PART V

CHEMICAL EXAMINATION OF THE ESSENTIAL OIL OF
TODDALIA ASIATICA
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OF TODDALIA ASIATICA

Toddalia asiatica (Hindi- janglikalmirch, Tamil- milakaranai, Telugu- konda kasivinda) a climbing shrub of the natural order Rutacea is found in the lower Himalayas, and in western and southern India. The whole plant, more especially the root bark is pungent and aromatic, and is used in the Ayurvedic medicine for its tonic, stimulant, and antipyretic properties; it is also reputed to be a specific for remittant fever. As a result of a few early trials, with an infusion of the drug in Madras Hospital it was considered superior to quinine as an antipyretic and to calumba as a tonic but recent investigations have disproved its utility in Malarial fever.

A considerable amount of chemical work has been done on the drug chiefly because of the celebrity it had attained at one time in European medicine under the name 'Lopez root'. From the essential oil of the Toddalia aculeata has been isolated citronellal, linalool and a camphoraceous substance m.p.96.5 - 97.

The bark has been found to contain resin, bitter stuff, tannins, citric acid, sugar, pectin, starch etc., and to yield

2. Hooper, Schimmel's Ber., 1893, April, 64.
on incineration an ash rich in manganese\textsuperscript{3}. An alkali soluble glucoside originally thought to be Hesperidine had also been isolated\textsuperscript{4} but Chesterle and Wander\textsuperscript{5} on investigating this more closely have identified this as glucoside diosmin. Perkin and Hummel\textsuperscript{6} examined the root bark and isolated from it a yellow alkaloidal hydrochloride; they considered this to be berberine hydrochloride from the results of the determination of platinum from its chloroplatinate and the general behaviour of the hydrochloride. They also obtained a resin from which no other crystalline material could be isolated. But in 1933, it was shown by Dey and Pillay\textsuperscript{7} that though the alkaloidal chloride isolated by these authors resembled berberine hydrochloride, and also gave very nearly the same platinic chloride, it was different from it and hence was named, Toddaline having a molecular formulae $C_{20}H_{21}NO_4$. This had properties entirely different from berberine hydrochloride. Dey and Pillay also reported the presence of one more alkaloid Toddalinine. No attempts, however, have so far been made to study the essential oil of Toddalia asiatica. Hence the present author undertook the work in the oil and obtained the essential oil in

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an yield of (0.7%) by the steam and water distillation of the leaves of the plant, collected from the hills at Vandalur near Madras (India). The oil, a light green mobile liquid with an orange like odour gave the following constants:

\[
\begin{align*}
D^{30} & = 0.9703 \\
\eta_{D}^{30} & = 1.4825 \\
\left[\alpha\right]_{D}^{30} & = 20^\circ 7' \\
\text{Acid value} & = 3.065 \\
\text{Saponification value} & = 94.11 \\
\text{Saponification value} \ (\text{after acetylation}) & = 312.59
\end{align*}
\]

Separation of the constituents of the oil by chromatography and their chemical studies supported by physical methods have revealed the presence of Limettin, m.p. 143° in the oil. In addition it was found to contain 39% of terpenic hydrocarbons, and a mixture of three azulenes (GLC), besides the presence of \(\beta\)-myrcene, \(\alpha\)-limonene, \(\Delta^3\)-carene, ocimene, citronellal, linalool, citronelloyl, geraniol, geranyl acetate and bisabolene. The details are given in the experimental portion.
EXPERIMENTAL

Steam and water distillation of the shade dried leaves of Toddalia asiatica in a galvanised still gave a light green mobile oil in an yield of 0.7% with a faint orange odour. The oil had the following properties:

- specific gravity at 30°: 0.9703
- refractive index at 30°: 1.4825
- optical rotation at 30°: 2° 7'
- acid value: 3.065
- saponification value: 94.11
- saponification value after acetylation: 312.59

Separation of the solid compound limettin from the essential oil.

The oil exhibited violet fluorescence on dilution with alcohol. Coumarones were suspected to be present. The coumarones were separated as follows:

70 g of the oil was shaken well with a 5% aqueous alcoholic solution of potassium hydroxide. The mixture was diluted with water and extracted with ether. The coumarones were dissolved in the alkaline layer and were liberated by acidifying with concentrated sulphuric acid and extraction with ether. Removal of

ether gave a solid which on repeated crystallisation with methyl alcohol gave shining needles (1.086 g) melting at 145° (Found: C, 65.2; H, 4.9; Calc. for C_{11}H_{10}O_{4}; C, 64.07; H, 4.88%; m. wt. 207.9; C_{11}H_{10}O_{4} requires m.wt. 206; methoxy 30%, C_{11}H_{10}O_{4} requires for 2 methoxy groups 31%).

The compound was identified to be Limettin.

**Formation of a bromo derivative**

0.5 g of the fraction was dissolved in glacial acetic acid (2 ml) to which an ice cold solution of bromine (2 g of bromine in 4 ml of glacial acetic acid) was added. A crystalline solid separated after a few minutes which when recrystallised with glacial acetic acid gave a m.p. and mixed m.p. 254°.

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a hydrocarbon.

(Found: C, 88.16; H, 11.76; Calc. for C_{10}H_{16}: C, 88.24; H, 11.76%; m.wt. 137.4; C_{10}H_{16} requires m.wt. 136.)

Preparation of maleic anhydride adduct.\textsuperscript{10,11}

3.2 g of the fraction were mixed with 2.45 g of maleic anhydride in 13 cc of dry benzene and refluxed for 3 hr. After the reaction was complete, benzene was distilled off and light petroleum (40-60) added. The precipitated adduct (2.02 g) recrystallised from methyl alcohol gave a m.p. and mixed m.p. 36\textdegree. Preparation of acid from maleic anhydride adduct.\textsuperscript{12}

2 g of the adduct prepared as above was dissolved in sodium hydroxide (20\%) solution and refluxed for an hour. Dilute hydrochloric acid was added to precipitate the acid, which when crystallised with alcohol gave m.p. and mixed m.p. 120-122\textdegree (with corresponding acid obtained from the hydrolysis of the maleic anhydride adduct of an authentic specimen of \(\beta\)-myrcene) (Found: C, 66.0; H, 7.8; Calc. for C_{14}H_{20}O_{4}, C, 66.6; H, 7.3; O, 25.5).

The I.R. spectrum of the fraction finally confirmed it to be myrcene (Fig. XVII).

Fraction (ii) Table I (\(\beta\)-myrcene and \(\delta\)-limonene)

8 g of the fraction was dissolved in light petroleum and

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>wt. in gms.</th>
<th>eluant</th>
<th>$n_D^{25}$</th>
<th>No. of TLC spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.8921</td>
<td>Light petroleum</td>
<td>1.4727</td>
<td>one</td>
</tr>
<tr>
<td>16</td>
<td>0.9017</td>
<td>&quot;</td>
<td>1.4727</td>
<td>&quot;</td>
</tr>
<tr>
<td>17</td>
<td>0.9321</td>
<td>&quot;</td>
<td>1.4728</td>
<td>&quot;</td>
</tr>
<tr>
<td>18</td>
<td>0.6017</td>
<td>&quot;</td>
<td>1.4728</td>
<td>&quot;</td>
</tr>
<tr>
<td>19</td>
<td>0.2091</td>
<td>&quot;</td>
<td>1.4728</td>
<td>&quot;</td>
</tr>
<tr>
<td>20</td>
<td>0.0127</td>
<td>&quot;</td>
<td>1.4728</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Fractions (i) to (vii) Table II. These were found to be identical and combined together. The combined fraction had the following constants:

$$D^{25} \quad 0.7892 \quad n_D^{25} \quad 1.4675.$$  

It was confirmed to be $\beta$-myrcene by Co. TLC with an authentic specimen of $\beta$-myrcene.

Fractions (viii) to (xx) Table II (d-limonene). Removal of the solvent gave a liquid, b.p. 117°, $D^{25} \quad 0.8404$, $n_D^{25} \quad 1.4723$

$$[\alpha D^{25}] \quad 127^\circ 5^\prime.$$  

The fraction had a pleasant orange like odour.

Colour test for limonene$^{13}$  

A small amount of the fraction was taken in benzene.

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To this solution Bezssonoff's reagent ($\text{K}_2\text{Cr}_2\text{O}_7$, $\text{WO}_3\text{(P}_2\text{O}_5)_3\text{H}_2\text{O}$ dissolved in $5\% \text{H}_2\text{SO}_4$) was added. Development of a blue colour showed the presence of $d$-limonene.

**Formation of tetrabromide**

2 ml of the fraction was dissolved in ether (5 ml) and cooled in ice. To this was added an ice cold solution of bromine in ether (2 ml of bromine in 5 ml of ether). A white precipitate was obtained after some time which when recrystallised with ethyl acetate gave m.p. and mixed m.p. 104°.

**Formation of an adduct with maleic anhydride**

The fraction formed an adduct with maleic anhydride, which had a m.p. and mixed m.p. 146°.

**Fraction (iii) Table I (d-limonene and $\Delta_3$-carene)**

4.8 g of the fraction was dissolved in light petroleum and chromatographed (table III) over a column of alumina (480 g, ratio 1 : 100) and eluted with petroleum ether (60-80°).

**Table III**

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Wt. in gms.</th>
<th>eluant</th>
<th>$n_D$</th>
<th>No. of TLC spots.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0986</td>
<td>Light petroleum</td>
<td>1.4722</td>
<td>one spot</td>
</tr>
<tr>
<td>2</td>
<td>0.1237</td>
<td>&quot;</td>
<td>1.4722</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>0.1915</td>
<td>&quot;</td>
<td>1.4722</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Contd. ..

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To this solution Bezssonoff's reagent (\(\text{WO}_3\cdot\text{WO}_3\left(\text{P}_2\text{O}_5\right)_{17}\cdot\text{H}_2\text{O}\)) dissolved in 5% \(\text{H}_2\text{SO}_4\) was added. Development of a blue colour showed the presence of d-limonene.

**Formation of tetrabromide**\(^{14}\).

2 ml of the fraction was dissolved in ether (5 ml) and cooled in ice. To this was added an ice cold solution of bromine in ether (2 ml of bromine in 5 ml of ether). A white precipitate was obtained after some time which when recrystallised with ethyl acetate gave m.p. and mixed m.p. 104\(^{0}\).

**Formation of an adduct with maleic anhydride**

The fraction formed an adduct with maleic anhydride, which had a m.p. and mixed m.p. 146\(^{0}\).

**Fraction (iii) Table I (d-limonene and \(\Delta^3\)-carene)**

4.8 g of the fraction was dissolved in light petroleum and chromatographed (table III) over a column of alumina (480 g, ratio 1 : 100) and eluted with petroleum ether (60-80).  

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Wt. in gms.</th>
<th>eluant</th>
<th>(n_D)</th>
<th>No. of TLC spots.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0986</td>
<td>Light petroleum</td>
<td>1.4722</td>
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</tr>
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<td>3</td>
<td>0.1915</td>
<td>&quot;</td>
<td>1.4722</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Contd. ..

Co TLC of the fraction with an authentic specimen confirmed it to be d-limonene.

Fractions (vii) to (xx) Table III ($\Delta^3$-carene). These were mixed together and purified by rechromatography over a column of alumina (grade I) and elution with light petroleum (60-80). Removal of the solvent gave a fraction $D^{25}_{25} 0.8639$, $n^25_D$ 1.4675.

Preparation of nitrosate

2 g of the fraction was mixed with an ice cold mixture of amyl nitrite (2 ml), glacial acetic acid (1 ml) and concentrated nitric acid (1 ml). The mixture was warmed for half an hour and diluted with alcohol. An oily layer was obtained which on cooling solidified and on recrystallisation gave a m.p. and mixed m.p. 146° (with an authentic specimen of $\Delta^3$-carene).

Formation of maleic anhydride adduct

The fraction formed an adduct with maleic anhydride in benzene which had a m.p. and mixed m.p. 186°.

Fraction (iv) Table I (ocimene) The fraction was purified by chromatography over a column of alumina (grade I) and elution with light petroleum (60-80) gave on removal of the solvent, a fraction $D^{25}_{25} 0.7854$, $n^25_D$ 1.4854.

The fraction gave a positive ferrox test for hydrocarbons.

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Preparation of bromoderivative

The bromoderivative of the above fraction gave a m.p. and mixed m.p. 85°. The fraction was confirmed to be ocimene by Co TLC with an authentic specimen of ocimene.

Fraction (v) Table I (citronellal)

2.9 g of the fraction was dissolved in 10 ml of light petroleum (40-60°) and chromatographed over alumina grade I (300 g). The fraction had the following constants:

\[ \begin{align*}
    D_{25}^25 & = 0.8497, \\
    n_D^{25} & = 1.4512
\end{align*} \]

It gave reactions of a carbonyl group.

Formation of a semicarbazone

To a solution of the fraction (2 g) in alcohol (4 ml) was added semicarbazide hydrochloride (1.5 g), sodium acetate (2 g) and water (4 ml). The mixture on refluxing gave on cooling a white crystalline solid which on recrystallisation from chloroform gave a m.p. and mixed (with an authentic specimen of citronellal) m.p. 95°.

Characterisation with TLC.

It was confirmed by Co TLC with an authentic specimen of citronellal.

A yellow spot characteristic of citronellal was obtained on

spraying the TLC plate with 2:4 dinitrophenyl hydrazine reagent (0.4% 2:4 DNP in 2N HCl).

**Fraction (4)** Table I (linalool)

The fraction was purified (TLC) by chromatography over a column of silica gel and elution with benzene. It had the following constants:

$$D_{25}^2 0.8601, \quad n_D^{25} 1.4607$$

and an odour resembling that of lily of the valley.

**Colour reaction**

The fraction gave Liberman's Burchard colour test for linalool.

**Preparation of phenyl urethane**

2 g of the alcohol were mixed with 3 g of phenyl isocyanate and allowed to stand in a stoppered conical flask for about a week in a dessicator. Recrystallisation of the phenyl urethane so obtained with light petroleum (60-80) gave m.p. and mixed m.p. 68°C.

The fraction was thus identified to be linalool which was further confirmed by oxidation.

**Oxidation of linalool**

A mixture of potassium dichromate (1 g) concentrated sulphuric acid (1.2 g) and water (13 ml) was added to 1.5 g of the fraction and warmed. The oxidised product was then distilled off and shaken with a saturated solution of sodium bisulphite. The addition product was then mixed with sodium bicarbonate solution
and steam distilled. The product so obtained was identified to be citral by Co TLC with an authentic specimen, thereby confirming the presence of linalool in the original fraction.

I.R. Spectrum: The I.R. spectrum of the fraction finally confirmed it to be linalool.\cite{viii}

Fraction (viii) Table I (citronellol)

The fraction was purified by chromatography over a column of alumina and eluted with benzene, gave after removal of the solvent a fraction b.p. 93°/4 mm, $D_{25}^0 0.8558$, $n_{D}^{25} 1.4569$, $[\alpha]_{D}^{25} 20° 13'$. It had a typical rose like odour.

Identification of the functional group by TLC

The fraction gave a reddish violet spot characteristic of citronellol, by spraying the TLC plate with a mixture of anisaldehyde and concentrated sulphuric acid.

It was further confirmed by Co TLC with an authentic specimen of citronellol.

I.R. Spectra of citronellol

The I.R. spectrum (Fig. \textsuperscript{vii}) of the fraction finally confirmed the presence of citronellol.

Fraction (viii) Table I (geraniol)

Geraniol was suspected to be present as the fraction gave a rose like odour. It was isolated as follows:

The fraction (12 g) was thoroughly mixed with anhydrous calcium chloride and left for 24 hr. in a dessicator. The product
formed was decomposed with water and steam distilled. The pure fraction (GLC Fig. ***) isolated in the usual way had the following constants:

\[ D_{25}^{25} = 0.8319, \quad n_D^{25} = 1.4768, \quad [\alpha]_D^{30} = 0. \]

**Oxidation of fraction** 18

The fraction (7.5 g) was oxidised with a mixture of potassium dichromate (5 g), concentrated sulphuric acid (6.25 g) and water (50 cc) by refluxing for 2 hr. The oxidised product was isolated by adding sodium hydroxide solution and steam distillation. The distillate was refluxed with saturated sodium bisulphite solution for 2 hr. On cooling a white addition product bi-separated, which when mixed with sodium carbonate and steam distillation gave a light yellow liquid (1.3 g) with a citrus smell.

α-Citryl, β-naphthyl cinchoninic acid, a derivative of the above light yellow liquid gave m.p. and mixed m.p. 191° thus showing the fraction to be geraniol.

**Fraction (ix) Table I (gerany acetate)**

The fraction on distillation gave a colourless liquid, b.p. 114°/10 mm, \( D_{25}^{25} = 0.9104, \quad n_D^{25} = 1.4647. \)

**Saponification with alcoholic potassium hydroxide**

3 g of the fraction was dissolved in 10 ml of alcohol. 10 ml of alcoholic KOH was added to it and the contents were refluxed on a water bath for 2 hr. Anhydrous calcium chloride (5 g)

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was added to this solution. The solution was allowed to stand for 3 hr. and filtered. The solid complex so obtained was taken in a beaker and 15 ml of water was added to it, and the separated oil extracted with ether. Removal of ether gave a liquid with a rose like smell, identified to be geraniol by Co TLC with an authentic specimen.

A Co TLC and Co GLC of the original fraction with an authentic specimen of geranyl acetate, confirmed its identity.

Fraction (x) Table I

The fraction on distillation over metallic sodium gave a fraction b.p. 130-133°/12 mm; \(D_{25}^2\) 0.8702, \(n_D^2\) 1.4929.

Preparation of trihydrochloride

1.5 g of the fraction was dissolved in 10 ml of ether. Hydrogen chloride gas was bubbled through it for 30 min., and the mixture allowed to stand overnight.

Crystals of trihydrochloride separated out which on recrystallisation from alcohol gave m.p. and mixed m.p. 81°.

Dehydrogenation with sulphur

Dehydrogenation of the fraction with sulphur did not produce naphthalene thereby distinguishing the identity of the fraction. Final confirmation of the fraction was achieved by Co TLC and Co GLC with an authentic specimen.

Fraction (xi) Table I (mixture of unidentified azulenes)

The fraction gave three peaks with GLC (Fig. XXI) showing
the presence of three components.

Chemical methods of analysis gave positive reactions for azulenes. But the mixture could not be separated into individual components either by chromatography or fractionation. I.R. studies (Fig. ××II) were carried out to study the various groups present in the mixture. But no definite conclusions were arrived at.

SUMMARY AND CONCLUSION

1) The essential oil of Toddalia asiatica was obtained from the leaves (shade dried) by steam distillation as a light green mobile liquid with a faint orange like smell in an yield of 0.7%.

2) The oil was found to contain 39% of terpenic hydrocarbon and a mixture of azulenes (three) which could, however not be separated either by fractionation or by chromatography.

3) The essential oil has been found to have the following percentage composition:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>geraniol</td>
<td>17.24</td>
</tr>
<tr>
<td>citronellol</td>
<td>14.4</td>
</tr>
<tr>
<td>P-myrcene</td>
<td>13.33</td>
</tr>
<tr>
<td>d-limonene</td>
<td>11.1</td>
</tr>
<tr>
<td>geranyl acetate</td>
<td>10.85</td>
</tr>
</tbody>
</table>
\[ ^3\Delta \text{-carene} \quad 5.75 \\
\text{linalool} \quad 5.74 \\
\text{citronellal} \quad 4.17 \\
\text{ocimene} \quad 4.1 \\
\text{bisabolene} \quad 3.98 \\
\text{limettin} \quad 1.55 \\
\text{azulenes} \quad 3.7 \\
\text{(unidentified)} \\
\]

4) The plant has been used in the indigenous medicine as a tonic, stimulant and antipyretic. It seems likely that the essential oil could be tried for these properties more profitably, though the antipyretic property has already been proved to be less than that of quinine.

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