td>
</tr>
</tbody>
</table>

ANalytical and spectral data of the compound - B

Found : C, 68.60; H, 7.18; calculated for $\text{C}_{19}\text{H}_{24}\text{O}_5$:

  C, 68.67; H, 7.22%.

UV : $\lambda_{\text{max}}$ EtOH 324 nm
IR : $2\nu_{\text{max}}^\text{KBr}$ 3455-3350, 2975, 2890, 1725, 1615, 1515, 1465, 1400, 1390, 1350, 1285, 1230, 1180, 1160, 1120, 1085, 1010, 990, 915, 840, 825, 775 and 640 cm$^{-1}$.

$^1$H-NMR : (CDCl$_3$, 220 MHz, TMS, $\delta$) : 7.60, 7.35, 6.80, 6.20, 5.50, 4.62, 3.35, 2.40, 1.80, 1.20 and 1.16.

MS : m/z 332, 273, 272, 171, 162, 153, 134, 81, 71 and 59.

**THERMAL DECOMPOSITION OF THE COMPOUND - B**

When the compound - B (500 mg) was heated at 185$^\circ$ a sublimate was obtained. It was crystallised from ethyl acetate as needles, m.p. 231-232$^\circ$ which was identical to umbelliferone (m.m.p. and co-TLC)$^{150}$.

**CATALYTIC HYDROGENATION OF THE COMPOUND - B**

The compound - B (300 mg) in ethanol (15 ml) was hydrogenated in the presence of PtO$_2$ (50 mg) which consumed 1 mole of hydrogen. The solution was diluted with water (50 ml) and extracted with ether. The ethereal extract was washed with aqueous sodium hydroxide (75 ml). The alkaline extract upon acidification produced dihydro umbelliferone, m.p. 132-133$^\circ$ (m.m.p. and co-TLC)$^{150}$.

Found : C, 65.80; H, 4.82; calculated for C$_9$H$_8$O$_3$:

C, 65.85; H, 4.87%.

The ether residue was washed with water and dried (Na$_2$SO$_4$) which gave a terpenaceous oil.
ACID HYDROLYSIS OF THE COMPOUND - B

(1) The compound (500 mg) in glacial acetic acid (2 ml) was treated with 2 drops of sulphuric acid in a 250 ml round bottomed jointed flask fitted with a water condenser. It was then refluxed for 1 hour, poured into ice-cooled water, made alkaline and extracted with ether. The alkaline mother liquor (designated as A) was worked up for umbelliferone as given below. The ethereal solution was freed from solvent and the residue warmed for 5 hours with 20% sulphuric acid (20 ml). The hydrolysate was extracted with ether which was washed with 10% alkaline solution, then water and dried. The ethereal solution on evaporation left some oil with an odour of geraniol. The alkaline washing was slightly fluorescent but on acidification and extraction with ether did not yield any product.

(II) The compound - B (500 mg) in a 250 ml round bottomed jointed flask was refluxed with glacial acetic acid (2 ml) for 2 hours on a water bath using a water condenser. The product was made alkaline and extracted with ether. The aqueous alkaline solution (designated as B) was worked for umbelliferone as given below. The ether extract was washed with water, dried and evaporated to give an oily product with a fragrant odour. This was refluxed with 0.5% methanolic potassium hydroxide for 1 hour. The cold solution was diluted with water and shaken with ether (150 ml). The ethereal extract on concentration, left an oil with a geraniol like smell.
The alkaline solution - A and B had a strong blue fluorescence. They were cooled and acidified with hydrochloric acid. A precipitate appeared which was taken up in ether. The ethereal solution was washed and dried. Evaporation gave umbelliferone (from both) which was crystallised from ethyl acetate as needles, m.p. 231-232° (m.m.p. and co-TLC).

DEHYDRATION OF THE COMPOUND - B

The compound - B (100 mg) in dry benzene (5 ml) was refluxed with phosphorus oxychloride (500 mg) in a 100 ml round bottomed jointed flask fitted with a water condenser for 2 hours. The benzene solution was decanted and concentrated to give umbelliferone (m.m.p. and co-TLC). The mother liquor on evaporation gave an oil having terpene like odour.

CHROMIC ACID OXIDATION OF THE COMPOUND - B

An acetic acid solution (15 ml) of the compound - B (1 g) in a conical flask was treated with chromic acid (1 g) in 50% acetic acid (15 ml) and kept at room temperature for 3 days. The solution was cooled, neutralized with 50% potassiumhydroxide solution and steam distilled. The distillate was collected in a 5% acetic acid solution of p-nitrophenyl hydrazine (50 ml) giving acetone-p-nitrophenyl hydrazone as reddish-yellow needles from methanol, m.p. 147-148° (m.m.p. and co-TLC).

Found : C, 56.65; H, 5.85; N, 21.00; calculated for C₉H₁₁O₂N₃:
   C, 55.95; H, 5.69; N, 21.76%.
In a similar experiment acetone was collected over 10% sodium hydroxide solution containing freshly distilled benzaldehyde (4-5 drops). The solution was kept at 0° over night, then extracted with ether, washed and dried. The ethereal extract on concentration gave dibenzylideneacetone as yellow coloured needles from methanol, m.p. 110-111°.  

Found : C, 87.10; H, 5.95; calculated for \( \text{C}_{17}\text{H}_{14}\text{O} \) :

\[
\begin{align*}
\text{C} & : 87.17; \\
\text{H} & : 5.98%.
\end{align*}
\]
SECTION III

STRUCTURAL STUDY OF THE COMPOUND - C

The compound - C, molecular formula, \( C_{14}H_{14}O_{4} \), m.p. 192-193\(^\circ\), (\( \alpha \))\(_D\) \( +26.0\)\(^\circ\) (in chloroform) gave a negative Molisch's test showing the absence of glycosidic nature of the compound - C.

The compound - C responded all the colour reactions (1-3)\(^{130}\) as described on page 60 indicative of the coumarin nature of the compound - C which was also supported by the following facts.

1. The compound - C dissolved in concentrated sulphuric acid with a yellow colour and gave a deep violet fluorescence.

2. The compound - C did not consume perphthalic acid and gave a negative Angeli-Rimini test showing that the compound - C did not contain any methoxy, methylenedioxy, ketone and aldehyde functions.

3. The compound - C did not react with diethylamine and potassium chloride, the presence of epoxide group was also excluded.

INFRA-RED SPECTRUM OF THE COMPOUND - C

The significant peaks obtained in the IR spectrum (KBr, cm\(^{-1}\)) (FIGURE - XXIII) of the compound - C alongwith
their structural assignments with the help of available literature\textsuperscript{108-110} are recorded in Table - 18.

**TABLE - 18**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peaks (KBr, cm(^{-1}))</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3450</td>
<td>Hydroxyl group</td>
</tr>
<tr>
<td>2.</td>
<td>2990</td>
<td>Methyl group</td>
</tr>
<tr>
<td>3.</td>
<td>1700 and 1630</td>
<td>Coumarin lactone</td>
</tr>
<tr>
<td>4.</td>
<td>1570</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td>5.</td>
<td>1130</td>
<td>Ethereal oxygen</td>
</tr>
<tr>
<td>6.</td>
<td>1450, 1400, 1370, 1265, 950, 890 and 720</td>
<td>Complex aromatic substitution pattern in coumarin skeleton of the compound - C</td>
</tr>
</tbody>
</table>

The compound - C on acetylation with acetic anhydride-sodium acetate formed a monoacetyl derivative, m.p. 129-130\(^\circ\) which was analysed for one acetyl group indicated the presence of one hydroxyl group. The compound - C did not react with ferric chloride or diazomethane indicating the nature of hydroxyl group as an alcoholic.

The compound - C on oxidation with chromic acid yielded a product, m.p. 259-260\(^\circ\) (dec.) which gave a violet colour with ferric chloride and readily dissolved in aqueous sodium bicarbonate with the evolution of effervescence. The product was found to be identical with umbelliferone-6-carboxylic
FIGURE - XXIII: INFRA-RED SPECTRUM OF THE COMPOUND - C.
Umbelliferone-6-carboxylic acid

This observation revealed that the compound - C possesses a coumarin nucleus which contain a ring system attached through an ethereal oxygen at position - 7 to position - 6.

The compound - C on mild oxidation with chromic acid afforded acetone which showed the presence of \((\text{CH}_3)_2\text{C-OH}\) group in the compound - C and it should be a part of \(\text{C}_4\text{H}_{10}^0\). The presence of \((\text{CH}_3)_2\text{C-OH}\) group in the compound - C was also supported by its dehydration reaction.

The compound - C on dehydration with phosphorus pentoxide yielded a compound, m.p. 138-139° which was found to be identical with anhydromarmesin (Lit. m.p. 138-140°)\(^{152}\), m.m.p. and co-TLC.
The above results for the compound - C were in agreement with the structure of marmesin. Thus the structure of compound - C can be represented as follows:

![Chemical Structure of Compound C]

The above structure of the compound - C as marmesin was further supported by its $^1$H-NMR and mass spectra.

$^1$H-NMR SPECTRUM OF THE COMPOUND - C

The $^1$H-NMR spectrum (CDCl$_3$, 90 MHz, TMS, $\delta$) of the compound - C (FIGURE - XXIV) was found to be in complete conformity with the proposed structure of the compound - C. The significant signals along with their structural assignments are given in Table - 19.
### TABLE - 19

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value (θ)</th>
<th>Nature</th>
<th>J (Hz)</th>
<th>Number of protons</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.60</td>
<td>d</td>
<td>9.50</td>
<td>1</td>
<td>H-4</td>
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<tr>
<td>2.</td>
<td>7.25</td>
<td>s</td>
<td>-</td>
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<tr>
<td>3.</td>
<td>6.75</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-5</td>
</tr>
<tr>
<td>4.</td>
<td>6.25</td>
<td>d</td>
<td>9.50</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td>5.</td>
<td>4.70</td>
<td>t</td>
<td>8.50 each</td>
<td>1</td>
<td>H-2'</td>
</tr>
<tr>
<td>6.</td>
<td>3.25</td>
<td>d</td>
<td>9.00</td>
<td>2</td>
<td>H-3'</td>
</tr>
<tr>
<td>7.</td>
<td>1.85</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>1 x OH</td>
</tr>
<tr>
<td>8.</td>
<td>1.35</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>1 x CH₃</td>
</tr>
<tr>
<td>9.</td>
<td>1.20</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>1 x CH₃</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM OF THE COMPOUND - C**

The mass spectrum (FIGURE - XXV ) of the compound-C (CHART - IX) showed fragment ions of m/z 246 (M⁺) 228, 213, 188, 187, 160, 159, 132, 131 and 59, respectively which supports the above structure of the compound - C.

On reviewing the literature it has been found that the compound - C has already been reported by other workers in nature which was finally confirmed by its m.m.p. and co-TLC with an authentic sample¹⁵².
FIGURE - XXIV: $^1$H-NMR SPECTRUM OF THE COMPOUND-C.
FIGURE - XXV : MASS SPECTRAL FRAGMENTATION PATTERN OF THE COMPOUND-C.
MASS SPECTRAL FRAGMENTATION PATTERN OF THE COMPOUND - C
EXPERIMENTAL

The isolation, purification and crystallisation of the compound - C has already been described on page 110.

The compound - C was found to be soluble in chloroform, ethyl acetate, acetone, methanol, ethanol, pyridine and dioxan.

COLOUR REACTIONS OF THE COMPOUND - C

The compound - C gave a negative reaction to Molisch's test and responded all the positive tests (1-3)\textsuperscript{130} and (1-3) as given on pages 60 and 127 respectively.

THIN LAYER CHROMATOGRAPHY EXAMINATION OF THE COMPOUND - C

TLC was done on silica gel 'G' plates using the following solvent systems as described on page 30. A single spot was observed in each case by developing the plates in iodine vapours.

<table>
<thead>
<tr>
<th>Solvent systems</th>
<th>R\textsubscript{f} values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Benzene : chloroform; 6:4 v/v</td>
<td>0.68</td>
</tr>
<tr>
<td>2. Benzene : methanol; 7:3 v/v</td>
<td>0.72</td>
</tr>
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</table>

ANALYTICAL AND SPECTRAL DATA OF THE COMPOUND - C

Found : C, 68.36; H, 5.75; calculated for C\textsubscript{14}H\textsubscript{14}O\textsubscript{4}:
C, 68.30; H, 5.69%.

IR : \[\nu_{\text{KBr}}^\text{max}\] 3450, 2990, 1700, 1630, 1570, 1450, 1400, 1370, 1265, 1130, 950, 890 and 720 cm\textsuperscript{-1}.
$^1$H-NMR: CDCl$_3$, 90 MHz, TMS, $\delta$: 7.60, 7.25, 6.75, 6.25, 4.70, 3.25, 1.85, 1.35 and 1.20.

MS: m/z 246, 228, 213, 188, 187, 160, 159, 132, 131 and 59.

ACETYLATION OF THE COMPOUND - C

The compound - C (100 mg) was acetylated with acetic anhydride (5 ml) and sodium acetate (200 mg) as usual (on page 31). The acetylated product was filtered and crystallised from chloroform-acetone mixture as colourless plates, m.p. 129-130$^\circ$.

Found: C, 66.70; H, 5.50; calculated for C$_{16}$H$_{16}$O$_5$: C, 66.66; H, 5.55%.

DETERMINATION OF THE ACETYL PERCENTAGE IN ACETYL DERIVATIVE OF THE COMPOUND - C

The acetyl percentage in the acetyl derivative of compound - C was determined by the method of Wisenberger's$^{126}$ as described by Belcher and Godbert$^{127}$.

Found: Acetyl 14.30; calculated for C$_{14}$H$_{13}$O$_4$ (COCH$_3$): 14.23%.

CHROMIC ACID OXIDATION OF THE COMPOUND - C

The compound - C (200 mg) was boiled with 3% sulphuric acid (40 ml) in a 100 ml round bottomed flask on a water bath. Potassium dichromate (600 mg) in distilled water (10 ml) was added slowly to the boiling mixture and refluxed
for 6 hours. The dark green solution thus obtained was cooled at room temperature. Then the reaction mixture was shaken with ether to remove unchanged compound - C and the ethereal layer was separated and the green aqueous filtrate was concentrated on a water bath to 10 ml and kept over night at 0° which deposited a pale yellow substance. It was then filtered, washed with cold water and crystallised from methanol as colourless needles, m.p. 259-260° (dec.).

Found : C, 58.20; H, 2.87; calculated for C_{10}H_{6}O_{5} :

C, 58.27; H, 2.95%.

MILD OXIDATION OF THE COMPOUND - C WITH CHROMIC ACID

The compound - C (300 mg) in glacial acetic acid (6 ml) was added to chromic acid (150 mg in 8 ml of 50% glacial acetic acid) and kept at room temperature for 60 hours in a closed conical flask. It was then neutralized completely with sodium hydroxide. The solution was distilled until 10 ml was collected in a receiver containing few drops of 10% sodium hydroxide. The mixture was left overnight in a refrigerator which deposited a semi-solid mass that was extracted with ether. The ethereal extract was evaporated and treated with 2 drops of benzaldehyde in dilute acetone and cooled in ice which afforded a needles shaped crystalline compound which was then crystallised from methanol as pale yellow coloured needles, m.p. 111-112°. The substance was identified as dibenzal acetone (m.m.p. and co-TLC)_{152}. 
DEHYDRATION OF THE COMPOUND - C

The compound - C (250 mg) dissolved in 50 ml of chloroform in a 100 ml round bottomed jointed flask fitted with a water condenser was refluxed with phosphorus pentaoxide (2.5 g) for 5 hours and filtered. The filtrate was concentrated to get a product which was filtered and crystallized as colourless plates from ether, m.p. 138-139°.

Found: C, 73.75; H, 5.30; calculated for C₁₄H₁₂O₃:

C, 73.60; H, 5.26%.