PART III: Phytochemical Investigation
1. PHYTOCHEMICAL INVESTIGATION OF CASSIA OBTUSA

1.1. General Introduction

In the next phase of our study, while making search on some medicinal plants for the phytochemical investigation, we came across an interesting report on the roots and its medicinal properties of Cassia obtusa (Indian Avarai).

The genus Cassia is comprised of 52 species\textsuperscript{1-10}, which are well known for its high medicinal properties\textsuperscript{11}. The 30 or more species that have been examined so far contain various types of compounds e.g. Polysaccharides\textsuperscript{12}, alkaloids\textsuperscript{13}, flavanoids\textsuperscript{13}, anthraquinones\textsuperscript{14}, sterols, anthocyanides and dimers of flavanoids\textsuperscript{15}.

Some of the structures of Anthraquinone derivatives isolated from Cassia are given in Table.

![Anthraquinone structure](image)

<table>
<thead>
<tr>
<th>Structure No.</th>
<th>Name</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>R&lt;sub&gt;3&lt;/sub&gt;</th>
<th>R&lt;sub&gt;4&lt;/sub&gt;</th>
<th>R&lt;sub&gt;5&lt;/sub&gt;</th>
<th>R&lt;sub&gt;6&lt;/sub&gt;</th>
<th>R&lt;sub&gt;7&lt;/sub&gt;</th>
<th>R&lt;sub&gt;8&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>1,5-dihydroxy-3-methylanthraquinone</td>
<td>-OH</td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
</tr>
<tr>
<td>b</td>
<td>1,3,5-trihydroxy-6-methoxy anthraquinone</td>
<td>-OH</td>
<td>H</td>
<td>-OH</td>
<td>H</td>
<td>-OH</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>c</td>
<td>1,3-dihydroxy-2-formylanthraquinone</td>
<td>-OH</td>
<td>-CHO</td>
<td>-OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>d</td>
<td>1,5,8-trihydroxy-3-methyl anthraquinone</td>
<td>-OH</td>
<td>H</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>-OH</td>
<td>H</td>
<td>H</td>
<td>-OH</td>
</tr>
<tr>
<td>e</td>
<td>1,8-dihydroxyanthraquinone</td>
<td>-OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>-OH</td>
</tr>
</tbody>
</table>

Continued...
### 1.2. Results and Discussion

Roots of *Cassia obtusa* plants (1 kg) were collected in the month of July 1996, in the Bharathiar University Campus which is located at the foot of Maruthamalai Hills, Western Ghats, S. India. They were dried in air (30 days). While drying, care was taken to avoid the decomposition of roots. Air dried roots were powdered and extracted with boiling methanol and the methanol concentrate was fractionated into water soluble and insoluble portions. The water insoluble portion was further extracted with boiling chloroform. The extract was column chromatographed over silica gel by eluting with ethyl acetate - petroleum ether (5:1) to afford, brown solid (100 m.g). It was recrystallised as needles from benzene, m.p.185°C. Formation of red colour with methanolic sodium hydroxide and also with methanolic magnesium acetate indicated the isolated compound to be an anthraquinone. The absorption

<table>
<thead>
<tr>
<th>Structure No.</th>
<th>Name</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>R₆</th>
<th>R₇</th>
<th>R₈</th>
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</thead>
<tbody>
<tr>
<td>f</td>
<td>7-methoxy-2-methyl-1,3,4,5-tetrahydroxy-anthraquinone</td>
<td>OH</td>
<td>-CH₃</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>g</td>
<td>2-methoxy-3-methylanthraquinone</td>
<td>H</td>
<td>OCH₃</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>h</td>
<td>1,2,4-trihydroxy-3-methylanthraquinone</td>
<td>OH</td>
<td>OH</td>
<td>CH₃</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>i</td>
<td>2,5-dihydroxy-1-methoxy-3 methyl anthraquinone</td>
<td>OCH₃</td>
<td>OH</td>
<td>CH₃</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>j</td>
<td>1,3,8-trihydroxy-4-formylanthraquinone</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>CHO</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>k</td>
<td>1,3,8-trihydroxy-2-methyl-6-methoxyanthraquinone</td>
<td>OH</td>
<td>CH₃</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>l</td>
<td>1,5-dihydroxy-8-methoxy-2,3-dimethylanthaquinone</td>
<td>OH</td>
<td>CH₃</td>
<td>CH₃</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>OCH₃</td>
</tr>
</tbody>
</table>
maximum at 440 nm in its UV spectrum and the generation of a red colour with methanolic magnesium acetate suggested the presence of chelated α-hydroxy group. A strong band at 3450 cm$^{-1}$ in its IR spectrum (Fig. 1) and the solubility in 5% aq. sodium bicarbonate solution confirms the presence of β-hydroxy group.

Phthalein fusion test* confirms the presence of 1,3-dihydroxy groups. It was finally confirmed from its NMR spectrum (Fig. 2.), which showed two singlets at δ 12.20 and 12.60 ascribable to non-chelated and chelated hydroxyl groups. A medium IR absorption bands at 2900 and 2850 cm$^{-1}$ indicated the presence of methoxy and methyl groups respectively. The β-methyl group was accustomed by a peak at 1460 cm$^{-1}$ in the IR-spectrum and further supported by a $^1$H NMR signal at δ 2.45. The 6-methoxy group was identified by colour reactions$^{21-23}$ and further it was confirmed by $^1$H NMR at δ 3.94. Strong IR bands at 1630 and 1660 cm$^{-1}$ suggested the presence of chelated and nonchelated carbonyl groups. The C$_2$- and C$_4$- protons appeared as two doublets at δ 7.37 and 6.69 with meta coupling constant J=2.37 Hz. The two singlets at δ 7.74 and 7.26 were ascribable to the remaining two protons C$_6$-H and C$_b$-H respectively. The elemental analysis C,67.56%; H,4.18% suggested the molecular formula C$_{16}$H$_{12}$O$_5$.

This was further confirmed by the presence of a molecular ion peak (M$^+$) at m/e 284, with other strong peaks at m/e 256[M-28]$^+$, 241[M-43]$^+$ due to loss of water and multiple loss of carbon monoxide in the mass spectrum of the compound typical of polyhydroxy anthraquinones$^{18}$.

On the basis of above spectral evidence the structure of the compound was assigned as 1,3-dihydroxy-6-methoxy-7-methyl anthraquinone (1m).

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* A pinch of the substance was heated with phthalic anhydride and concentrated sulfuric acid. Then it was cooled and poured into a beaker containing water and sodium hydroxide solution. Green fluorescence colour was produced, which confirms the presence of 1,3-dihydroxy groups.
Fig. 2

$^1$H NMR: 270 MHz
Solvent: CDCl$_3$
The water soluble portion was further extracted with hot solution of ethylacetate. The extract was concentrated in to small bulk and column chromatographed over silica gel and elution with ethyl acetate afforded compound 1n, m.p. >300°C. The compound forms red colour with methanolic sodium hydroxide and also with methanolic magnesium acetate indicated the anthraquinone nature\textsuperscript{16,17}. The wavelength at 426 nm in its UV spectrum and also strong broad band at 3450 cm\textsuperscript{-1} in its IR spectrum and the solubility in 5% sodium hydroxide solution confirmed the presence of hydroxyl group\textsuperscript{24}. The IR spectrum (Fig. 3) of the compound indicated the presence of chelated and non-chelated carbonyl absorptions at 1630 cm\textsuperscript{-1} and 1660 cm\textsuperscript{-1} and the formyl group C-H stretching at 2950 cm\textsuperscript{-1}.

Its \textsuperscript{1}H NMR spectrum (Fig. 4) taken, in dimethyl sulphoxide exhibited as follows,

(i) One proton singlet at \( \delta \) 12.97 was due to the presence of one chelated hydroxy group.

(ii) Two singlets for two protons at \( \delta \) 9.58 and 9.19 were due to the presence of two formyl groups.

(iii) Two doublets at \( \delta \) 7.40 and 6.76 were due to C\textsubscript{5}- and C\textsubscript{6}-protons with ortho coupling constant \( J=8.23 \) Hz.

(iv) Three singlets with one proton intensity at \( \delta \) 6.19, 6.95 and 6.99 were due to C\textsubscript{2}-H, C\textsubscript{4}-H and C\textsubscript{8}-H respectively.

The elemental analysis C,68.50% and H,2.78% suggested the molecular formula C\textsubscript{16}H\textsubscript{8}O\textsubscript{5}. 

![Compound 1n](image-url)
**Fig. 4**

$^1$H NMR: 270 MHz  
Solvent: CDCl$_3$
This was further confirmed by the presence of a molecular ion peak (M\(^-\)) at m/e 280, with other strong peaks at m/e 262 \([\text{M-18}]^+\), 234[\text{M-46}]^+ due to loss of water and carbon monoxide in the mass spectrum of the compound typical of hydroxy anthraquinones\(^2\).

![Chemical Structure](image)

On the basis of spectral and elemental analysis the structure of the compound was assigned as 1-hydroxy-3,7-diformyl anthraquinone (1n)
REFERENCES


