PART III

CHEMICAL EXAMINATION OF THE SEEDS OF

PSORAlea CORYLIFOLIA LINN.
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The curative action of the seeds of Psoralea species in the treatment of leucoderma was known in India since very ancient times. The genus psoralea belongs to the family Leguminosae comprising of more than 100 strongly aromatic species. The most important among the species of the genera psoralea is Psoralea corylifolia Linn., also known as 'Maley tea' or 'Bawchang seed'. It is indigenous to India and is a common herbaceous weed growing throughout the plains. In Hindi it is commonly known as Babchi or Bakuchi. The seeds of this plant have been in use in the Ayurvedic system of medicine since a long time. Mention of its use has been made by ancient Hindu physicians, and according to some they are laxative, stimulant and aphrodisiae. The seeds have been specially recommended in the treatment of leprosy internally and also applied externally in the form of paste or ointment. The drug was so effective in leprosy that it was known as "Kusthanashini" meaning "leprosy destroyer". In other inflammatory diseases of the skin such as psoriasis and in leucoderma it has been recommended both orally as well as locally. The seeds are also said to be anthelmintic,
diuretic and diaphoretic in febrile conditions\textsuperscript{3,4}. Psoralea seeds are really fruits with pericarp adhering to seed coat, 3.5 - 4.5 mm. long, 2.3 mm. broad, pods one seeded, ovoid - oblong, somewhat compressed, glabrous giving the appearance of a bath sponge\textsuperscript{5}, colour dark chocolate to almost black.

Psoralea corylifolia seeds give an orange red fixed oil in a yield of 11.2\%. Menon\textsuperscript{6} obtained a fixed oil from the seeds and reported some of its physical and chemical constants. Sen et al\textsuperscript{7} isolated from the unsaponifiable matter a compound of the formula $C_{17}H_{24}O$ b.p. 180-190\(^\circ\)C/11-15mm., a yellow acid $C_{40}H_{45}O_{10}$ and a methyl glucoside m.p. 105-7\(^\circ\)C., and found the oil to be pharmacologically active. Chopra and Chatterjee\textsuperscript{8} also examined the seeds chemically and isolated a crystalline material from its fixed oil, melting at 135\(^\circ\)C. This was later identified by Jois et al\textsuperscript{9} as psoralen, $C_{11}H_{6}O_{3}$, m.p. 162\(^\circ\)C, by repeated crystallisation of the impure compound melting at 135\(^\circ\)C. Psoralen was found by them to be sparingly soluble in cold petroleum ether and ether, but readily soluble in alcohol and chloroform and to crystallise in long needles from water. Jois and Manjunath\textsuperscript{10}, however, found psoralen to be accompanied by another
compound isopsoralen also, both having similar properties and forming similar derivatives. They assigned the structures I and II to psoralen and isopsoralen respectively.

![Chemical structures](image)

(I)  \( \text{C=O} \)  

(II)  \( \text{C=O} \)

Isopsoralen was identical with the furocoumarin, angelicin\(^{11}\) isolated by Späth and co-workers\(^{12}\) from the roots of Angelica archangelica. Amongst the other workers who chemically examined the seeds, mention may be made of (i) Seshadri and Venkatrao\(^{13}\), who obtained a terpenoid oil, psoralen, isopsoralen and a sterol (probably phytosterol), m.p. 126-128\(^{\circ}\)C, (ii) Chakravarti et al\(^{14}\), who isolated from the pericarp of the seeds a new crystalline substance, psoralidin\(^{15}\) \( \text{C}_{16}\text{H}_{14}\text{O}_{4} \), m.p. 315\(^{\circ}\)C, decompr.), and (iii) Khastgir', Duttagupta and Sengupta\(^{16,17}\), who also isolated psoralen and isopsoralen and studied the sterols from the unsaponifiable matter of its fixed oil.
In addition to these Dey\textsuperscript{18} examined the therapeutic uses of the oleo resinous extract and Mukherjee\textsuperscript{19} studied in detail its use in leucoderma.

The present author studied the fixed oil obtained from the seeds, and found the acids present in it to be composed of 19.6\% of saturated and 69\% of unsaturated ones. These acids were found to be palmitic, stearic, and lignoceric. The unsaponifiable matter obtained from the oil consisted of \( \gamma \)-sitosterol (0.03%) and a new compound \( \text{C}_{20}\text{H}_{28}\text{O}_{2}, \) (0.3\%) which appeared to be a diterpene. In previous investigations, however, Seshadri and Venkatarao reported the probable presence of phytosterol, whereas Khastgir et al (loc cit.) found the presence of stigmasterol. The finding of the present author in this connection seem to be more reliable as the sterol has been isolated and purified by chromatography of the unsaponifiable matter. An acid, m.p. 117\textdegree C, was also isolated from the aqueous extract of the defatted seeds. Lastly a crystalline glycoside m.p. 154\textdegree C, was also isolated, which on hydrolysis gave a flavone, m.p. 212-214\textdegree C and glucose.
EXPERIMENTAL?

The material employed for the investigation consisted of the seeds of psoralea corylifolia obtained from the local market and identified from the Botany department of the University of Saugar.

Analysis of ash:

20 gms. of the seeds were crushed and incinerated completely on a porcelain dish when 1.2 gm. of dirty sticky ash was obtained which on analysis gave test for calcium, magnesium, sodium, potassium, chloride, carbonate and sulphate.

About twenty five gms. of the crushed seeds were extracted with boiling water. The extract did not give any test with ferric chloride, but gave precipitate with lead acetate solution and did not reduce felling solution.

Absence of alkaloids:

A small amount of the crushed material was then extracted with hydrochloric acid solution but the extract gave no test for alkaloid with the usual alkaloidal reagent. This was further confirmed when the proliiou's fluid extract
also gave no test for alkaloids.

Extraction of the seeds with various solvents:

Twentyfive grams of the powdered seeds were extracted in a soxhlet with a number of solvent in the given order when the following results were obtained:

1. **Petroleum ether (60-80)** - An orange yellow extract was obtained which on removal of the solvent gave an orange yellow oil (9-10%) having a characteristic smell.

2. **Benzene** - An orange yellow extract was obtained as in the case of petroleum ether, having the same characteristic smell; on removal of the solvent it gave an orange yellow oil (11.12%).

3. **Ether** - Yellowish orange extract (2%) was obtained.

4. **Chloroform** - Thick dark orange mass (7-8%) was obtained. On removal of solvent the thick orange mass consisted mostly of oleo resins.

5. **Ethyl acetate** - An orange yellow extract was got which on removal of the solvent, yielded negligible quantity of semi solid sticky resin.
(6) **Rectified spirit** - An orange red extract giving a syrupy mass (13-14%) on removal of the solvent was obtained consisting of resins.

Thus on the basis of the above observation the seeds were extracted with petroleum ether (60-80°C) for 18 hours in lots of 100 gms. in a soxhlet apparatus, when an orange yellow extract was obtained. This extract on cooling deposited a crystalline solid, m.p. 163-164°C. Filtration of this solid, and removal of the solvent from the filtrate gave a viscous yellowish orange oil, which on cooling in a frigidaire gave another crop of crystals which on repeated crystallisation from petroleum ether melted at 164-165°C. (Found: C, 69.05; H, 7.58. C₁₂H₁₅O₃ requires C, 69.5, H, 7.24%). The infrared absorption spectrum (graph No.4) shows the presence of a 6 membered lactone ring. This was identical with the previous product m.p. 163-164°C (as was shown by comparison of their infrared spectra - graph No.5).

The oil remaining after the separation of the crystalline solid gave the following constants (p.90).
<table>
<thead>
<tr>
<th>Constants</th>
<th>Author's values.</th>
<th>Values obtained by Seshadri, Manjunath.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref. Index</td>
<td>1.4928 at 30°C</td>
<td>1.4739 at 30°C</td>
</tr>
<tr>
<td></td>
<td>1.5132 at 25°C</td>
<td></td>
</tr>
<tr>
<td>Specific gravity</td>
<td>0.9297 at 25°C</td>
<td>0.9283 at 25°C</td>
</tr>
<tr>
<td></td>
<td>0.9692 at 25°C</td>
<td></td>
</tr>
<tr>
<td>Acid value</td>
<td>12.60</td>
<td>8.01</td>
</tr>
<tr>
<td></td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>Saponification value</td>
<td>179.2</td>
<td>194.7</td>
</tr>
<tr>
<td></td>
<td>117.2</td>
<td></td>
</tr>
<tr>
<td>Iodine value</td>
<td>97.1</td>
<td>96.4</td>
</tr>
<tr>
<td></td>
<td>96.9</td>
<td></td>
</tr>
<tr>
<td>Unsaponifiable matter</td>
<td>2.3</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>2.70</td>
<td></td>
</tr>
</tbody>
</table>

As suggested by Seshadri, the high value for the unsaponifiable matter, may be due to the alkali insoluble terpenoids. Also it has been suggested that due to the presence of resin and hydrocarbons, the acid value of the oils is high while the saponification values is low.

**Saponification of the oil:**

100 gms. of the oil was saponified with alcoholic potassium hydroxide in the usual manner. The soap formed was dissolved in water and the aqueous soap solution was extracted with ether. The alkaline aqueous solution so left was acidified with dilute sulphuric acid. The acids
(93 g.) so liberated were found to have:

Iodine value 115.1
Mean molecular weight 302

This mixture of fatty acids was separated into saturated and unsaturated acids as below by Twitchell's method.  

<table>
<thead>
<tr>
<th>Acids</th>
<th>Amount in gms.</th>
<th>Percentage</th>
<th>Iodine value</th>
<th>Mean molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>17.9</td>
<td>19.6</td>
<td>0.56</td>
<td>294</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>65.6</td>
<td>69</td>
<td>138.6</td>
<td>312</td>
</tr>
</tbody>
</table>

The saturated and the unsaturated fatty acids were identified in the usual way (page 63) and consisted of:

**Saturated**: Palmitic acid 11.4%
Stearic acid 6.2%

**Unsaturated**: Oleic acid 38%
Linoleic acid 31%

Traces of lignoceric acid were also found to be present.  

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The ethereal extract obtained from the aqueous soap solution contained mostly unsaponifiable matter. It was thoroughly washed with water and dried over anhydrous sodium sulphate. On removal of solvent it gave a sweet smelling oily liquid, which was subjected to column chromatography over a column of alumina using successively petroleum ether (40-60°) and (60-80°), benzene, ether and methanol as eluents, when the following fractions were collected:

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluent</th>
<th>Volume collected</th>
<th>Residue on removal of solvent</th>
<th>Weight of residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Petroleum ether (40-60)</td>
<td>10 ml.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4-8</td>
<td>Petroleum ether (40-60) &amp; (60-80) (1:1)</td>
<td>10 ml. Sweet smelling oily mass</td>
<td>0.2 g.</td>
<td></td>
</tr>
<tr>
<td>9-16</td>
<td>Petroleum ether &amp; ether (60-80)</td>
<td>10 ml. Sweet smelling oily mass</td>
<td>0.4 g.</td>
<td></td>
</tr>
<tr>
<td>17-22</td>
<td>Ether</td>
<td>15 ml. Sweet smelling oily mass</td>
<td>0.8 g.</td>
<td></td>
</tr>
<tr>
<td>23-30</td>
<td>Methanol</td>
<td>10 ml. Non crystalline mass</td>
<td>0.1 g.</td>
<td></td>
</tr>
</tbody>
</table>

On removal of the solvent from the petroleum ether and ether fractions (4-22) of the unsaponifiable
GRAPH No. 6

PHASE: CHCl₃
THICKNESS: ~5 m.m.

Wavelength range: 4000 to 700 cm⁻¹
matter, sweet smelling oily product was obtained. Sen et al (loc cit) have also reported the presence of this unsaponifiable oil. The unsaponifiable oily product has the following constants:
Specific gravity at 30°C. 0.9132; refractive index, 1.5012; acid value, 5.06; saponification value, 15.29;
( found: C, 80.78; H, 9.33. C_{20}H_{28}O_{2} requires C, 80.12; H, 9.33 % ).

It gave a crystalline nitroso derivative, m.0. 181°C, and a nitro derivative, m.p. 248°C (decomp.)
( found: C, 53.1; H, 5.81; N, 5.21 % ).

The infrared spectrum (graph No. 6) showed the sharp stretching at 1625 (cm^{-1}) for non-conjugated \( \text{C=C} \)

The other fraction (0.03 %) of the unsaponifiable matter eluted from methanol gave the test for sterols.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Colour developed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Liebermann Buchard reaction</td>
<td>Yellow to blue to green.</td>
</tr>
<tr>
<td>In acetone</td>
<td>Yellow to blue green.</td>
</tr>
<tr>
<td>2. Lifschutz reagent</td>
<td>Red colouration.</td>
</tr>
<tr>
<td>3. Salkowski reagent</td>
<td>First yellow then pink.</td>
</tr>
<tr>
<td>4. Tschugazeff reagent</td>
<td>Yellow colouration.</td>
</tr>
</tbody>
</table>
On recrystallisation from methanol a colourless crystalline product was obtained ( unlike Seshadri and Venkatrao and Khastgir et al ) was definitely identified to be \( \gamma \)-sitosterol by the preparation of suitable derivatives and comparison with those obtained from authentic specimen of the sterol.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>M.P.</th>
<th>Optical rotation</th>
<th>Found and calculated Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>140-41°</td>
<td>-45</td>
<td>C, 80.92; H, 11.29 Calc. for C_{31}H_{52}O_{2} C, 81.52; H, 11.48%</td>
</tr>
<tr>
<td>Sterol (from hydrolysis of acetate)</td>
<td>139-40°</td>
<td>-38</td>
<td>C, 83.39; H, 11.82 gives Calc. for C_{29}H_{50}O_{2} Libermann-C, 83.99; H, 12.15% Buchard reaction purple blue green</td>
</tr>
<tr>
<td>Benzoate</td>
<td>149-50°</td>
<td>-16.5</td>
<td>C, 83.80; H, 10.62. Calc. for C_{36}H_{54}O_{2} C, 83.34; H, 10.49%</td>
</tr>
<tr>
<td>Dibromide</td>
<td>141-42°</td>
<td>-47.5</td>
<td></td>
</tr>
</tbody>
</table>

It may be pointed out that mixed m.p. with authentic specimen is not much helpful for the identification of the sterols particularly in sitosterol. As such the present author confirmed its presence by the comparison of the analysis, and m.p. of sterol and its acetate and benzoate.
Chromatography of the glycoside over alumina:

The chloroform extract of the defatted seeds was subjected to chromatographic separation by adsorption on a column of alumina eluting successively with petroleum ether, benzene, ether, ethyl acetate and methanol, when yellowish brown fractions were obtained. On removal of solvents these did not give any crystalline mass. The alumina however separated into three bands as below:

(i) Top - brown coloured - may be ketals
(ii) Middle - yellowish brown
(iii) Bottom orange - may be oxidised material.

The bands were extruded, but all attempts to extract the adsorbed material from these with solvents failed.

Chromatography of the flavonoid compound:

The alcoholic extract of the defatted seeds, after removal of the solvent was dissolved in acetone and poured over a column of magnesol (prepared from a slurry of magnesol in acetone). Elution with acetone removed the chlorophyll. Further elution with ethyl acetate and water (1:1) gave a yellow band on the
column, which was extruded and extracted with methanol. On removal of the solvent from the methanol extract, a yellow coloured solid was obtained, which on recrystallisation from methanol and ethyl acetate melted at 124°C. This could, however, not be worked up further due to small quantity available.

Isolation of glycoside$^{30}$:

Adopting the procedure of Piccard, a hot alcoholic extract of the defatted seeds was treated with lead acetate, and the yellow precipitate filtered off. On removal of lead from the filtrate by hydrogen sulphide, evaporation gave a solid which on several crystallisations from methanol and ethyl acetate (1:1), gave a yellow crystalline gritty mass, m.p. 153-54°C. (Found: C, 55.69; H, 6.28. \( \text{C}_{21}\text{H}_{23}\text{O}_4 \), \( 3\text{H}_2\text{O} \) requires C, 55.38; H, 5.93 %). This was found to be a glycoside and was extremely hygroscopic. On drying it over phosphorus pentaoxide for eight hours under reduced pressure it gave the anhydrous product (Found: C, 59.98; H, 5.32. \( \text{C}_{21}\text{H}_{23}\text{O}_2 \) requires C, 60.14; H, 5.4%). It reduced fehling's solution only after hydrolysis. It dissolved in caustic alkalis and alkali carbonates.
giving a deep yellow solution, which on boiling changed to deep yellowish orange. It did not reduce silver nitrate, nor did it give any colouration with alcoholic ferric chloride. It also did not show the presence of any methoxyl or ethoxyl groups.

Hydrolysis of the glycoside:

Hydrolysis of the above glycoside in alcohol in the usual manner with a mixture of hydrochloric and acetic acids and extraction with ether, gave on removal of the solvent, a yellowish brown solid, which on recrystallisation with ethyl acetate and methanol gave a yellowish crystalline solid, m.p. 212-214°C, (Found: C, 66.73; H, 4.24. C_{15}H_{11}O_{5} requires C, 66.36; H, 4.05%). This was found to be a flavone, as with excess of alcoholic hydrochloric acid and magnesium turnings, it gave an intensive violet colouration which turned brown on dilution with water.

The aqueous solution obtained after the extraction of the flavone with ether contained the sugar. It was identified in the usual manner to be glucose and confirmed by the formation of an osazone, m.p. and mixed m.p. 205-206°C.
Isolation of acid

The powdered seeds (100 gms.) were extracted with water (1500 ml.). The yellow filtrate so obtained was extracted for 18 hours with caustic soda (2%, 350 ml.). The slimy product was pressed through linen and centrifuged. The clear yellowish orange solution was acidified to congo red with hydrochloric acid, when a precipitate was obtained. The precipitate was washed with water and dried m.p. 117°C. This acid was insoluble in all solvents and was purified by repeated crystallisation of its ammonium salt from water. The acid burnt with a sooty flame and did not give any colouration with ferric chloride (Found: C, 71.05; H, 6.23. C₄₀H₄₃O₁₀ requires C, 70.32; H, 6.44 %). The acid could not be assigned a structure due to paucity of material.
REFERENCES


27 Lifschutz, *Ber.*, 1908, **41**, 252.

28 Salkowski, *Z. Physiol.*, 1908, **57**, 523.


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

Carrisa spinarum Linn
Wild growth
Carissa spinarum Linn
Spikelet with fruits and stipules
Carissa spinarum Linn
Dry berries