CHAPTER II - BARK OF PROSOPIS SPICIGERA -
STUDY OF SAPONINS & SAPOGENINS
Fig. 1: Prosopis spicigera.
Fig. 2: Bark of Prosopis spicigera.
Prosopis spicigera\(^1\)–\(^4\) (Fig. 1) (Hindi: Jhand; Telugu: Jammi) belongs to the family Leguminosae and subfamily Mimosae. The plant is widely distributed throughout India, particularly in the southern regions. The bark of the plant (Fig. 2) is dry, acrid, bitter with a sharp taste, anthelmintic; cures dysentery, bronchitis, asthma, leucoderma, tumors of the muscles and wandering of the mind. The bark is used in Central provinces as a remedy for rheumatism. The smoke of the leaves is good for eye troubles. The fruit is dry and hot with a flavour; indigestible, causes biliousness, destroys the nails and the hair (Ayurveda). The pod is considered astringent.

Kidwai and his coworkers\(^5\) have isolated patulitrin, a flavone glycoside from the ethanolic extract of the flowers. Bhattay et al.\(^6\) has analysed the saw dust of heart-wood and reported the presence of five flavones. The plant yields a


\(^{2}\) Chopra, R.N., Nayar, S.L. and Chopra, I.C.; Glossary of Indian Medicinal plants (1956) 204, CSIR publication, New Delhi.


\(^{4}\) Chopra, R.N.; Indigenous drugs of India (1933) 519.


gum, which was found to be an acidic polysaccharide freely soluble in water and contains sugars, proteins and peroxidase. The emulsifying properties of the gum were studied by Mithal et al.\textsuperscript{8}

In the year 1969 Varshney\textsuperscript{9} has reported that the plants of the family Leguminaceae are a good source of saponins and most of the plants contain triterpenoid sapogenins. The plants of the sub-family Papilionaceae yield steroidal as well as triterpenoid sapogenins, whereas the plants of the sub-family Mimoseae are a rich source of triterpenoid sapogenins only. The seed saponin of Pithacoelobium dulce\textsuperscript{10} has been found to consist of oleanolic and echinocystic acids as sapogenins. The seeds of Albizia procera\textsuperscript{11} contain a new sapogenin namely, proceric acid. Saponin from the seeds\textsuperscript{12} of Albizia lebbek obtained from J.P. has been found to contain echinocystic acid as sapogenin, and glucose, rhamnose (1:1) as the sugar moiety. All the

---


\textsuperscript{8} Khaigiwal, P.C., Mithal, B.M.; Indian J. Pharm (1970) 32(4), 82-5 (Eng.).

\textsuperscript{9} Varshney, I.P.; Indian J. Chem (1969) 7(5), 446-9 (Eng.).


sapogenins isolated from this family belongs mostly to 
B - amyrin group.

It was noted that although considerable amount of work 
has been done on the seed saponins of various members of the 
sub-family Mimosae, the bark saponins of only few plants has 
been studied in detail. Acacic acid occurs in the bark of 
Acacia intia Wild, along with two neutral sapogenins, lupeol 
and acaciol. The bark of Albizia lebbek from U.P. 
was found to contain Acacic acid as sapogenin. Musenin, an 
echinocystic acid saponin has been isolated from the bark 
of Albizia anthelmintica. As no work on the saponin and 
sapogenin of Prosopis spicigera has been reported, it was 
taken up for investigation.

The coarsely powdered bark of Prosopis spicigera was 
found to contain moisture (2.16%), ash (12.07%), acid 
insoluble ash (7.14%) and sulphated ash (15.38%). The 
methanol soluble extract of the bark gave all the tests for 
saponin. The extract was concentrated and precipitated in 
ether repeatedly, which gave light brown coloured powder of 

France (1959) 17, 442.
14 Farooq, M.O., Varshney, I.P., and Naim, Z; Arch. der 
Pharm (1961) 294, 133.
15 ibid; (1961) 294, 197.
16 Varshney, I.P., Hasan, H., and Ahmed, S.A; Indian J. 
Pharm (1961) 23, 331.
17 C.A; (1958) 52, 10137a.
saponin. The saponin so obtained was found to be a mixture of saponin, A and B, together with free sugars. These were separated by column chromatography and hydrolysed. The saponin, A and B consists of echinocystic acid and oleanolic acid as sapogenins and the sugar moiety in the sequence of arabinose, glucose and xylose, arabinose, glucose respectively. The identification was carried out by comparison of the physical constants of the genin and all its derivatives with all the known acids of B - amyrin group. The I.R. and mass spectra of the sapogenins has further confirmed the identity.

**EXPERIMENTAL**

The bark of Prosopis spicigera was collected from Chinoaogirala (Andhra Pradesh) after proper identification. The general analysis of the air dried bark was carried out according to methods recommended by the Pharmacopoeia of India. The average values are recorded below.

<table>
<thead>
<tr>
<th>Moisture</th>
<th>2.16%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>12.07%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>7.14%</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>15.88%</td>
</tr>
</tbody>
</table>

The coarsely powdered bark (100 gms) was extracted successively with solvents from non-polar to polar (viz., petroleum ether (60-80⁰), benzene, solvent ether, acetone and methanol) in a soxhlet for 20 hours. The solvent was distilled

---

18 Pharmacopoeia of India, 2nd Edn., Ministry of Health, Government of India (1953), 947.
off from all these extracts under reduced pressure and the residue dried in a vacuum desiccator and weighed to a constant weight. The physical characteristics such as colour of each extract are given in Table 1. These extracts obtained by

### Table 1

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>Colour of the extract</th>
<th>Yield of the extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Petroleum ether</td>
<td>Yellow</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>(50-80°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Benzene</td>
<td>Orange red</td>
<td>1.25</td>
</tr>
<tr>
<td>3.</td>
<td>Solvent ether</td>
<td>Reddish brown</td>
<td>2.00</td>
</tr>
<tr>
<td>4.</td>
<td>Acetone</td>
<td>Dark brown</td>
<td>3.20</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol</td>
<td>Dark red syrup</td>
<td>4.32</td>
</tr>
</tbody>
</table>

selective extraction were examined qualitatively for common plant constituents.19-22 The petroleum ether and benzene extracts gave test for phytosterols and fat. The solvent


20 Robinson; The organic constituents of higher plants (1934), Burgess Minn.


ether extract responded to the tests of phenolic compounds and tannins, whereas acetone extract showed the presence of glycosides, besides these constituents. The methanol extract gave positive test for saponin.

(1) **Foam test**: The methanolic extract of the plant (1 ml) was diluted with distilled water (20 ml) and shaken in a graduated cylinder for fifteen minutes. The presence of saponin was indicated by a one cm layer of foam.

A little of the residue from methanolic extract was boiled with about 1 ml of distilled water and shaken. Appearance of characteristic honey comb indicated the presence of saponin.

A little of the residue from methanolic extract was taken with 2 ml of distilled water. A small quantity of sodium carbonate was added and shaken well. The characteristic foam formation indicated the presence of saponin.

(ii) **Haemolysis test**: One gm of the residue from the methanolic extract was dissolved in 5 ml of normal saline. In series of 5 test tubes, doses of 0.2, 0.4, 0.6, 0.8 and 1.0 ml saline solution of the crude drug was taken and volume was made upto 1 ml in all test tubes with normal saline. 0.5 ml of rabbits blood was diluted to 25 ml with normal saline. 1 ml of the diluted blood was added to each of the test tubes. A drop of the mixed solution, from each of the test tubes was viewed under microscope to confirm the absence of red blood cells, due to haemolysis of the red blood corpuscles by the
saponin present in the methanolic extract.

Study of Saponins and Sapogenins:

The powdered bark (500 gms) was extracted with ethanol (90%) in a soxhlet for 20 hours. The ethanol extract was concentrated under reduced pressure to a dark brown syrupy mass which was then refluxed successively with different solvents of increasing polarity (i.e., petroleum ether, benzene, solvent ether and acetone) to remove the impurities soluble in these solvents. The remaining gummy mass was dissolved in minimum quantity of methanol and filtered. The methanolic filtrate was precipitated by the addition of a large volume of ether. The process of dissolution and precipitation was repeated a number of times to obtain amorphous light brown powder which gave all the tests for saponin.

The saponins are commonly identified by haemolysis and foam tests. The following colour reactions commonly used for quick identification and differentiating the classes of saponins were found to be positive.

<table>
<thead>
<tr>
<th>Colour test</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liebermann - Burchard test</td>
<td>Positive</td>
</tr>
<tr>
<td>Stannic chloride reagent</td>
<td>&quot;</td>
</tr>
<tr>
<td>Noller test</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

23 Liebermann, C; Ber; (1855) 18, 1803.
The crude saponin was soluble in methyl, ethyl alcohols and completely insoluble in solvent ether, benzene and petroleum ether. The saponin was decolourised by activated charcoal.

Preparative TLC of the crude saponin

The preparative TLC of the crude saponin was carried out on thick layers of silica gel-G using chloroform-methanol-water solvent system (55:35:10). The chromatogram showed the presence of three spots, when sprayed with concentrated sulphuric acid. It was therefore separated into the different constituents by column chromatography.

Column chromatography of saponin

The separation of the individual components of saponin was achieved by column chromatography on silica-gel. The column was eluted with chloroform, methanol and water in different proportions. The eluted fractions were tested by TLC and all the fractions having similar Rf values were mixed together. The details are given in Table 2.

Fractions 1 to 33 gave a saponin, named as saponin A whereas the fractions from 40-55 consisted of saponin B. The last fractions 62-74 showed the presence of free sugars. Saponin A was present in a higher percentage (major quantity) than saponin B (minor quantity).

Saponin A: The homogeneity of the saponin was established by descending paper chromatography on Whatman No. 1 paper using
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Fraction</th>
<th>Eluant</th>
<th>TLC of fraction</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1 - 33</td>
<td>Chloroform : Methanol : water(35:35:10)</td>
<td>Single spot (Major)</td>
<td>Saponin A</td>
</tr>
<tr>
<td>2.</td>
<td>34 - 39</td>
<td></td>
<td>Two spots</td>
<td>Saponin A+B</td>
</tr>
<tr>
<td>3.</td>
<td>40 - 55</td>
<td></td>
<td>Single spot (minor)</td>
<td>Saponin B</td>
</tr>
<tr>
<td>4.</td>
<td>56 - 61</td>
<td></td>
<td>Two spots</td>
<td>Saponin B + free sugars</td>
</tr>
<tr>
<td>5.</td>
<td>62 - 74</td>
<td>80% Methanol</td>
<td>One spot</td>
<td>Free sugars</td>
</tr>
</tbody>
</table>

n - butanol : acetic acid : water (4 : 1 : 5) solvent system.
The chromatogram was sprayed with stannic chloride reagent and dried at 100°C for five minutes. It gave a single spot; m.p. = 221.2°C.

Hydrolysis of Saponin A:

Saponin A (500 mg) was treated with sulphuric acid
Figure 3: I.R. Spectrum of Sapogenin-A
(70% w/v; 5 ml) and kept for half an hour at 5°C and another half an hour at room temperature. The above solution was diluted to 2N sulphuric acid with distilled water and refluxed for 4 hours. The separated sapogenin was filtered and washed with water until the washings are neutral to litmus and dried (180 mg). The filtrate containing the glycone portion was analysed for the sugars.

**Isolation of pure genin A**:

The crude genin was dissolved in methanol and refluxed with methanolic solution of sodium hydroxide (5%; 75 ml) for two hours. The reaction mixture was concentrated to half its volume and diluted with water (300 ml). The solution was extracted with ether to remove the impurities. The aqueous extract was acidified with hydrochloric acid to precipitate the acid sapogenin. The pure genin was filtered, washed with water, dried and recrystallised from methanol as colourless plates melting at 306 – 308°C; $^{28}_{\text{D}}$ $^{[a]} + 37.6$ in ethanol. (Found: C, 75.6; H, 10.04; Calc. for C$_{30}$H$_{48}$O$_{4}$: C, 75.24; H, 10.24 %). The I.R. spectrum (Fig. 3) showed characteristic peaks at 3460, 1685, 1370, 1365 and 820 cm$^{-1}$.

**Methyl ester of Sapogenin A**:

Sapogenin A (50 mg) was suspended in solvent ether (25 ml) and an ethereal solution of diazomethane (100 ml) was added. The container was left over night in the frigidaire.
The ether was distilled off leaving the methyl ester of the sapogenin A. The methyle ester was recrystallised from methanol and dried; m.p. 213-14 °C.

**Diacetate of Sapogenin A**

Sapogenin A (50 mg) was mixed with anhydrous sodium acetate and acetic anhydride. The mixture was refluxed (two hours) and poured in ice-water. The acetate of sapogenin A, so obtained was extracted with ether and evaporated to dryness. The residue was recrystallised from methanol a number of times; m.p. 275-76 °C.

**Diacetate methyl ester**

The diacetate was methylated with an ethereal solution of diazomethane and the product was recrystallised three times from methanol; m.p. 198-200 °C.

The physical constants of the sapogenin A and all its derivatives were compared with those of known compounds (Table 3). The available data of the echinocystic acid is in complete agreement with that of sapogenin A. The sapogenin A has therefore been confirmed to be echinocystic acid, a triterpenoid sapogenin.

The identity of echinocystic acid (1) was finally confirmed by the mass spectra of methyl ester of sapogenin A. The methyl ester gave molecular ion peak at m/e 486, which undergoes a reverse Diels-Alder fragmentation,26 to furnish

---

Comparison of the physical constants of Sapogenin A and its derivatives with echinocystic acid from other Saponins.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bergstein and Noller</th>
<th>Tschesche and Frostmann</th>
<th>Varshney et al.</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>305-310°</td>
<td>305-310°</td>
<td>296-299°</td>
<td>306-308°</td>
</tr>
<tr>
<td>Methyl ester</td>
<td>213-214°</td>
<td>216-217</td>
<td>213-215</td>
<td>213-214°</td>
</tr>
<tr>
<td>Diacetate</td>
<td>272-275°</td>
<td>-</td>
<td>265-276°</td>
<td>275-276°</td>
</tr>
<tr>
<td>Diacetate methyl ester</td>
<td>200-201°</td>
<td>200-201</td>
<td>201-202</td>
<td>198-200°</td>
</tr>
</tbody>
</table>

Characteristic peaks at m/e 207 and m/e 279 respectively. The rest of the fragments observed are typical of \( \Delta^{12} \)oleanane series.

![Molecular ion peak diagram](image)

Molecular ion peak at m/e 496

---


Study of glycone:

Saponins generally contain the glycosidic chain attached at one or more hydroxyl groups and one or more carboxyl groups. The partial structure of the saponin can be established by determining the position of attachment of glycosidic chain to the aglycone and the sequence of sugars in the glycosidic chain.

Identification of sugars:

The hydrolysate obtained after the hydrolysis of saponin A, was neutralised with barium carbonate, followed by deionisation with ion exchange resin (Amberlite IRA 120H+). The neutral sugar solution was concentrated to a golden yellow syrup. It consists of sugars involved in glycosidic linkage with the aglycone. The sugars were subjected to paper chromatography (descending) with authentic specimens. The chromatogram was developed in n-butanol - acetic acid - water (4:1:5) solvent system and sprayed with aniline hydrogenphthalate reagent. The Rf values of the spots were compared with those of authentic specimens and the sugars identified as
arabinose and glucose.

Graded hydrolysis of Saponin A:

Saponin A was subjected to graded hydrolysis with Killiani mixture of reduced hydrochloric acid concentration to determine the sugar moiety directly attached to genin.

Saponin A (50 mg) was kept at 80°C with 4 ml of Killiani mixture of reduced hydrochloric acid strength (hydrochloric acid - acetic acid - water: 5 : 35 : 60) in glass tube fitted with ground glass stopper. The test solution was spotted on paper at regular intervals of 15 minutes during the course of hydrolysis for three hours. The paper was irrigated with n-butanol-pyridine-water (6:4:3) solvent system. Glucose was the first sugar to be released (after 30 minutes), while the spot due to arabinose started to appear only after 75 minutes.

This experiment showed that glucose is present as the end sugar and arabinose should be directly attached to the genin. The position of attachment of glycosidic chain was determined after identifying the sugars.

In echinocystic acid there are three possible places for attachment of glycosidic chain to aglycone i.e., at C3, C15 hydroxyl groups and a carboxyl group at C28. The attachment of sugars at C3 hydroxyl is the most common position in the case of triterpanoid saponins. Saponin A was subjected to alkaline hydrolysis with 5% methanolic sodium hydroxide under reflux for 6 hours. The hydrolysate on TLC showed the
presence of two spots; one due to prosapogenin and the other of sugar chain. The solvent system used consisted of n-butanol-pyridine-water (6:4:3). The I.R. spectrum of saponin A also showed a sharp absorption at 1740 cm⁻¹, indicating the presence of an ester linkage. Therefore the sugars are attached at C₂₈ carboxyl group also. The attachment of sugars at C₁₆ hydroxyl group is not possible due to its strongly hindered position.

The sugar chains are therefore attached at C₃ hydroxyl group and C₂₈ carboxyl group in saponin A. The saponin A has, therefore, been assigned the partial structure (2) on the basis of the above chemical and physical data.

![Diagram](image.png)

R' + R'' = Arabinose, Glucose.

(2) Saponin A

**Saponin B**

The saponin B was present in minor quantity (180 mg), m.p. 207-9°.

**Hydrolysis of Saponin B**

Saponin B was hydrolysed with 2N sulphuric acid as in
FIG-NO: 4  I.R. SPECTRUM OF SAPOGENIN-B.
the case of saponin A. The genin was separated and purified as sodium salt of the genin. The sodium salt was decomposed into pure sapogenin by dilute hydrochloric acid and crystallised from methanol as colourless needles, m.p. 295-98.

Acetylation of Sapogenin B:

Sapogenin B was acetylated with acetic anhydride and pyridine. The acetylated genin was recrystallised from methanol and dried in vacuum, m.p. 288° (Found: C, 75.7; H, 9.89. Calc. for C_{32}H_{50}O_{4}: C, 77.06; H, 10.01%)

Methylation of Sapogenin B:

Sapogenin B was dissolved in ether and an ethereal solution of diazomethane was added. The methyl derivative formed was recrystallised from chloroform-methanol mixture and dried, m.p. 198°.

The I.R spectrum (Fig. 4) of the sapogenin B showed absorption bands at 1655, 825. The above physical data of the sapogenin was compared with that of known compounds. These values are in agreement with those of oleanolic acid reported by Djerassi et al. The sapogenin B has therefore been confirmed to be oleanolic acid. The identity of oleanolic acid was further confirmed by taking mass spectrum of the methyl ester of sapogenin B. The compound showed molecular ion peak at m/e 468; which suffered

retro-Diels-Alder fragmentation leading to the formation of characteristic peaks at m/e 205 and m/e 262 respectively. The rest of the peaks due to further cracking were similar to that of $\Delta^{12}$-oleanane series.

Identification of sugars:

The filtrate, after hydrolysis of saponin B, was neutralised and deionized by passing through a column of ion-exchange resin (Amberlite I.R 120 $H^+$). The neutral solution was concentrated and tested for the sugars by paper chromatography (descending). The sugars have been identified as xylose, arabinose and glucose by comparing the $R_f$ values with those of authentic specimens.

The saponin B thus consists of oleanolic acid as sapogenin and the sugars as xylose, arabinose and glucose.
SUMMARY

The bark of Prosopis spicigera has been found to contain a mixture of two saponins. These saponins have been separated by column chromatography and named as saponin A and B.

Saponin A present in major quantity has been found to consist of the sapogenin echinocystic acid with the sugars, arabinose and glucose. The sugar chains are attached to the aglycone at $C_3$ hydroxyl group and $C_{28}$ carboxyl group.

Saponin B which is present as the minor constituent of the bark saponins consists of the sapogenin oleanolic acid with the sugars xylose, arabinose and glucose.