# CHEMICAL EXAMINATION OF *SOLANUM NIGRUM* LINN.

## Part I. The Component Fatty Acids and the Probable Glyceride Structure of the Fatty Oil from Seeds

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[The fatty oil from the seeds of *Solanum nigrum* Linn. has been examined and found to have Sp. Gr. 0·9198 (30° C.); refractive index 1·4712 (25° C.); Acid value 11·62; Sap. value 184·0; Acetyl value 25·7; Hehner value 92·9; unsaponifiable matter 1·4%; R.M. value 0·66; Iodine value (Hanus) 123·2; Thiocyanogen value (24 hours) 84·4. The component fatty acids of the oil are Linoleic 46·63%, Oleic 49·73%, Palmitic 1·76%, Stearic 1·88%. The unsaponifiable matter consists of a phytosterol, m.p. 133° C. The component glycerides of the oil are palmitostearo-olein 0·63%, Dipalmito-olein 2·43%, Palmito-oleolinolein 2·48%, Stearo-oleolinolein 3·4%, Oleodilinolein 44·84% and Dioleolinolein 46·22%. The optical rotation could not be found as the oil was too dark to read.]

*Solanum nigrum* is a small-sized shrub belonging to the natural order, *Solanaceæ*. It is distributed throughout India, Ceylon, and all temperate and tropical zones of the world. The chief product of the tree is its fruit, which is spherical and about five millimeters in diameter. It is called 'Chhoti Makoi' in Hindustani. Its English equivalent is 'Black nightshade', 'Hound Berry', etc. The fruit is described as highly medicinal[1, 2, 3] being used as "laxative, alterative, aphrodisiac, tonic, diuretic; improves appetite and taste; useful in diseases of heart and eye, pains, piles, inflammation, '*tridosh*', leucoderma, itch, worms in ears, dysentery, hiccough, vomiting, asthma, bronchitis, fever, urinary discharges; improves the voice, favours conception and facilitates delivery; useful in erysipelas and rat-bite, diarrhœa, hydrophobia; mixed with honey it is administered for pulmonary tuberculosis, and with other drugs in snake-bite and scorpion-sting."

An analysis of the oil has been reported by Pendse.[4] He has determined the constants of the oil and also the percentage of the constituent fatty acids. His results differ from ours and are included in Tables I and II for the sake of comparison. The present investigation was undertaken in the first

instance to determine the glyceride structure of the oil as it had not been done so far. We found that the percentage composition of oleic (49·0%) and linoleic (47·22%) acids, as found by us (Tables V and XI) was markedly different from that found by Pendse, *viz.*, oleic acid 68·45% and linoleic acid 25·66%. Further the quantity of solid acids found by Pendse was higher than that found by us. It was therefore, necessary to redetermine the percentage composition of these acids by several methods. Pendse does not describe the locality from which he collected the material; our plants were collected from two villages (Rajapur and Mumfordganj) near Allahabad. A glance at Tables I and II will show that there is a marked difference in our oil and that analysed by Pendse. It may, however, be pointed out that our results, as obtained by different methods, are concordant and, therefore, may be taken as correct.

The investigation has shown the actual number of glycerides to be six whereas the maximum number of individual glycerides, which may be obtained by any combination of four different acids with the glyceryl radical, $CH_2-CH-CH_2$ is forty.

Another species of *Solanum*, viz., *S. indicum*, had been previously investigated by one of us[5] for the fatty acid composition and the glyceride structure of oil. The available data of the seed oils of the *Solanaceæ*, which are more abundant than for many other plant families, disclose certain broad features: the seed fat content of palmitic acid is usually between 5 to 10% of the total acids; both oleic and linoleic acids are present in abundance; stearic acid is present in smaller amount than palmitic acid, but other saturated acids are generally absent or present only in very small quantities. In this respect the seed oil of *Solanum nigrum* conforms, in its fatty acid composition, to the abovementioned general type, except that the saturated acids form only about 4% of the total acids, palmitic and stearic acids occurring in equal amounts.

Whereas the fatty acid composition of the oils of *Solanum indicum* and *Solanum nigrum* fairly conforms to the above mentioned type, their glyceride composition, on which the physical and chemical properties depend, is markedly different. They are similar in one respect, *viz.*, the content of oleodilinolein: it is 51% in *Solanum indicum* and 45% in *Solanum nigrum*. In other respects there are marked differences not only in the percentage amounts of the glycerides, but also in their nature: the percentage of dioleolinolein is 10·4 in *Solanum indicum* and 46·2 in *Solanum nigrum*; trilinolein occurs to the extent of 1·6% in oil of *Solanum indicum*, but it is absent in that of *Solanum nigrum*.

## Experimental

The material employed for this investigation consisted of authentic seeds of *Solanum nigrum* Linn. The preliminary examination of the seeds was conducted and the following results obtained:—

| | |
|---|---|
| Average weight of seed .. | 0·0006 gms. |
| Ash content of the seed.. | 7·6% |
| Water insoluble in ash .. | 36·69% |
| Water soluble in ash .. | 63·31% |

The following is the qualitative composition of ash:—

| | |
|---|---|
| Water soluble .. | Sodium, Potassium, Chloride, Carbonate, Sulphate. |
| Water insoluble .. | Calcium, Magnesium, Carbonate, Sulphate. |

Fifty gm. of the crushed seeds were extracted in a Soxhlet apparatus with various solvents in succession as given below:—

| | | |
|---|---|---|
| 1. | Petroleum ether (40–60°) .. .. | A clear dark green fatty oil was obtained. Yield 21·5%. |
| 2. | Absolute ether .. | A similar product as above. Yield 2·64% |
| 3. | Chloroform .. | A dark green residue. Yield 2·35% |
| 4. | Ethyl acetate .. | A green residue. Yield 2·04% |
| 5. | Absolute alchol .. | A brown residue. Did not give any test for glucoside or sugar. Yield 5·6% |
| 6. | 70% Alcohol .. | A similar residue as above. Yield 1·76% |

For the purpose of complete examination four kilograms of the seeds were crushed and extracted with petroleum ether (40–60°). It was a dark green transparent clear oil.

### *Examination of Fatty Oil*

The oil was purified with animal charcoal and Fuller's earth. The physical and chemical constants of the oil were determined as given in Table I.

Five hundred grams of the oil were saponified with alcoholic sodium hydroxide, the unsaponifiable matter removed with ether and the fatty acids liberated. The constants of these mixed fatty acids are given below in Table II.

The mixed fatty acids were then separated into solid and liquid acids by Hilditch's[6] modification of Twitchell's lead salt-alcohol process. The mixed fatty acids (200 gm.) are dissolved in 1000 c.c. of 95% alcohol and

TABLE I

| Constants | Present authors' results | Pendse's results |
|---|---|---|
| Specific gravity .. | 0·9125 (30° C.) | 0·8964 (30° C.) |
| Refractive index .. | 1·4712 (25° C.) | 1·4436 (30° C.) |
| Angle of rotation .. | Oil was too dark to be read | $[\alpha]_D^{28}$ —6·61° |
| Acid value .. | 11·62 | 2·4 |
| Saponification value .. | 184·0 | 184·7 |
| Acetyl value . | 25·7 | 9·97 |
| Hehner value .. | 92·9 | 93·1 |
| Unsaponifiable matter .. | 1·4% | 1·4—1·6% |
| R.M. value .. | 0·66 | .. |
| Iodine value (Hanus) .. | 123·2 | 111·7 |
| Thiocyanogen value (24 hours) .. | 84·4 | .. |

TABLE II

| Constants | Present authors' results | Pendse's results |
|---|---|---|
| Neutralization number .. | 200·0 | 183·4 |
| Saponification Equivalent .. | 280·5 | 305·2 |
| Iodine value (Hanus) . | 129·0 | 112·4 |
| Thiocyanogen value (24 hours) .. | 86·6 | .. |

the solution boiled and then mixed with a boiling solution of lead acetate (140 gm.) in 1000 c.c. of 95% alcohol containing 1·5% of glacial acetic acid. The lead salts which are deposited on cooling at 15° C. overnight are recrystallised from two litres of alcohol. The solid acids are regenerated from the crystallised lead salt, and the liquid acids recovered from the lead salts left on evaporation of mixed alcoholic filtrates from both operations. The constants of solid and liquid fractions are given in Table III.

TABLE III

| Constants | Sold acids | Liquid acids |
|---|---|---|
| Percentage .. | 4·37 | 95·63 |
| Neutralization number .. | 201·2 | 199·4 |
| Saponification equivalent .. | 278·9 | 281·4 |
| Iodine value (Hanus) .. | 3·73 | 134·9 |
| Thiocyanogen value (24 hours) .. | 2·0 | 91·5 |

### *Examination of Liquid Acids*

A known weight of liquid acids was brominated[7,8] in dry ether at —10° C. and kept at this temperature for two hours. No solid separated thus showing the absence of linolenic acid. The excess of bromine was destroyed

with sodium thiosulphate solution, washed with distilled water and dried over anhydrous sodium sulphate. Ether was distilled off. The residue was dissolved in 500 c.c. of petroleum ether (40–60°) and kept in frigidaire overnight. Some tetrabromide separated. It was filtered on a weighed Gooch crucible. The filtrate was concentrated to 250 c.c. and again kept in frigidaire overnight. Some more tetrabromide separated. This also was filtered into the same Gooch crucible. The solvent from filtrate was removed and the residue (di- and tetrabromide) weighed and the bromine content of the mixture determined. The results of bromination and analysis are given below in Table IV:

TABLE IV

| | |
|---|---|
| Weight of acid brominated .. .. . | 2·2696 gm. |
| Weight of linoleic acid tetrabromide .. .. .. | 0·4530 gm. |
| M. P. of tetrabromide .. .. .. .. | 113·0° C. |
| Weight of residue (di-and tetrabromide) .. .. .. | 3·7310 gm. |
| Bromine content of residue .. .. .. | 44·82% |
| Weight of linoleic acid tetrabromide in residue .. .. | 1·8790 gm. |
| Total weight of tetrabromide .. .. .. | 2·3320 gm. |
| Weight of oleic acid dibromide in residue .. .. .. | 1·8520 gm. |
| Weight of Linoleic acid .. .. .. .. | 1·0880 gm. |
| Weight of oleic acid .. .. .. .. | 1·1820 gm. |
| Percentage of linoleic acid in liquid acids .. .. .. | 47·96 |
| Percentage of oleic acid in liquid acids .. .. .. | 52·04 |

Five grams of the mixed acids were oxidized with alkaline permanganate by Bertram's method[9] and 3·84% of solid acids were obtained. This was repeated with five grams of oil and solid acids thus obtained were 3·48%.

The percentage of linoleic, oleic and solid acids as calculated by different methods is given below in Table V.

TABLE V

| Method | Oleic acid | Linoleic acid | Solid acids |
|---|---|---|---|
| | % | % | % |
| 1. From thiocyanogen value of mixed acids (Table II) | 49·2 | 47·8 | 3·0 |
| 2. From thiocyanogen value of liquid and solid acids (Table III) | 49·82 | 45·91 | 4·27 |
| 3. By bromination of liquid acids (Table IV) | 50·16 | 46·20 | 3·64 |
| 4. Average of 1 to 3 .. | 49·73 | 46·63 | 3·64 |
| 5. Solid acids by Bertram's method .. | .. | .. | 3·84 |
| 6. By glyceride structure (Table XI) .. | 49·00 | 47·22 | 3·78 |
| 7. Pendse's results .. | 68·45 | 25·66 | 5·89 |

The percentage weights of the component acids obtained by the different methods agree fairly closely amongst themselves as well as with those deduced from the experiments on the glyceride structure of the oil.

## *Examination of Solid Acids*

The solid acids were converted into methyl esters. 10·52 grams of the esters were fractionally distilled under reduced pressure, and the results of distillation and calculation[10] are given in Tables VI and VII.

TABLE VI

| Fraction No. | Pressure | Temperature range | Weight in grams |
|---|---|---|---|
| $S_1$ .. | 6 m.m. | 175°–180° C. | 5·295 |
| $S_2$ .. | 6 m.m. | 180°–185° C. | 2·356 |
| $S_3$ .. | 6 m.m. | 185°–190° C. | 1·460 |
| $S_4$ (residue) .. | .. | .. | 1·149 |
| Total .. | | | 10·260 |
| Loss .. | | | 0·260 |

*Fractions $S_1$ and $S_2$.*—By repeated crystallizations from dilute acetone of the acids liberated from these fractions gave palmitic acid m.p. 61° C., which was not depressed on adding an authentic sample of palmitic acid.

*Fraction $S_3$.*—On crystallizing from dilute acetone of the liberated acids, stearic acid m.p. 62° C. was obtained. The m.p. was raised by adding an authentic sample of stearic acid and lowered by palmitic acid.

*Fraction $S_4$ (Residue).*—The acid was liberated and extracted with petroleum ether and crystallized from dilute acetone. Stearic acid m.p. 63° C. was obtained which was raised by adding an authentic sample of stearic acid. The molecular weight was found to be 282·4.

TABLE VII

| Fraction No. | I. V. | S. E. | Palmitic acid % | Palmitic acid Gm. | Stearic acid % | Stearic acid Gm. | Unsaturated acid % | Unsaturated acid Gm. |
|---|---|---|---|---|---|---|---|---|
| $S_1$ .. | 1·0 | 278·4 | 67·17 | 3·557 | 27·09 | 1·432 | 0·074 | ·003934 |
| $S_2$ .. | 1·01 | 284·5 | 46·69 | 1·100 | 47·65 | 1·123 | 0·074 | ·201735 |
| $S_3$ .. | 2·25 | 298·4 | .. | .. | 93·64 | 1·368 | 1·666 | ·02434 |
| $S_4$ (Residue) .. | 4·96 | 300·7 | .. | .. | 91·62 | 1·053 | 3·676 | ·04225 |
| | | | | 4·657 | | 4·976 | | ·07226 |

| | |
|---|---|
| Palmitic acid in solid acids .. .. | 48·37% |
| Stearic acid in solid acids .. .. | 51·63% |
| Palmitic acid in mixed acids .. .. | 1·76% |
| Stearic acid in mixed acids .. .. | 1·88% |

The component acids of the oil from the seeds of *Solanum nigrum*, therefore, consist of oleic, linoleic, palmitic and stearic acids. The percentages are given below in Table VIII.

TABLE VIII

| | Wt.% | Mol. % |
|---|---|---|
| Oleic acid .. | 49·73 | 49·57 |
| Linoleic acid .. | 46·63 | 46·79 |
| Palmitic acid .. | 1·76 | 1·84 |
| Stearic acid .. | 1·88 | 1·80 |

*Examination of Unsaponifiable Matter*

The unsaponifiable matter extracted from the soap solution with ether was a pale yellow sticky mass, which on crystallisation from absolute alcohol gave a compound m.p. 133° C. the acetyl derivative of which melted at 121–22° C. It gave all colour reactions of a phytosterol. Therefore, it is sitosterol.

*The Component Glycerides*

The oil was neutralized with sodium carbonate and purified with animal charcoal and Fuller's earth.

One hundred grams of the purified oil was dissolved in six times its weight of dry acetone[11,12] and kept in frigidaire for a week at 0° C. 4·02 grams of solid separated showing the presence of trisaturated or disaturated monounsaturated glycerides or both.

Twenty-five grams of the neutral oil was dissloved in ten times its weight of dry acetone and oxidized[13] with powdered permanganate. The process was repeated twice and in the end nothing remained showing the absence of trisaturated glycerides.

Acetone was distilled off from the filtrate of acetone-chilled oil and the oil, thus left, was dissolved in ten times its weight of dry petroleum ether (40–60° C), cooled to −5° C. and excess of bromine added till the brown colour persisted. It was left in frigidaire overnight. No solid separated. Excess of bromine was destroyed with sodium thiosulphate solution and washed with distilled water, dried over fused calcium chloride and petroleum ether distilled off. The brominated oil was extracted with absolute alcohol and alcohol and acetone (1 : 1) successively. The scheme of separation is given below:

Fractions $F_2$ and $F_3$ were debrominated by taking them in methyl alcohol, adding zinc dust (equal in weight to the fraction), passing dry hydrochloric acid gas to saturation and refluxing for eight hours. All the three fractions were then saponified, unsaponifiable matter removed with ether and acids liberated. The saponification values, and thiocyanogen values

Neutral oil (100 gm. (Chilled in acetone)

- Insoluble $F_1$ (4·02 gm.)
  - Insoluble (Nil)
    - Insoluble (extracted with alchohol and acetone (1:1)
      All dissolved ($F_3$)
      104·8 gm. (brown viscous) liquid
    - Soluble ($F_2$) 67·76 gm. (dark viscous liquid)
  - Soluble (Washed with thiosulphate solvent removed; extracted with absolute alcohol)
- Soluble (Filtered and solvent removed, brominated in petroleum ether)

of acids were determined. The acids were oxidized with dilute potassium permanganate, extracted with petroleum ether and their saponification values determined.

The results calculated from these values are given in Tables IX, X, XI and XII. These results are in fair agreement with those obtained for the component fatty acids and are given below in Table XI.

TABLE IX

| | $F_1$ | $F_2$ | $F_3$ |
|---|---|---|---|
| Wt. of brominated product in gm. .. | ..* | 67·76 | 104·8 |
| Wt. of debrominated product in gm. .. | 4·02 | 37·97 | 57·97 |
| Wt. of unsaponifiable matter in gm. .. | 1·0 | 0·25 | 0·002 |
| Wt. percent of glyceride (free from unsaponifiable matter) | 3·06 | 38·21 | 58·73 |
| Sap. Equivalent of liberated acids .. | 279·7 | 279·1 | 280·6 |
| Iodine value of liberated acids .. | 32·4 | 130·2 | 134·0 |
| Thiocyanogen value of liberated acids .. | 32·14 | 86·76 | 89·6 |
| Sap. Equivalent of solid acids .. | 259·2 | 270·3 | 280·6 |
| Mol. per cent. of mixed acids. .. | 3·06 | 38·21 | 58·73 |

* *N.B.*—Fraction $F_1$ was got by chilling from acetone and hence was not brominated.

TABLE X

*Mol. per cent, of acids in each fraction*

| | $F_1$ 3·06% | $F_2$ 38·21% | $F_3$ 58·73% |
|---|---|---|---|
| Linoleic acid .. | 0·27 | 48·06 | 49·19 |
| Oleic acid .. | 33·56 | 47·93 | 50·09 |
| Solid acids .. | 66·17 | 3·91 | 0·72 |

TABLE XI

*Mol. per cent. of acids on total acids*

| | $F_1$ 3·06% | $F_2$ 38·21% | $F_3$ 58·73% | Mean 100% |
|---|---|---|---|---|
| Linoleic acid .. | 0·008 | 18·36 | 28·89 | 47·25 (46·79) |
| Oleic acid .. | 1·027 | 18·31 | 29·42 | 48·76 (49·57) |
| Solid acids .. | 2·025 | 1·54 | 0·42 | 3·99 (3·64) |

*N.B.*—In fraction $F_1$ the amount of linoleic acid being too small, it may be considered as oleic acid.

The figures in brackets are obtained from component fatty acids (Table VIII).

TABLE XII

*Probable component glycerides of the oil of* Solanum nigrum *(Mol. per cent.)*

| Glycerides in | $F_1$ 3·06% | $F_2$ 38·21% | $F_3$ 58·73% | Mean 100·0% |
|---|---|---|---|---|
| 1. Fully saturated glycerides .. | Nil | Nil | Nil | .. |
| 2. Disaturated-monounsaturated glycerides | 3·06 | Nil | Nil | 3·06 |
| 3. Monosaturated-Diunsaturated glycerides | .. | .. | .. | .. |
| (*a*) Monosaturated oleolinolein .. | Nil | 4·62 | 1·26 | 5·88 |
| 4. Triunsaturated glycerides— | | | | |
| (*a*) Oleodilinolein .. | Nil | 16·87 | 27·97 | 44·84 |
| (*b*) Dioleolinolein .. | Nil | 16·72 | 29·50 | 46·22 |

*N.B.*—1. By oxidation of the neutral oil with potassium permanganate in acetone.

2. By chilling the neutral oil in acetone at 0° C.

3 & 4. By calculating from the component fatty acids of the brominated glycerides in the oil.

In these calculations the solid acids have been considered as one acid. In view of the fact that the acids distribute themselves evenly in proportion to their amounts, we are justified in assuming that the unsaturated acids in fractions $F_2$ and $F_3$ are combined in the glycerides of the *Solanum nigrum* seed oil as monosaturated oleolinolein rather than as monosaturated diolein and monosaturated-dilinolein. The same may be assumed in case of saturated acids in these fractions as well as in fraction $F_1$, and as such the probable glyceride structure may be given as follows:—

Dipalmito-olein 2·43%; Palmitostearo-olein 0·63%

Palmito-oleolinolein 2·48%; Stearo-oleolinolein 3·40%; Oleodilinolein 44·84% and Dioleolinolein 46·22%.

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## References

1. Kirtikar and Basu .. *Indian Medicinal Plants*, Vol. III, 1748.
2. Dey .. *Indigenous Drugs of India*, 300.
3. Dymock, Warden and Hooper *Pharmacographic Indica*, Vol. I, 549.
4. Pendse .. *J. Ind. Chem. Soc.*, 1937, 367–70.
5. Saran, B. M., and Singh, B. K. *Proc. Nat. Acad. Sci. (India)*, 1942, **12**, 219.
6. Hilditch, T. P. .. *Chemical Constitution of Natural Fats*, 1941 Edition, 371.
7. Jamieson and Boughmann *J. Amer. Chem. Soc.*, 1920, **42**, 1197.
8. Dean, H. K. .. *Utilization of Fats*, 72.
9. Eibner and Muggenthalor *Chem. Tech. & Analysis of Oils, etc.*, 5th Edn., Vol. I, 568.
10. Jamieson and Boughmann *J. Amer. Chem. Soc.*, 1920, **42**, 152.
11. Singh, B. K., and Tewari, R. D. *Proc. Nat. Acad. Sci. (India)*, 1943, **13**, 128.
12. Hilditch, T. P. .. *Chemical Constitution of Natural Fats*, 1941 Edition, 412–13.
13. ———— .. *Ibid.*, 405–06.