PART IV

CHEMICAL EXAMINATION OF THE BERRIES OF CARISSA SPINARUM LINN.
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Such cooling fruits, as the kind hospitable
wood supply - Milton

Carissa spinarum or Karaunda or Karamadika as it is known in Hindi and Sanskrit respectively, is a small
spinous ever green shrub met with throughout India in dry
regions especially in Madhya Pradesh and Punjab. The
berries are edible and are juicy sweet when ripe. The juice
is of violet blue colour. The shrub spreads upto 3.6 m.
high, armed, where the stem branches, with opposite straight
or forked thorns, which arise from between. The shrub
makes exceedingly strong fence and its number of sharp
spreading thorns render such hedges almost impassable.
The leaves are fairly rich in tannins. Its roots in
combination with other roots are used in rheumatism by
Mundas ( wild tribe ) of Chota Nagpur (India). If taken
internally, causes purging which can not be stopped.

The presence of an alkaloid and salicylic acid
has been reported in the allied plant Carissa carandas Linn.
No work, however, seems to have been done on Carissa
spinarum. As such the plant has been taken up for detailed
chemical examination. A fixed oil has been extracted
from the dried fruits and its composition determined. The fixed oil gives a white crystalline deposition melting at $224^\circ_C$ (Graph 7). The unsaponifiable matter of the oil has shown the presence of three sterols, $\beta$ sitosterol, stigmasterol and $\gamma$ sitosterol. The defatted berries have given a steroidal glycoside 'Carissonin', which has been hydrolysed to the aglucone carissogenin and two sugars glucose and rhamnose. Free glucose has also been isolated from the fresh berries which are found to contain phlobotannin.

**EXPERIMENTAL**

The fresh berries were collected in winter and were dried in shade, and these were used for all investigations.

A small portion of berries was burnt in a porcelain dish whereby a whitish -gray ash (4.32 %) was obtained which on analysis was found to contain calcium, iron, sodium, silica, traces of potassium, carbonate, sulphate, chloride and phosphate.

**Extraction with different solvents:**

100 gms. of the powdered material was extracted successively in soxhlet with the various solvents in the
order given below when the following results were obtained (the extracts were dried at 100°C), on removal of the solvent.

1. Petroleum ether (60-80): A dark green oil (6.07%) was obtained.

2. Chloroform: A dark greyish green mass (1.3%) consisting mostly of chlorophyll was obtained.

3. Ether: Thick sticky resinous mass (1.72%) consisting mostly of chlorophyll was obtained.

4. Ethyl acetate: A light green extract (1.1%) consisting of chlorophyll and tannins was obtained.

5. Absolute alcohol: A dirty brown mass (16.32%) was obtained.

6. Water: A reddish violet coloured residue (9.24%) was obtained which gave tests for tannins and reducing sugars.

Fixed oil:

5 Kg. of the dried berries were powdered and extracted in lots of 100 gms. with petroleum ether. A green coloured extract was obtained which gave a dark green oil on removal of the solvent. The oil had the following properties: (p. 105)
Specific gravity at 30°C
$n_D^{30}$
Iodine value
Saponification value
Acetyl value
Acid value
Unsaponifiable matter

Preparation of mixed fatty acids from the oil and separation of the unsaponifiable matter:

100 gms. of the oil dissolved in absolute alcohol was saponified in the usual manner with potassium hydroxide by refluxing for four hours. After distilling off the alcohol, the soap was dissolved in water and the unsaponifiable matter extracted with a mixture of equal amounts of ether and petroleum ether (40-60°C). The last traces of the solvent were removed from the soap solution, and it was decomposed with sulphuric acid. The mixed fatty acids so liberated were extracted with ether. On removal of the ether, these had the following characteristics:

<table>
<thead>
<tr>
<th>Consistency</th>
<th>Semi solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity at 30°C</td>
<td>0.8933</td>
</tr>
<tr>
<td>Mean molecular weight</td>
<td>293.8</td>
</tr>
<tr>
<td>Iodine value (Hanu's)</td>
<td>95.3</td>
</tr>
</tbody>
</table>
The mixed fatty acids were separated as given below in to saturated and unsaturated portion by lead salt method of Twitchell.

<table>
<thead>
<tr>
<th>Acids</th>
<th>% in mixed fatty acid</th>
<th>% in oil</th>
<th>I.V.</th>
<th>M.Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated (solid)</td>
<td>18.2%</td>
<td>19.6</td>
<td>0.57</td>
<td>284.9</td>
</tr>
<tr>
<td>Unsaturated (liquid)</td>
<td>74.54</td>
<td>77.8</td>
<td>135.59</td>
<td>280.79</td>
</tr>
</tbody>
</table>

Bromination of the liquid acids:

A solution of bromine (1 ml.) in glacial acetic acid (3 ml.) was added with constant stirring and cooling to an ice-cold solution of the liquid acids (5g.) in ether. The addition of bromine was continued till a yellow colour persisted. The solution was kept in the frigidaire overnight, no crystalline bromide separated thus indicating the absence of linolenic acid. On removal of the solvent a viscous bromide was obtained which on being freed from bromine gave a solid residue which was dissolved in petroleum ether (40-60°) and again cooled in the frigidaire, when crystals of linoleic tetra bromide (confirmed by m.p. and mixed m.p.) separated out from the solution, showing the presence of linoleic acid. The filtrate on evaporation gave oleic dibromide, which on
recrystallisation from rectified spirit gave m.p. and mixed m.p. 29-30\(^{\circ}\)C.

1. Weight of unsaturated acids taken 5.029 gm.
2. Weight of linoleic tetrabromide insoluble in petroleum ether 2.78 gm.
3. Weight of residue (consisting of above dibromide and tetrabromide) 6.21 gm.
4. Weight of oleic dibromide in the residue (3) 4.89 gm.

**Oxidation of liquid acids**: Further confirmation of the presence of oleic acid and linoleic acid was done by oxidation method.

6 gms. of the liquid acids was dissolved in dilute caustic soda, the volume was made up to 500 ml. and a concentrated solution of potassium permanganate (40 gm.) added slowly with constant stirring during the course of 15-20 minutes. The excess of potassium permanganate was destroyed by passing sulphur dioxide. The oxidised oil floated as a white suspension. It was filtered and washed with a little ether to remove the adhering unoxidised oil, and dried. The dried solid was extracted with ether (250 ml.). By this process dihydroxy acid if any present, is removed. Removal of ether gave a solid acid which on recrystallisation from petroleum ether gave a m.p. 130\(^{\circ}\)C.
undepressed when mixed with an authentic specimen of
dihydroxy stearic acid. The insoluble oxidised material
left after extraction with ether was extracted with a
large volume of hot water. The aqueous solution thus
obtained was kept in ice bath, when a solid separated. This
on recrystallisation from rectified spirit melted at
163°C. undepressed when mixed with an authentic specimen of
tetrahydroxy stearic acid. The formation of di and
tetrahydroxy stearic acids shows the presence of oleic and
linoleic acids, which were found on calculation to be 45.5%
and 32.3 % respectively.

**Methyl esters of solid acids:**

The saturated acids were dried and esterified by
refluxing with 100 ml. of absolute methyl alcohol saturated
with dry hydrogen chloride for six hours. After keeping
the contents to 24 hours, methanol was distilled off and
the esters formed extracted with ether. The ether extract
was washed with water followed by dilute sodium carbonate
and finally again with water, dried over anhydrous sodium
sulphate and fractionally distilled, when two main fractions
were collected (i) 210 - 228°C and (ii) 230 - 236°C.
These fractions on cooling deposited solids which on recrystallisation from dilute acetone melted at 60-62° and 68-69° respectively and were identified to be palmitic and stearic acids by mixed melting points with authentic specimens. Thus the oil contained:

<table>
<thead>
<tr>
<th>Acid</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>7.2%</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>12.1%</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>45.5%</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>32.3%</td>
</tr>
</tbody>
</table>

**Unsaponifiable matter:**

The unsaponifiable matter from the saponified oil was extracted with ether. The ethereal solution was thoroughly washed with water and dried over anhydrous sodium sulphate. Removal of ether gave a whitish yellow wax 3.21g. Chromatography\(^\text{10-11}\) of this wax (2 gm.) in light petroleum ether (40-60°) over a column of alumina using successively light petroleum, ether, ethyl acetate and methanol gave the following fractions on removal of the solvents. Each fraction was eluted of 10 ml.
<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluent</th>
<th>Colour of the fraction</th>
<th>Residue on removal of the solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 2</td>
<td>Light petroleum ether (40-60°)</td>
<td>Colourless</td>
<td>Nil</td>
</tr>
<tr>
<td>3 – 6</td>
<td>Petroleum ether &amp; ether (3:1)</td>
<td>Yellowish white</td>
<td>Yellowish white solid 0.2 g.; 0.08%</td>
</tr>
<tr>
<td>7 – 12</td>
<td>Petroleum ether &amp; ether (1 : 1)</td>
<td>Yellowish white</td>
<td>Yellowish white solid 0.231 g.; 0.089%</td>
</tr>
<tr>
<td>13 – 18</td>
<td>Ether</td>
<td>Colourless</td>
<td>Colourless solid 0.192 g.; 0.079%</td>
</tr>
<tr>
<td>19 – 24</td>
<td>Ethyl acetate</td>
<td>Colourless</td>
<td>Colourless solid 0.339 g.; 0.1%</td>
</tr>
<tr>
<td>25 – 30</td>
<td>Methanol</td>
<td>Yellowish</td>
<td>Yellowish solid 0.127 g.; 0.05%</td>
</tr>
</tbody>
</table>

All the above fractions gave a positive test for sterols, and were identified as given in the table II (page 111).
<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Crystallised from</th>
<th>m.p.</th>
<th>$\left(\alpha\right)_D^{30}$</th>
<th>Found</th>
<th>Calculated for</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 - 6</td>
<td>Ethyl alcohol</td>
<td>147-148°C</td>
<td>-41°</td>
<td>C 83.30%</td>
<td>C_{29}H_{50}O</td>
<td>$\gamma$-sitosterol</td>
</tr>
<tr>
<td>7 - 12</td>
<td>Ethyl alcohol</td>
<td>170-171°C</td>
<td>-49°</td>
<td>H 11.90%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-24</td>
<td>Ethyl alcohol</td>
<td></td>
<td></td>
<td>C 85.36%</td>
<td>C_{29}H_{48}O</td>
<td>Stigmasterol</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td></td>
<td></td>
<td>H 12.27%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-30</td>
<td>Methyl alcohol</td>
<td>135-136°C</td>
<td>-37.4°</td>
<td>C 84.1%</td>
<td>C_{29}H_{50}O</td>
<td>$\beta$-sitosterol</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td></td>
<td></td>
<td>H 11.95%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Derivative</td>
<td>Found C</td>
<td>Found H</td>
<td>Calculated for</td>
<td>Recrystallised from</td>
<td>m.p.</td>
<td>(\alpha^3_{D})</td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------</td>
<td>---------</td>
<td>----------------</td>
<td>---------------------</td>
<td>------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>(\gamma)-sitosterol acetate</td>
<td>80.94%</td>
<td>10.97%</td>
<td>C 81.52%</td>
<td>Ethanol</td>
<td>140-141°C</td>
<td>-48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 11.48%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\gamma)-sitosterol benzoate</td>
<td>83.72%</td>
<td>10.23%</td>
<td>C 83.34%</td>
<td>Ethanol</td>
<td>150-151°C</td>
<td>-17.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 10.49%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\gamma)-sitosterol dibromide</td>
<td></td>
<td></td>
<td></td>
<td>Methanol</td>
<td>142-143°C</td>
<td>-44.12</td>
</tr>
<tr>
<td>Stigmasterol acetate</td>
<td>80.87%</td>
<td>11.53%</td>
<td>C 81.75%</td>
<td>Ethanol</td>
<td>210-213°C</td>
<td>-52.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 11.20%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stigmasterol benzoate</td>
<td>83.81%</td>
<td>9.98%</td>
<td>C 83.72%</td>
<td>Ethyl acetate</td>
<td>160-161°C</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 10.07%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\beta)-sitosterol acetate</td>
<td>81.3%</td>
<td>11.21%</td>
<td>C 81.57%</td>
<td>Methanol</td>
<td>130°C</td>
<td>-41.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 11.47%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\beta)-sitosterol benzoate</td>
<td>83.74%</td>
<td>10.93%</td>
<td>C 83.34%</td>
<td>Methanol</td>
<td>142°C</td>
<td>-16.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 10.49%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\beta)-sitosterol 3:5 dinitrobenzoate</td>
<td></td>
<td></td>
<td></td>
<td>Methanol</td>
<td>202-203°C</td>
<td>-12.4</td>
</tr>
</tbody>
</table>
From the above results and these derivatives the presence of \( \beta \)-sitosterol, \( \gamma \)-sitosterol and stigmasterol has been confirmed in the unsaponifiable matter of the fixed oil obtained from the berries of carissa spinarum.

**Examination of the cardiac glycoside:**

The defatted berries were extracted with rectified spirit. On removal of the solvent from the alcoholic extract, the residue was treated with refreshly prepared lead hydroxide, when a precipitate was obtained, which was filtered off.

The excess of lead was removed from the filtrate by passing hydrogen sulphide. After removing the lead sulphide, the filtrate was concentrated under reduced pressure and extracted successively with the following solvents, giving residues on removal of the solvents as given below: (p. 114)
<table>
<thead>
<tr>
<th>Solvents used</th>
<th>Residue after removal of Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Petroleum ether</td>
<td>Yellowish green oily mass.</td>
</tr>
<tr>
<td>2. Ether</td>
<td>Negligible residue.</td>
</tr>
<tr>
<td>3. Chloroform</td>
<td>Darkbrown mass (0.5 g.)</td>
</tr>
<tr>
<td>4. Chloroform &amp; ethanol(9:1)</td>
<td>Brown gummy mass (1.35 g.)</td>
</tr>
<tr>
<td>5. Chloroform &amp; ethanol(1:1)</td>
<td>Dirty brown, gummy sticky mass (2.7 g.)</td>
</tr>
</tbody>
</table>

The residues were tested for the presence of cardiac glycosides by the Legal's$^{12}$ colour reaction, when the presence of cardiac glycosides could be detected in the 3, 4, and 5 fractions by the development of dark red colour.

This was further confirmed by paper chromatography$^{13}$ of the residue using a n-butanol-water system when a blue spot appeared on spraying with antimony trichloride in chloroform. The Rf. value was found to be 0.81.

**Separation of the glycoside$^{14}$:**

Chromatography of an alcoholic extract of the defatted berries on a column of cellulose powder, using
a mixture of ethyl acetate - pyridine - water and benzene as the eluent (5:3:3:1) gave a brownish red amorphous powder which on recrystallisation from a mixture of methanol and ethyl acetate (1:1) gave the glycoside as a brownish crystalline solid, m.p. 258° C. (Found: C, 52.93; H, 7.80. C₃₅H₅₉O₁₉ requires: C, 53.63; H, 7.52%)

Hydrolysis of the glycosides:

The glycoside obtained above was hydrolysed by refluxing it with a mixture of 35 ml. acetic acid, 55 ml. water and 10 ml. hydrochloric acid for 8 hours, when the aglucone separated as a solid which on recrystallisation from methanol and ethyl acetate melted at 208 - 210° C. (Found: C, 65.92; H, 8.67. C₂₃H₃₄O₇ requires: C, 65.40; H, 8.9%)

The aglycone has been provisionally named 'Carissogenin' and the glycoside as 'Carissonin'.

Both the glycoside and aglycone were found to be saturated compounds having a steroidal skeleton (as evidenced by the Libermann Buchards reaction and Salwoskis reaction).

Acetylation of the aglycone:

Carissogenin (0.5 g.) was dissolved in acetic
anhydride (5 ml.) and a drop of pyridine added to it and the mixture heated under reflux for 3-4 hours. The hot mixture was poured into cold water and the acetyl derivative so obtained filtered, washed, and dried. Recrystallisation from alcohol, gave a crystalline solid m.p. 178°C. (Found: C, 62.76; H, 6.82. C_{23}H_{29}O_7(COCH_3)_5 requires: C, 62.65; H, 6.96%).

Benzoylation of carissagenin:

To carissagenin (0.5 g.) in pyridine (10 ml.) was added benzoyl chloride (5 ml.). The mixture was heated under reflux for about one and half hour and on cooling poured with constant stirring into cold water and kept over night. A crystalline mass settled down which was washed with water and then with sodium carbonate solution. Crystallisation from ethyl acetate gave a crystalline benzoyl derivative, m.p. 126°C. (Found: C, 72.38; H, 6.01. C_{23}H_{29}O_8(COCH_3)_5 requires: C, 72.65; H, 5.72%).

Selenium dehydrogenation of carissagenin\textsuperscript{18}:

1 g. of carissagenin was intimately mixed with 5 g. of selenium metal powder and the mixture was taken in a tube. The mixture was heated at first at low
temperature. The temperature was gradually increased to 320 - 325°C (lead bath), and maintained for about an hour. The reaction mixture after cooling was extracted with ether. The ethereal extract was washed with water and dried over anhydrous sodium sulphate. Removal of ether gave a reddish orange compound m.p. 122°C identified to be Diel's hydrocarbon \textsuperscript{19} ($C_{18}H_{16}$).

On the basis of the above observations the following probable structure has been assigned for carissogenin.

\[
\text{\includegraphics{structure.png}}
\]

**Sugars:**

Fresh berries were lixiviated with water and the aqueous extract concentrated under reduced pressure. The
concentrated extract was treated with phenylhydrazine in glacial acetic acid, when on heating for half an hour a yellow osazone was obtained, which on recrystallisation from acetic acid, gave a m.p. and mixed m.p. 206°C, and was identified to be glucosazone.

Chromatographic separation of the osazones of the sugars isolated on hydrolysis of the glucoside by circular paper chromatography:

The sugars obtained after hydrolysis of the glycoside were converted into a mixture of osazones which was identified to consist of glucosazone and rhamnosazone following the method of Barbarin Arreguin. The presence of glucose and rhamnose was thus confirmed in the glycoside.
REFERENCES


15 Kiliani, *Ber.*, 1930, 63, 2866.

16 Liebermann, Ber, 1885, 18, 1803.
18 Diels and Karstens, Annalen, 1930, 478, 129.
Lobelia pyramidalis Wall
Flowering Head