STUDIES IN SESQUITERPENES

PART II.

SESQUITERPENES OF THE ESSENTIAL OIL FROM THE OLC-SEED OF

HARDWICKIA PINNATA.

ORGANIC CHEMISTRY LABORATORIES.
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CHAPTER I.

THE ESSENTIAL OIL FROM THE OLEO-BRIN OF HARDWICKIA PINNATA.
Hardwickia Pinnata.

Tamil: Kolavu, Madayan, Sampurani
Malayalam: Skurali, Kiyaru, Kella.
Kanada: Yennemara.
Marathi: Aanja.

(Ref: Indian Medicinal Plants: Khatkar & Basu. 1993 Ed. W.I.)
THE ESSENTIAL OIL FROM THE OLEO-RESIN OF HARDWICKIA PINNATA.

*Hardwickia Pinnata* (N.C. Caesalpiniacea) is a very large and handsome tree found in the evergreen forests of western Ghats from South Kanara to Travancore. It is also known as the Malabar Mahogany (Kanada: Yennemara; Tamil: Enneykolavu; Malayalam: Shurali; Marathi: Anjana). It grows to a height of about 100 ft. with a trunk of about 4-8 ft. in diameter. Its wood is moderately hard and yields a valuable timber; the sapwood is large and the heartwood is dark red or reddish brown, exuding a red sticky resin. (1, & 2).

The Oleo-resin.

The tree, when tapped, yields a dark oleo-resin. The yield of the resin is about 4 to 16 gallons for a single tree. Sometimes a tree is found which yields no oil at all (3). Though the oleo-resin has the smell and taste of Copaiba, its characteristics are sufficiently distinct to prevent any confusion (4).

**Medicinal Uses:** The balsam of Hardwickia has been used in India for the treatment of Gonorrhoea (1). The resin exuding from the heartwood is used for dressing the sores of elephants (5).

**Composition.** According to G. Seigel (6), the oleo-resin contains 48.5% of volatile oil, 48.3% of resin acids and 3.2% of resene. Schimmel and Co. (7) got 44% of the oil. Iyer and Sudborough (2) determined the percentage of the volatile oil as 43-47%. The latter authors prepared caryophyllene alcohol from the oil by Wallach and Walkers method (8). They also prepared a nitrosochloride m.p.161° (α-caryophyllene nitrosochloride, m.p.
176°), and a nitrosate, m.p. 153-54° (α-caryophyllene nitrosate, m.p. 168°) and concluded that the oil chiefly consists of α-caryophyllene and that the β-caryophyllene is absent. But as is evident from the comparison of the m.p. of the derivatives given above, their derivatives were not sufficiently pure and as such their conclusion about the present of α-caryophyllene was based on unsound grounds. Further, their statement about the absence of β-caryophyllene is erroneous in view of the facts now established.

The essential Oil.

In order to study the constituents of the volatile oil, we have steam-distilled two lots of the oleo-resin obtained from Tinnevelly district in May 1946 and October 1947. It has been found that the yields of the essential oil and the relative proportions of the chief constituents vary considerably with the season. The first sample gave us only 26% of the oil, which boiled over a comparatively wider range. The second specimen yielded 48.3% of oil consisting mostly of the lower boiling sesquiterpenes. The essential oil obtained from the second lot of the oleo-resin has been investigated fully.

The oil has a characteristic resinous odour and a pungent bitter taste. The oil as obtained on steam-distillation from a large copper still had a clear green colour, but the redistilled oil is colourless. Its various constants are given in Table I and have been compared to those recorded by other investigators.

The oil on careful and repeated fractionation furnished
six fractions. The physical properties of the various fractions obtained are recorded in Table II. A survey of these properties indicates that the oil consists entirely of bicyclic sesquiterpenes. This is confirmed by the analyses of the separate fractions.

**Fraction I.**

Fraction I, which constitutes about 80% of the oil, has been shown to consist essentially of \( \beta \)-caryophyllene. The presence of this hydrocarbon was established by the preparation of the characteristic blue nitrosite, m.p.110\(^\circ\). The m.p. of the compound as given in the literature (9) is 115\(^\circ\). Since we were unable to raise the m.p. of our product, an authentic sample of \( \beta \)-caryophyllene nitrosite was prepared from genuine caryophyllene (obtained from the British Drug House, London) which also melted at 110\(^\circ\) and did not depress the m.p. of our product. The fraction

<table>
<thead>
<tr>
<th>Constant</th>
<th>Present sample</th>
<th>Schimmel &amp; Co. (7)</th>
<th>Iyer and Sudborough (2)</th>
<th>Travancore</th>
<th>Mysore</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d )</td>
<td>0.9045(^{30.8})</td>
<td>0.9062(^{15})</td>
<td>0.921(^{15})</td>
<td>0.908(^{15})</td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>1.4949(^{30.8})</td>
<td>-</td>
<td>1.506(^{26})</td>
<td>1.506(^{26})</td>
<td></td>
</tr>
<tr>
<td>([\alpha]_D)</td>
<td>-9.4</td>
<td>+7(^\circ) 42'</td>
<td>-1.78(^\circ)</td>
<td>-7.96(^\circ)</td>
<td></td>
</tr>
<tr>
<td>Acid value</td>
<td>Nil.</td>
<td>0.86</td>
<td>trace</td>
<td>trace</td>
<td></td>
</tr>
<tr>
<td>Saponification value</td>
<td>Nil.</td>
<td>2.88</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>Acetyl value</td>
<td>Nil.</td>
<td>-</td>
<td>12.6</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>
also yields \( \beta \)-caryophyllene alcohol on hydration with Bertram-Halbaum reagent. Further, the presence of the hydrocarbon has been confirmed by the preparation of caryophyllene dihydrochloride in a 60\% yield. The physical constants, especially the optical rotation, of the fraction III agree more closely with those recorded for \( \beta \)-caryophyllene. This fraction yields the dihydrochloride in practically the same yield, but the nitrosite is obtained in about double the yield. This indicates that probably the fraction I contains another strongly laevorotatory hydrocarbon capable of yielding the same dihydrochloride. It is not unlikely that some \( \gamma \)-caryophyllene is present in the oil but its presence could not be confirmed by the preparation of its nitrosocarbom chloride.

**Reaction with Maleic Anhydride.**—In 1935, Ruzicka and Zimmermann (10) made the interesting observation that the caryophyllene mixture as obtained from clove oil is capable of reacting with maleic anhydride in benzene to yield a crystalline adduct (m.p. 98\(^\circ\)) corresponding to about 50\% of the terpene. Since, the chief constituent of the caryophyllenes is supposed to be \( \beta \)-caryophyllene, it was considered likely that the product has originated from this hydrocarbon, which, however, does not contain a conjugated system of double bonds. A careful reinvestigation of the problem in 1941 by Ruzicka, Plattner and Balla (11) revealed, that the compound is probably formed by diene synthesis by addition to a relatively strongly laevorotatory monocyclic sesquiterpene. They examined several different samples of caryophyllene and found that the yields of the adduct are highly variable, and that samples giving higher yields of the dihydro-
chloride, give poor yields of the maleic anhydride addition product.

The reaction of maleic anhydride on fraction I has also been studied. But the hydrocarbon does not give the product, m.p. 98°. Instead, another crystalline product m.p. 170-20° has been isolated in a poor yield. This supports Mazicka's results that the adduct m.p. 98° is not derived from α-caryophyllene.

Fraction V.

Fraction V does not yield any picrate forming material on dehydrogenation with selenium and also did not give the blue nitrosite. The fraction consists chiefly of humulene (α-caryophyllene) as shown by the preparation of the crystalline nitrosodchloride and nitrosate.

Fraction VI.

Fraction VI gives cadalene and a trace of an unidentified azulene on dehydrogenation with selenium. 1-Cadinene dihydrochloride is obtained in a poor yield by passing dry hydrochloric acid gas through its ethereal solution. The fraction is slightly dextro-rotatory and does not give the characteristic colour reaction of cadinene. The cadinenic hydrocarbon present in this fraction may be either 1-cadinene or some other sesquiterpene capable of giving cadinene dihydrochloride. (See Chapter II).

EXPERIMENTAL.

The Oleo-resin.

The oleo-resin was collected by the courtesy of the Forest
Range Officer, Ambasamudram, Tinnevelly, in August 1947 and was received by us in October 1947. The specimen was a thick viscid liquid of a dark brown (almost black) colour. When viewed in thin layers, the oleo-resin looked transparent and was of a greenish yellow colour. It had a characteristic odour which was not unpleasant.

Fractionation of the Essential Oil.

The Essential Oil.

The oleo-resin (29 lbs.) was steam-distilled from a large copper still, when 14 lbs. (48.3%) of a light green oil were obtained. The oil was dried over anhydrous sodium sulphate and distilled from an ordinary Claisen's flask under vacuum. The oil was obtained as a colourless, mobile liquid, bp. 100-105°/2 mm. (125-129°/10 mm.). The various constants of the redistilled oil as recorded in Table I were determined by standard procedures.

Fractionation.

The oil was carefully fractionated in vacuum first at 2 mm. The various fractions, thus obtained, were then systematically and repeatedly fractionated at 30-40 mm. in order to have sharper cuts. A final distillation of each fraction was carried out at 2 mm. over sodium. The results of the fractionation are given in Table II.

A fractionating column of the partial condensation take-off type, as illustrated in the adjoining figure, was used. Various
types of packings, including single-turn copper helices made from 18 B.S. gauge copper wire, were tried, but flooding could not be controlled, hence the column was indented (Vigreux type). Asbestos rope 3" in diameter was wound round the column which was finally covered with several rounds of asbestos paper to serve as insulation.

Table II.

<table>
<thead>
<tr>
<th>No.</th>
<th>E.P.</th>
<th>Weight in gms.</th>
<th>Percent yield</th>
<th>30.8°</th>
<th>30.8°</th>
<th>24°</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>d</td>
<td>n D</td>
<td>[a] D</td>
<td>MR D</td>
</tr>
<tr>
<td>I</td>
<td>150°/36 mm.</td>
<td>233.8</td>
<td>77.9</td>
<td>0.8976</td>
<td>1.4940</td>
<td>-10.5</td>
</tr>
<tr>
<td></td>
<td>100°/2 mm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>150-2°/36 mm.</td>
<td>86.0</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>152-3°/36 mm.</td>
<td>176.0</td>
<td>5.9</td>
<td>0.8990</td>
<td>1.4365</td>
<td>- 9.3</td>
</tr>
<tr>
<td>IV</td>
<td>153-5°/36 mm.</td>
<td>20.0</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>155-7°/36 mm.</td>
<td>93.0</td>
<td>3.1</td>
<td>0.8997</td>
<td>1.4960</td>
<td>- 6.0</td>
</tr>
<tr>
<td>VI</td>
<td>114-8°/3 mm.</td>
<td>80.0</td>
<td>2.6</td>
<td>0.9026</td>
<td>1.4960</td>
<td>+ 0.5</td>
</tr>
<tr>
<td>VII</td>
<td>Residue</td>
<td>80.0</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Loss</td>
<td>127.0</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

tation. The column is a slight modification of the one described by Thompson (12; cf. 13). The column was operated at a maximum reflux ratio so that there was just no flooding. The rate of distillation was maintained at about 0.5 c.c./min. The reflux ratio was adjusted by the manipulation of the dephlegmator and
by wrapping or unwrapping some cotton wool over the portion of the column just above the insulation. The oil was heated in a metal-bath with proper control of the bath temperature.

The oil (3000 gms.) on fractionation gave seven fractions (vide Table II) on which different physical measurements were made.

**Identification of β-Caryophyllene.**

The evidences for the identification of β-caryophyllene in *Hardwickia Finnata* oil are summarised in Table III.

**The Nitrosite.**

The fraction I (10 c.c.) was dissolved in petroleum ether (10 c.c.; b.p.60-80°) and an aqueous saturated solution of sodium nitrite (10 c.c.) was added. The mixture was chilled in ice and salt mixture to below -10° and glacial acetic acid (10 c.c.) was introduced slowly under shaking during 10-15 minutes. The petroleum ether layer slowly turned blue and towards the end blue needles started separating out. The reaction mixture was kept in the ice bath for another 15 minutes and the crystals that separated out were filtered off and washed with a little petrol and further purified by recrystallising twice from dilute alcohol in long, blue needles, m.p.110° with effervescence. Yield from fraction I was 1.4-1.6 g. (10-12%); fraction III gave nearly double this yield.

Further recrystallisations of the product from dilute alcohol or petroleum ether (b.p.40-60°) failed to raise the melting point.
Table III.

<table>
<thead>
<tr>
<th>Property</th>
<th>( \beta )-Caryophyllene</th>
<th>Fraction I</th>
<th>Fraction III</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.p.</td>
<td>103-103.5(^\circ)/4 mm.*</td>
<td>100.0(^\circ)/2 mm.</td>
<td>102-103(^\circ)/2 mm.</td>
</tr>
<tr>
<td>Density.</td>
<td>d(20^\circ) 0.9075*</td>
<td>d(20^\circ) 0.8976</td>
<td>d(20^\circ) 0.8990</td>
</tr>
<tr>
<td>Refractive Index.</td>
<td>n(20^\circ) 1.5496*</td>
<td>n(D20^\circ) 1.4040</td>
<td>n(D20^\circ) 1.4055</td>
</tr>
<tr>
<td>Optical Rotation.</td>
<td>([\alpha]_D -8.16^\circ)</td>
<td>([\alpha]_D -10.5^\circ)</td>
<td>([\alpha]_D -9.3^\circ)</td>
</tr>
<tr>
<td>% C.</td>
<td>88.16 (Calc.)</td>
<td>88.01</td>
<td>88.22</td>
</tr>
<tr>
<td>% H.</td>
<td>11.84 (Calc.)</td>
<td>11.90</td>
<td>11.75</td>
</tr>
<tr>
<td>M.p. of Nitrosite.</td>
<td>115.0(^\circ)*</td>
<td>110.0(^\circ)</td>
<td>110.0(^\circ)</td>
</tr>
<tr>
<td>(110.0(^\circ))**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.p. of Dihydrochloride.</td>
<td>69-70(^\circ)</td>
<td>68-8(^\circ)</td>
<td>68-8(^\circ)</td>
</tr>
<tr>
<td>M.p. of ( \beta )-Caryophyllene Alcohol.</td>
<td>94-96(^\circ)</td>
<td>95-6(^\circ)</td>
<td>95-8(^\circ)</td>
</tr>
</tbody>
</table>

The mixed melting points of the nitrosite, dihydrochloride, and \( \beta \)-caryophyllene alcohol prepared from genuine caryophyllene (S.D.H.) with the corresponding derivatives prepared from these fractions were 110\(^\circ\), 68-8\(^\circ\) and 95-96\(^\circ\) respectively.

* Naves and Perrottet, (14).

** The m.p. 110\(^\circ\) is of the product prepared by the author from genuine caryophyllene. Deussen (9) has recorded the m.p. as 115\(^\circ\).

† Schreiner and Kremers (15).

‡ Wallach and Walker (8); Asahina and Tsukamoto (16).
= 10 =

The dihydrochloride.

The hydrocarbon (10 g.) was mixed with anhydrous ether (10 c.c.) and cooled in an ice-salt bath (-20°). The well-cooled solution was saturated with dry hydrochloric acid gas and the practically colourless solution, thus obtained, was kept in the freezing mixture for sixteen hours. The solvent and the excess of the hydrochloric acid gas were sucked off in vacuum with a feeble current of dry air being passed through it. The residual syrup was chilled in a freezing mixture (-20°) for half-an-hour with occasional scratching, when the product solidified. This was taken up in 7 c.c. of ether and 15 c.c. of alcohol were added. The clear solution was chilled as before for half-an-hour. The white crystals were filtered off and washed with a little cold alcohol. The product was recrystallised from alcohol-ether mixture in the same way as white needles, m.p. 68-69°; yield 50-60%.

β-Caryophyllene Alcohol.

The fraction I (10 g.) was mixed with 98% acetic acid (20 g.) and 50% sulphuric acid (1 g.). The reaction mixture was heated at 50-60° for eight hours. The dark violet reaction mixture was poured into excess of water and the crude acetyl β-caryophyllene alcohol was extracted with ether and then hydrolysed with alcoholic potash by refluxing for two hours. The hydrolysed product was worked up in the usual manner with ether. The solvent was removed and the residue was fractionated in vacuum. The β-caryophyllene alcohol distilled at 120-125°/1 mm. The distillate which had crystallised out was recrystallised from petrol ether (40-60°) at 10° to a constant melting point, as white silky needles, m.p. 95-96°.
[cf. Bertram and Walbaum (17), Wallach and Walker (8) and Asahina and Tsukamoto (16)].

Reaction with Maleic Anhydride.

The fraction did not give the colour reaction with diazotised p-nitroaniline (Biester's test for conjugation, 18, cf. 19). β-Caryophyllene as isolated from clove oil gives an orange brown colour in a few minutes (14).

A mixture of the terpene (85 g.), maleic anhydride (15 g.) and dry benzene (75 c.c.) was refluxed for twenty-four hours. A clear yellow solution resulted. The solvent was removed under a slight vacuum and the residue was fractionated at a low pressure. Maleic anhydride distilled over first, followed by the unreacted terpene (18 g., 72%). A higher boiling fraction b.p. 180-200°/2 mm. (4 g.) was obtained and a polymer was left in the flask. The fraction (b.p. 180-200°/2 mm.) was obtained as a syrup which crystallised on trituration with light petrol. The crystals were filtered off and recrystallised from a mixture of benzene and petrol, m.p. 177-80°. Further recrystallisations from the same mixture of solvents raised the m.p. to 179-80° with slight shrinking at 177-78°. The product was obtained in white silky needles, yield 0.6 g. (cf. 11).

Identification of Humulene.

The evidences for the presence of Humulene in the essential oil from Hardwickia Finnata oleo-resin are summarised in Table IV.
Table IV.

<table>
<thead>
<tr>
<th>Property</th>
<th>Humulene.*</th>
<th>Fraction V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.P.</td>
<td>263-65°</td>
<td>155-57°/36 mm.</td>
</tr>
<tr>
<td>Density.</td>
<td>d$^{15°}$ 0.9001</td>
<td>d$^{30.8°}$ 0.8997</td>
</tr>
<tr>
<td>Index of Refraction.</td>
<td>n$^{19°}$ 1.5021</td>
<td>n$^{30.2°}$ 1.4960</td>
</tr>
<tr>
<td>Optical Rotation.</td>
<td>Inactive.</td>
<td>-6.0</td>
</tr>
<tr>
<td>M.P. of Nitrosate.</td>
<td>163°, 161°</td>
<td>160°</td>
</tr>
<tr>
<td>M.P. of Nitrosochloride.</td>
<td>176°, 174°*</td>
<td>175°</td>
</tr>
<tr>
<td>M.P. of Nitropiperidide.</td>
<td>153°</td>
<td>153°</td>
</tr>
</tbody>
</table>

* Chapman (20).

† Simonsen and Todd (21).

The Nitrosate.

The terpene (10 c.c.) was dissolved in glacial acetic acid (10 c.c.) and freshly prepared (undistilled) iso-amyl nitrite (11 c.c.) was added. The resulting green solution was chilled in a ice-salt bath. A well-cooled mixture of glacial acetic acid (10 c.c.) and concentrated nitric acid (d, 1.42; 10 c.c.) was then slowly added to the above solution with shaking. The temperature was not allowed to go beyond 0°. After 15 minutes, 50 c.c. of alcohol were added and the clear green solution was left aside in
the ice-bath for 3-4 hours. The white solid which had separated out was filtered off, washed with some cold alcohol and dried. The product was recrystallised several times from a mixture of benzene and alcohol as fine silky needles, m.p. 160° with decomposition.

The Nitrosochloride.

A well cooled alcoholic solution of hydrochloric acid gas (20 c.c.) was added slowly to a chilled mixture of the hydrocarbon (20 c.c.), absolute alcohol (20 c.c.), ethyl acetate (20 c.c.) and iso-amyl nitrite (20 c.c.). The deep bluish green mixture was left in the ice-bath for an hour and was then kept at room temperature for another two hours. The white crystalline solid was filtered off and washed with ice-cold alcohol. The product was repeatedly crystallised from a mixture of chloroform and benzene till the m.p. became constant. The product was obtained in colourless prisms, m.p. 175° (decomp.), yield 5.0%.

The nitrolpiperidide was prepared by the action of piperidine on the nitrosochloride in the usual manner. The compound was recrystallised from dilute alcohol as fine white needles, m.p. 153°.

Presence of a Cadinenic Sesquiterpene.

Dehydrogenation.

The fraction VI (2 g.) and selenium (4 g.) were heated together at 300-315° for thirty-two hours when the evolution of hydrogen selenide had practically ceased. In the beginning the reflux slowly acquired deep blue colour, which vanished towards
the end. The product was worked up in the usual manner. The cadalene was identified as its picrate, m.p. 114-15°. Mixed m.p. with an authentic sample remained undepressed.

The Dihydrochloride.

The fraction VI (1 c.c.) was dissolved in dry ether (1 c.c.) and saturated with dry hydrochloric acid gas at below 0° and the mixture kept at 0° for twenty-four hours. The ether and excess gas were then sucked off and the residue was taken up in 1 c.c. of ethyl acetate. The solution was chilled to -10° and seeded with a speck of cadinene dihydrochloride when crystals began to separate out. After sometime the product was filtered off and recrystallised from ethyl acetate in white needles m.p. 117-18°. Cadinene dihydrochloride, m.p. 117-18° did not depress the m.p.

SUMMARY.

The essential oil from the oleo-resin of *Hardwickia Pinnata* has been examined and previous work has been shown to be erroneous.

The oil has been found to consist essentially of ρ-caryophyllene (85%), humulene (10%) and a cadinene sesquiterpene (5%).

Support has been lent to Kuzicka's view that the maleic anhydride adduct m.p. 92° is not derived from ρ-caryophyllene, by the inability of ρ-caryophyllene from *Hardwickia Pinnata* to yield this product on interaction with maleic anhydride.

REFERENCES.

7. Schimmel and Co., Reports April 1905 and April 1907.
11. Ruzicka, Plattner and Balla, ibid., 1941, 24, 1219.