PART II

CHEMICAL EXAMINATION OF THE BERRIES OF SOLANUM NIGRUM LINN.
CHEMICAL EXAMINATION OF THE BERRIES OF SOLANUM NIGRUM LNN.

"This very common plant has followed the foot steps of man all over the world"—Lindley.

Solanum nigrum Linn, or Gurkamai or Chhoti makoi as it is known in Sanskrit and Hindi respectively, is a variable annual herbaceous or suffrutescent plant found throughout India at altitudes up to 7,000 ft. It has berries, 6mm. in diameter, globose, usually purplish black. In Sanskrit works of medicine, berries of this plant are described as having tonic, alterative and diuretic properties and are said to be useful in anasarca and heart diseases. The juice of the plant is also considered of medicinal importance and is given in doses of 6 to 8 ounce in chronic enlargement of the liver. In Bengal (India) the berries are employed in fever, diarrhoea, eye diseases and various other ailments.

Genevill and Defosse of Besancon isolated the active principle of these fruits and named it Solanine. The presence of saponin in the berries, and the composition of fixed oil from the seeds has also been reported. The optical activity and structural pattern of the glycerides of the oil has also been studied. Amongst the other varieties only Solanum indicum and Solanum
Xanthocarpum have been investigated for their fixed oil. No detailed examination, however, seems to have been done on Solanum nigrum. As such the plant has been taken up for detailed chemical examination. A fixed oil has been extracted from the berries and its glyceride structure studied by the oxidation method of Kartha.

Further the unsaponifiable matter of the oil on chromatography has yielded two sterols in a crystalline form, m.p. 134°C and 128°C. The defatted berries gave a crystalline saponin, a glucoside and a glucoalkaloid which on hydrolysis have yielded sapogenin, an aglucone, and an alkaloid. The sugars produced on hydrolysis have been identified to be glucose, galactose and rhamnose by the formation of osazones and paper chromatography. A fourth new sugar has also been isolated in the free state and characterised by the formation of an osazone m.p. 120°C.

**EXPERIMENTAL**

The occurrence of saponin in the plant was indicated by the formation of permanent foam on shaking its aqueous solution. Moreover, the plant was found to be highly toxic to fish, obviously due to the presence of a saponin.
The berries were dried in shade at 40-50°C and powdered. 25 gm. of the powdered material was extracted successively in soxhlet by the following solvents in the given order and the following results were obtained:

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Weight of extract obtained gm.</th>
<th>Percentage of extract %</th>
<th>Composition of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Petroleum ether</td>
<td>1.75</td>
<td>7%</td>
<td>Mostly fat.</td>
</tr>
<tr>
<td>(60-80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Absolute ether</td>
<td>0.275</td>
<td>1.1%</td>
<td>Resinous mass.</td>
</tr>
<tr>
<td>3 Chloroform</td>
<td>0.245</td>
<td>0.98%</td>
<td>Greenish mass.</td>
</tr>
<tr>
<td>4 Ethyl acetate</td>
<td>0.16</td>
<td>0.64%</td>
<td>Greenish mass.</td>
</tr>
<tr>
<td>5 Rectified spirit</td>
<td>5.66</td>
<td>22.64%</td>
<td>Dirty brown.</td>
</tr>
</tbody>
</table>

The crushed material was, therefore, first extracted with petroleum ether (60-80°C) to remove the fat. On removal of the solvent from the petroleum ether extract a dark green fixed oil with characteristic smell of the berries was obtained in a yield of 7%. The oil has the following physical and chemical constants.

<table>
<thead>
<tr>
<th>Constants</th>
<th>Authors values</th>
<th>Values reported by Pendse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Optical rotation in CHCl₃</td>
<td>16.2°</td>
<td>- 6.61</td>
</tr>
<tr>
<td>2. Sp. gravity at 30°C</td>
<td>0.9098</td>
<td>0.8964</td>
</tr>
<tr>
<td>3. Refractive index at 30°C</td>
<td>1.4560</td>
<td>1.4436</td>
</tr>
<tr>
<td>4. Acid value</td>
<td>6.8</td>
<td>2.4.7</td>
</tr>
<tr>
<td>5. Saponification value</td>
<td>181.6</td>
<td>184.7</td>
</tr>
<tr>
<td>6. Unsaponifiable matter</td>
<td>1.8 %</td>
<td>1.4-1.6%</td>
</tr>
<tr>
<td>7. Iodine value</td>
<td>128.6</td>
<td>111.7</td>
</tr>
</tbody>
</table>
Preparation of mixed fatty acids from the oil and separation of the unsaponifiable matter:

In a round bottom flask 100 gm. of the oil was saponified by a solution of 30 parts by weight of potassium hydroxide in about 500 ml. of absolute alcohol. The solution was boiled under reflux for about 4 hours and then most of the alcohol was removed by distillation. The soaps so formed were dissolved in water, and the unsaponifiable matter was extracted with ether and the ethereal solution washed with water several times. On removal of the solvent, the unsaponifiable matter was obtained in pure condition. The free fatty acids were liberated from the soap solution by warming with dilute sulphuric acid in an atmosphere of carbon dioxide, and were extracted with ether. On removal of solvent from the dried ethereal extract a mixture of the acids was obtained. The mixed acids were resolved into solid and liquid acids by Twitchell's method, when 16.81 gms. (18.13%) of solid and 75.94 gms. (81.87%) of liquid acids were obtained.

Liquid acids:

The liquid acids were obtained as chocolate coloured viscous liquid having mean molecular weight 280 and iodine value - 124.31.
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 a cooled solution of the liquid acids (3 gms.) in ether. The addition of bromine was continued till a yellow colour persisted. The solution was kept in the frigidaire over-night. On removal of the solvent, a viscous bromide was obtained, which when kept in petroleum ether (60-80°) and cooled in a frigidaire deposited a brown solid. This on recrystallisation from light petroleum, melted at 114 C, undepressed when mixed with an authentic sample of linoleic tetra bromide. After separation of the above tetrabromide the filtrate was freed from bromine and removal of the solvent gave a liquid bromide which was found identical with oleic dibromide by comparison of the infrared absorption spectra.

Oxidation of liquid acids

5 gms. of the liquid acids was dissolved in dilute alkali (caustic soda), the volume was made up to 500 ml. and a concentrated solution of potassium permanganate (36 gms.) added slowly with constant stirring during the course of 15 minutes. The excess of potassium permanganate was destroyed by passing sulphur dioxide. The oxidised oil floated as a white suspension. It was filtered and the
residue washed with a little ether to remove adhering unoxidised oil, and dried. The dried solid was extracted with ether (250 ml.). By this process dihydroxy acid, if present, is removed. Removal of the solvent from the ethereal solution gave a solid acid which on recrystallisation from petroleum ether gave m.p. 130°C, undepressed when mixed with an authentic specimen of dihydroxy stearic acid. The insoluble oxidised material left after ethereal extraction was extracted with a large volume of hot water. This aqueous solution was kept in ice bath, when a solid separated. This on recrystallisation from rectified spirit gave a solid m.p. 163°C, undepressed when mixed with an authentic specimen of tetrahydroxy stearic acid. The formation of the di and tetrahydroxy stearic acids show the presence of oleic and linoleic acids.

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 for 24 hours, methanol was distilled off and the esters so formed extracted with ether. The ethereal solution was washed with water followed by dilute sodium carbonate and finally again with water, dried over anhydrous sodium sulphate
and fractionally distilled. Two main fractions were collected: (i) 210-228°C, and (ii) 230-236°C, which on cooling deposited solids, which on recrystallisation from dilute acetone melted at 60-62°C and 68-69°C respectively, and were identified to be palmitic and stearic acids by mixed melting points with authentic specimens. Thus the oil contained the following acids:

1. Palmitic
2. Stearic
3. Oleic
4. Linoleic.

**Determination of glyceride structure**:

Following the method of Kartha, 10 gms. of the neutral oil was dissolved in 400 ml. of anhydrous acetone and treated with 70 gms. of powdered potassium permanganate. The addition of permanganate was so regulated that the acetone was kept gently boiling. Glacial acetic acid was added in such a way that the quantity was maintained at 5%. After complete addition when the reaction subsided the reaction mixture was refluxed on the water bath. The addition of potassium permanganate continued till a permanent pink colour was obtained. After removal of the acetone under reduced pressure the residue was dissolved in 250 ml. of water, and th
the mixture was shaken with small quantities of sodium bisulphite and sulphuric acid (15%) to remove manganese dioxide. An oil floated on the surface, which was extracted with ether and the ethereal solution washed with distilled water till free from acid. It was then washed with sodium bicarbonate (10%) followed by water and dried over anhydrous sodium sulphate. This appeared to be trisaturated glyceride. It was saponified and the acid liberated was confirmed to be stearic acid. The sodium carbonate extract was acidified with dilute sulphuric acid and the azeleo glyceride mixture thus separated, extracted with ether. Removal of ether gave a residue having a saponification value of 294.0. The percentage of saturated acids, the trisaturated glyceride, the disaturated glyceride and the monosaturated glyceride was calculated and found to be as below:

1. Disaturated oleins 3%
2. Saturated diolein Nil
3. Saturated olee linoleins 6%
4. Tri olein Nil
5. Dioleo linolein 46%
6. Olee-dilinolein 45%
**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 yellowish waxy material (1.4 gm.), chromatography\textsuperscript{22,23} which in light petroleum (40-60\(^{\circ}\)) over a column of alumina gave the following (p.69) fractions on removal of solvents:
<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluent</th>
<th>Colour of the fraction</th>
<th>Residue on removal of the solvent</th>
<th>Yield</th>
<th>Found</th>
<th>Values calculated for molecular formulae</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether (40-60°)</td>
<td>Colourless</td>
<td>Nil.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Petroleum ether (40-60°)</td>
<td>Colourless</td>
<td>Crystalline deposit m.p. 75-76°</td>
<td>0.0115%</td>
<td>C 85.31% H 14.08%</td>
<td>C_{35}H_{72}O</td>
<td>Pentatriacontane_{24}</td>
</tr>
<tr>
<td>3-6</td>
<td>Petroleum ether (40-60°)</td>
<td>Yellowish</td>
<td>Crystalline deposit m.p. 65-66°</td>
<td>0.01%</td>
<td>C 85.29% H 13.95%</td>
<td>C_{30}H_{62}O</td>
<td>Tricontane_{25}</td>
</tr>
<tr>
<td>7-15</td>
<td>Petroleum ether (60-80°) &amp; Ether (3:1)</td>
<td>Yellowish</td>
<td>Crystalline deposit m.p. 128°</td>
<td>0.15%</td>
<td>C 80.58% H 11.29%</td>
<td>C_{27}H_{46}O,H_{2}O</td>
<td></td>
</tr>
<tr>
<td>16-20</td>
<td>Petroleum ether and Ether (1:1)</td>
<td>Yellowish</td>
<td>Crystalline deposit m.p. 128°</td>
<td></td>
<td></td>
<td></td>
<td>Dihydrosterol</td>
</tr>
<tr>
<td>21-24</td>
<td>Methanol</td>
<td>Yellowish</td>
<td>Crystalline deposit m.p. 134°</td>
<td>0.3%</td>
<td>C 83.17% H 11.50%</td>
<td>C_{29}H_{50}O</td>
<td>β-sitosterol</td>
</tr>
</tbody>
</table>
### Derivatives of Sitosterol Prepared

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Found C</th>
<th>Found H</th>
<th>Calculated for</th>
<th>Recrystallised from</th>
<th>Melting point (°C)</th>
<th>( \gamma^D )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )-sitosterol acetate</td>
<td>C 81.80%</td>
<td>H 11.63%</td>
<td>( C_{31}H_{52}O_2 )</td>
<td>Methanol</td>
<td>127-128</td>
<td>-42°</td>
</tr>
<tr>
<td></td>
<td>C 81.57%</td>
<td>H 11.47%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-sitosterol benzoate</td>
<td>C 83.2%</td>
<td>H 11.1%</td>
<td>( C_{36}H_{54}O_2 )</td>
<td>Ethanol ether</td>
<td>143-144</td>
<td>-14°</td>
</tr>
<tr>
<td></td>
<td>C 83.34%</td>
<td>H 10.49%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-sitosterol -3:5- dinitrobenzoate</td>
<td></td>
<td></td>
<td>Ethyl acetate and petroleum ether (60-80°)</td>
<td></td>
<td>203-204</td>
<td></td>
</tr>
</tbody>
</table>
Isolation of crude saponin:

The crude saponin gave the following tests:

The defatted berries were extracted with alcohol (70%). A dark brown extract was got, which gave a syrup on removal of the solvent. The syrup was kneaded with successive quantities of ether until it gave a greenish solid. The solid was redissolved in absolute ethanol and again treated with dry ether, and the process repeated several times till the saponin was free from impurities. If the solid at this stage was allowed to remain in contact with the atmosphere it became sticky and was difficult to manipulate. It was, therefore, kept under reduced pressure in a vaccum dessicator. The yield of the crude saponin was about 4.1% of the weight of berries employed. It formed light greenish amorphous powder, easily soluble in water to a strongly forthing solution which on heating deposited saponin as syrup.

1. Foaming test\(^2\): The saponin (50 mg.) on being shaken vigorously with 50 ml. of water produced lather with characteristic honey comb structure, which persisted even when the solution was heated.

As noted above a charge of 8 kg. in a soxhlet
in lots of 100 gms. was extracted. The total alcohol needed for extraction was 50-60 litres. The alcoholic extract was distilled in vacuum to remove the solvent when a thick syrup was got.

2. Toxicity to fishes:

The crude saponin (0.5 gm.) was dissolved in 500 ml. of water and fishes from half to one inch in length were introduced into this solution. Within ten minutes the fishes began to die and after 15 minutes, not a single fish was found alive in the solution.

Another lot of the same type of fishes was introduced into a solution containing 0.5 gm. of saponin in 2500 ml. of water. All fishes were found dead in half an hour in this case.

Purification of the crude saponin:

Two successive procedures were used to free the saponin (i) on one hand from the sugars and (ii) on the other from such fatty materials as might have escaped the treatment of ether.

(i) The crude saponin was dissolved in about five times its weight of chloroform and the solution shaken to an
emulsion with half its volume of water. Alcohol was now added until the mixture separated fairly rapidly into two layers. After shaking, the lower layer was separated and again shaken with water and sufficient alcohol to avoid emulsification. Finally it was again separated and evaporated. The residue was heated on a water bath until free from the solvent. The material thus obtained was about 90% by weight of crude saponin. The aqueous alcoholic washings on evaporation gave a dark hygroscopic syrup which strongly reduced Fehling's solution and gave an osazone which on crystallisation from alcohol (75%) melted at 120°C. This seems to be a new sugar - Found: C, 64.30; H, 6.36; N, 18.18%.

(ii) The material obtained on evaporation of chloroform layer, as above, was dissolved in three times its weight of alcohol and an equal volume of benzene added. An amount of water slightly less than that of the benzene was introduced and the mixture shaken and allowed to settle. The two layers formed were separated and each was washed with fresh quantities of the complementary solvent. Each layer was evaporated separately. 85% of the substance was found in the aqueous alcoholic layer. In order to remove
the last traces of benzene soluble substances, this product was boiled with successive quantities of benzene. The residue was freed from benzene under reduced pressure, when an ash free greenish yellow product was obtained having a molecular weight - 976. (Found : C, 56.80; H, 8.70; C_{45}H_{84}O_{22} requires : C, 56.20; H, 8.50 per cent.)

Hydrolysis of saponin and identification of the sugar components and the sapogenin:

**Sapogenin**: Saponin (5 gms.) was dissolved in 70% ethanol (300 ml.) containing 75 ml. of concentrated hydrochloric acid. The mixture was refluxed over a water bath for six hours, cooled and diluted with water (about 100 ml.), when a yellowish solid separated which could not be obtained in a crystalline condition by crystallisation. However, its chromatography in benzene solution over a column of alumina gave the following fractions:

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Solvent</th>
<th>Residue on removal of solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-6</td>
<td>Benzene</td>
<td>Greenish oil</td>
</tr>
<tr>
<td>7-15</td>
<td>Ether</td>
<td>Crystalline white solid</td>
</tr>
<tr>
<td>16-20</td>
<td>Methanol</td>
<td>Nil.</td>
</tr>
</tbody>
</table>

The crystalline mass was recrystallised from ethyl acetate when it melted at 198-99°C. (Found : C, 73.37; H, 9.49.)
The infra red spectrum of the pure sapogenin (graph No. 1) showed the presence of free hydroxyl group.

**Sugars:** (a) The acidic solution i.e., the filtrate from which the sapogenin was removed, was concentrated to the consistency of a syrup. It was heated with three times its weight of phenyl hydrazine in glacial acetic acid. The separated osazones were filtered, dried and refluxed for some time with acetone. The acetone insoluble osazone on crystallisation from alcohol melted at 205°C, and was identified to be glucosazone by mixed melting point with an authentic specimen. On removal of the solvent from the acetone soluble osazone it melted at 183°C, and was identified as rhamnosazone by mixed melting point with an authentic specimen.

(b) 5 ml. of the concentrated syrup of the sugars obtained on hydrolysis of saponin (above) was evaporated with 20 ml. of dilute nitric acid (sp. gr. 1.15) on water bath to a small bulk. To this was added 5 ml. of water, the next morning, when a crystalline deposit was obtained, which on recrystallisation from water decomposed
at 214-150°C. It was identified as mucic acid, which is generally obtained by the oxidation of galactose.

(c) In another method29 the solution after hydrolysis was neutralised with barium carbonate, the precipitate so obtained was filtered and the filtrate evaporated. The residual syrup was exhaustively extracted with boiling methanol and the filtrate concentrated to a syrup. The solid amorphous residue left after methanol extraction was the barium salt of the degraded polysaccharide. The methanol insoluble barium salt was found to be of an aldobiouronic acid. 0.1 gm. of the aldobiouronic acid was dissolved in 100 ml. sulphuric acid (100 ml.), and was heated on a boiling water bath for 36 hours. The solution was neutralized with barium carbonate, filtered and evaporated. The residue was extracted exhaustively with boiling methanol, filtered, and evaporated to a syrup. On cooling the syrup a crystalline rhamnose hydrate was obtained which was confirmed by the formation of an osazone and also by paper chromatography. The methanol insoluble barium salt was identified to be that of hexauronic acid. The uronic acid gave a positive basic lead acetate test, for galactouronic acid and was further confirmed by oxidation to mucic acid and mixed m.p. 213°C.
Chromatographic separation of the osazones of sugars by circular paper chromatography:

This method also confirmed the presence of glucosazone, galactosazone and rhamnosazone. Following the method of Dr. Barbarin Arreguin the sugars were identified by paper chromatography to be glucose (Rf. 0.41), galactose (Rf. 0.42), and rhamnose (Rf. 0.54).

Isolation of glucoalkaloid:

The presence of glucoalkaloid solanine (loc. cit.) has already been reported. To isolate it from the herb, 2 kg. of herb was defatted by extraction with petroleum ether. The defatted berries were extracted with 90% ethyl alcohol. On removal of the solvent from the alcoholic extract a syrupy mass was got. To this was added 1% acetic acid solution. The acidified solution was filtered to remove acid insoluble impurities (resinous). The clear filtrate was extracted with chloroform to remove all the acid soluble impurities. The aqueous layer then was basified gradually with ammonia, when voluminous precipitate was got which was filtered. The precipitate which gave positive test with alkaloidal reagents was divided into two portions.
GRAPH No. 2

PHASE - CHCl₃
THICKNESS ~5 m.m.
One half of it was kept for drying so as to get the glucoalkaloid, and the other half was hydrolysed by dissolving it in alcohol containing sulphuric acid (7%) and acetic acid in traces. It was refluxed for eight hours, when a solid resinous mass separated out, which on filtration gave positive tests with alkaloidal reagents.

The dried glucoalkaloid and the alkaloid obtained on hydrolysis were crystallised from methanol and ethyl acetate respectively when they melted at:

275-279 C (glucoalkaloid) I.R. graph No. 2 and 201-202 C (alkaloid) I.R. graph No. 3.

The alkaloid gave a crystalline acetate, melting at 189-190 C. The melting points of the glucoalkaloid and alkaloid indicated them to be solasonine and solasodine.

Colouring matter:

The berries left after the complete extraction of saponin and glucoalkaloid were extracted with alcohol containing 4% hydrochloric acid. The alcoholic extract on concentration gave a syrup, which on being redissolved in absolute alcohol and treatment with ether several times
gave a reddish brown colouring matter, decomposing at 260°C. This was recrystallised from a mixture of methanol and ethyl acetate (1:1), when it melted at 314°C. (Found: C, 48.70; H, 5.90. Calculated for C_{27}H_{30}O_{16}:3H_{2}O: C, 48.80; H, 5.40%). These results confirmed it to be glucorhamnoside 'Rutin', which has been shown to crystalline with 3 molecules of water which are sometimes difficultly eliminated on heating.

**Hydrolysis of Rutin**

The colouring matter 'Rutin' (above) was hydrolysed by refluxing it with a mixture of 35 ml. acetic acid, 55 ml. water, and 10 ml. hydrochloric acid for eight hours, when a solid separated, which on crystallisation with a mixture of methanol and ethyl acetate gave yellow needles melting at 314°C undepressed with an authentic specimen of quercetin (Found: C, 59.20; H, 3.50. C_{15}H_{10}O_{7}: C, 59.60; H, 3.30%) and yielded a penta acetyl derivative melting at 194°C. The sugar moiety obtained from the hydrolysed solution was identified in the usual way by chromatography to be a mixture of glucose and rhamnose.
REFERENCES


   Zenlralh, 1892, 712.


   cf. C.A. 1946, 40, 3279.


22 Thorbjarnarson & Drummond, Analyst, 1935, 60, 382.
23 Gridgeman, Gibson & Savage, Analyst, 1948, 73, 662.
24 Beilstein, Handbuch der Organischem Chemie, 1918, I, 177.
31 Kiliani, Ber., 1930, 63, 2866.
Psoralia corylifolia Linn

Wild growth
Psoralia corylifolia Linn

Branch bearing leaves
Psoraria corylifolia Linn
Seeds