Subject: The Chemistry of Olee Harpsa

From Melia Azadirachta.
THEORETICAL
Margosa oil, commonly known as Neem oil, is the oil obtained from the seed of Melia Azadirachta. The latter is a large evergreen tree growing wild throughout the greater part of the Indian peninsula, and frequently planted as a homestead or avenue tree as it is believed to purify the air.

The fruit gives an oil of a yellowish brown colour which appears dark in a thick layer and has a disagreeable odour resembling that of garlic.

The seeds contain a high percentage of oil. It has got a high reputation for medicinal value from early times.

The physiological actions of all the parts of this plant employed as drugs may be arranged as follows:

1. The root-bark, bark and young fruits — tonic and antiperiodic.
2. The oil, nuts and leaves — local stimulant, insecticide and antiseptic.
3. The flowers — stimulant and stomachic.
4. The gum — demulcent tonic.
5. The toddy — refrigerent, nutrient and alterative tonic.

The oil, according to the opinion of Major T. B. Spencer, I.M.S. (Kirtikar and Basu, Indian medicinal products p. 307), is the most active part of the plant. Externally it is
stimulant and antiseptic and is very useful in chronic syphilitic sores and indolent ulcers. It is also extremely useful as a parasiticide in various cutaneous affections, such as ringworm, scabies and others.

The natural yield of the seed per annum in India is a considerably large quantity, but the only demand for the oil is being for medicinal purposes, and that, too being very small, by far the larger part of the annual yield is never collected at all or run to waste. A nominal quantity is also being used by a few soap makers in India to make medicinal soaps possessing the odour of the original oil.

The chemical nature of the oil was first investigated by Warden (Phar. Indica, Vol. I, p. 223; J. Indian Chem. Soc. 1931 - 8 - 773), who found the oil to contain sulphur and removed the odorous bitter constituents by shaking the oil with alcohol.

Chatterjee (Indian Med. Gazettee 54, 171 - 4 (1919); C.A. 1920 - 14 - 2218) studied the bitter compound in detail and found the characteristic acid of the oil to be \textit{marmacic} acid belonging to the linoleic acid series.

Watson, Chatterjee and Mukerjee (J. Soc. Chem. Ind. 42, 387 - 9 T (1923); C.A. 1923, 18, 224), undertook the investigation primarily to determine whether neem oil could be refined to compete with other oils for edible purposes.
soap making, paint and varnish making, lubrication and the like and incidentally to study the chemistry of the odorous and bitter constituents. They ascribed the objectionable odour to the presence of slightly volatile organic sulphur compounds in the oil. The bitterness was also attributed to the presence of resinous acid in the form of esters in the oil. Watson, Chatterjee and Mukerjee (loc. cit.) removed the bitter principle of the substance by alcoholic extraction which they found to be $C_{15}H_{20}O_5$.

With a view to have a closer examination of the bitter principle, Sen and Banerjee (J. Indian Chem. Soc. 1931, 8, 773) extracted the oil with hot water and the bitter compounds isolated by them had been found to differ entirely from those of Watson and co-workers in the fact that it contained sulphur in the molecule. Their conclusion was that the bitterness of the oil was partly due to the presence of the sodium salt of some acid of mol. weight 281 and partly due to the presence of free acid which was held in solution in the oil.

Roy and Dutt (J.A. 1930, 24, 685) from a careful examination of the oil, came to the conclusion that the so-called 'margaric acid' was not a chemical entity and according to their opinion, it was the impure oleic acid found in the oil which was responsible for the bad odour.
Quadrati-Khuda, Ghosh and Mukerjee (J. Indian Chem. Soc. 1940 - 17 - 189-194) undertook a closer examination of the oil. They ascribed the objectionable odour of the oil to a substance containing sulphur in the molecule having the molecular formula \( \text{C}_{16}\text{H}_{30}\text{O}_3 \). The bitter compound, according to their opinion, was present in the residual oil (after the removal of the odoriferous constituent) and was free from sulphur having the molecular formula \( \text{C}_{23}\text{H}_{48}\text{O}_{10} \), thus differing from the observations of Sen and Banerjee (loc.cit).

An examination of the oil was undertaken by the Imperial Institute (London) to explore the possibility of removing the objectionable features of the oil, so as to render it fit for common soap making industry, but with no success.

A further examination for the deodourisation of the oil was undertaken by the Industries Department, Government of Bengal (Neem oil and its treatment for application in making washing soaps, Bulletin No.47, Industries Department, Government of Bengal). After several attempts to that end it was found that the oil did not lend itself to the usual chemical and other treatments with success.

Quadrati-Khuda, Ghosh and Mukerjee (loc.cit) found out four acids — two saturated and two unsaturated — having the molecular formulae \( \text{C}_{14}\text{H}_{28}\text{O}_2 \) and \( \text{C}_{16}\text{H}_{32}\text{O}_2 \) for the saturated ones and \( \text{C}_{15}\text{H}_{28}\text{O}_2 \) and \( \text{C}_{18}\text{H}_{32}\text{O}_2 \) for the unsaturated acids.
It appears that no work has yet been done on the unsaponifiable matter of the oil — the percentage of the unsaponifiable matter being too small. No detailed study has been made as regards the determination of the physical and chemical constants of the oil.

**Aim of the present work:**

The present work was undertaken with a view

1. To determine all the physical and chemical constants of the oil to have the preliminary ideas about the contents of the oil.

2. To study the bitter and the odoriferous constituents of the oil and to correlate the conflicting data so far mentioned in the literature by different investigators.

3. To institute a close examination of the fatty acids present in the oil.

4. To examine the unsaponifiable matter of the oil and

5. To study the medicinal properties of the constituents of the oil.

Genuine samples of fresh neem oil were procured from the local market.

A dry and purified representative sample of the oil gave the following physical and chemical constants:

**Physical:**

1. Consistency: Thick
2. Density at 15°C: 0.9132
3. Refractive Index at 31.3°C: 1.4662
4. Specific rotation at 30.7° C (10% solution in chloroform) \(-10.66\)
5. Solubility in 95% alcohol \(2.05\%\)

Chemical:
1. Saponification value \(198.5\)
2. Iodine value (Mij's solution) \(69.92\)
3. Hypochlorous acid value (Goswami and Basu) \(17.82\)
4. Thermal value \(14.2°C\)
5. Maumene test (Specific temperature reaction) \(78.7°C\)
6. Acid value \(2.73\)
7. Acetyl value \(16.56\)
8. Total saturated acids \(30.5\%\)
9. Total unsaturated acids \(64.49\%\)
10. Total unsaponifiable matter \(0.9\%\)

An attempt has been made to correlate the above data and explain their significance:

The thermal value and the Maumene test indicate that the oil is non-drying. This inference is further supported by the iodine value.

The solubility of the oil in alcohol is not much above the ordinary value (a little less than 2%). This shows the presence of little amount of glycerides of hydroxy acids.

The high acetyl value therefore suggests the presence of free alcohols (such as cholesterol or phytosterol) as also monoglycerides and di-glycerides. The high acid value is always associated with rancidity. As the value found in this case is too low -- it shows definitely the absence of rancidity and decomposition of the oil. The absence of
tetra-, hexa-, and octabromides in the bromination products of fatty acids indicates the absence of poly-unsaturated acids.

The oil contains a volatile odoriferous constituent together with a bitter substance.

A quantity of fresh neem oil was subjected to a process of prolonged steam distillation until all volatile substances were removed. The odoriferous constituent was found present in the distillate, whereas the bitter compound was present in the residual oil.

The distillate was cooled in ice, saturated with common salt and extracted with ether. After the removal of the solvent, the product, was distilled carefully under reduced pressure. On repeated distillation, a mobile yellow liquid was obtained which had a very objectionable smell. This product has been found to contain sulphur in the molecule and did not taste bitter. One gram of the product was obtained from 1000 grams of the oil.

The residual oil after the steam distillation was not free from the unpleasant odour. This oil was then extracted for a number of times by hot water.

The water extract was found to be extremely bitter. It was dried under reduced pressure. A gummy residue was obtained which was triturated with petroleum ether and extracted with chloroform. After the removal of the solvent, a yellow product (granular) was obtained which was treated with charcoal.
The product thus obtained was found free from sulphur and the yield was small.

These experiments definitely proved that the odoriferous constituent is a substance having sulphur in the molecule and the bitter principle—a product without sulphur and most probably a glucoside. The observations of Don and Banerjee (loc. cit) and Watson et al (loc. cit) are thus disproved.

The oil after the removal of the odoriferous constituent and the bitter principle—was saponified in the usual manner and the unsaponifiable matter extracted by means of ether in a Soxhlet.

The acids liberated from the sodium soaps by the treatment with strong HCl were extracted with ethyl ether. A part of it was dried with anhydrous sodium sulphate and purified.

A dry sample thus obtained gave the following values:

- Mean molecular weight: 293
- Iodine value: 64.49 %

The mean molecular weight showed the predominance of $C_{18}$ and $C_{20}$ acids and the iodine value indicated the percentage of the unsaturated acids in the oil.

The mixed acids, free from the unsaponifiable matter, were separated into saturated and unsaturated acids by Twitchell's lead salt alcohol method (Ind. Eng. Chem. 1921, 13, 906), and were found to possess the following constants:

<table>
<thead>
<tr>
<th>Acids</th>
<th>Iodine value</th>
<th>Mean molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S) solid</td>
<td>1.5</td>
<td>291</td>
</tr>
<tr>
<td>(L) liquid (unsaturated)</td>
<td>120.3</td>
<td>278</td>
</tr>
</tbody>
</table>
The mean molecular weight and the iodine value of the saturated (solid) acids showed the predominance of $C_{18}$ and $C_{20}$ acids and the total separation of the saturated and the unsaturated acids while those of the unsaturated (liquid) acids showed the predominance of $C_{18}$ and $C_{16}$ acids and that there was at least one acid having two double bonds.

Methyl esters of the saturated acids thus obtained were prepared in the usual manner (refluxing the acids with methyl alcoholic hydrochloric acid), purified, dried and fractionated under reduced pressure.

The fractions obtained were saponified separately and the acids liberated by strong hydrochloric acid. From the liberated acids three acids, having molecular formulae $C_{16}H_{32}O_2$, $C_{20}H_{40}O_2$ and $C_{14}H_{28}O_2$ could be isolated and identified. The first two acids were definitely palmitic acid, m.p./61-63°. and Arachidic acid m.p./77-78°. The molecular weight of the two acids respectively were found 257 and 310. The two acids require m.w. 256 and 312.

The most interesting result was obtained from the fraction (b.p. methyl ester 200-202°). The m.p. of the acid obtained from this fraction was 66-67°. Though the m.p. of the acid and the b.p. of the methyl ester were in fair agreement with those of stearic acid, it was not found possible to get the characteristic tests of the stearic acid. The molecular weight when carefully determined was
found to be 228. A duplicate experiment confirm the 
molecular weight. C\textsubscript{14}H\textsubscript{28}O\textsubscript{2} requires molecular weight 228. 
So the acid appeared to be C\textsubscript{14}H\textsubscript{28}O\textsubscript{2}. 
The three acids enumerated above were found to be present by a duplicate experiment of the acids by fractional 
crystallisation. 
The unsaturated (liquid) acids were saponified to break up any ethyl esters that might have been formed during 
Twitchell's separation. A portion of the de-esterified acids was converted into potassium soap and oxidised in 
cold alkaline solution with dilute potassium permanganate according to the modified method of Hazura (Lewkowitsch, 
dihydro stearic acid m.p. 131-32° a molecular weight 315 
was obtained. The residue on further crystallisation gave a product melting over a wide range of temperature 155-171° 
but having a molecular weight of 348. This was not considered strange as many investigators (Power and Borrowaliff, 
Katti and Puntambekar J.Indian Chem. Soc.1930,7,223; S. 
Krishna and S. V.Puntambekar J.Indian Chem. Soc.1931,8,301) have found that acids agreeing in composition and character 
with tetra hydro stearic acid possessed melting points
varying from 155-173 ° and it is probable, therefore, that these compounds represent isomeric acids of the formula 
\[ \text{C}_{18} \text{H}_{36} \text{O}_{6} \].

No hexa hydro stearic acid was found in the aqueous filtrate from the products of oxidation thus showing the absence of linolenic acid.

Freshly prepared mixed fatty acids were brominated according to the method of Eibner and Magyenthaler (Lewkowitzch, loc. cit. p. 585) crystallised from petroleum ether the brominated product gave tetra bromo stearic acid (m.p. 113-14 °, M.W. 597). It gave no hex or higher bromides.

The oxidation and the bromination results thus clearly showed that the unsaturated acids consisted mainly of oleic and linoleic acids.

The presence of the above two acids was further substantiated by the preparation of the methyl esters of the mixture of the acids and by their fractionation at reduced pressure.

The acids were liberated from the two main fractions and their iodine values determined. The values were 86.17 and 110.2 which were evidently those of oleic and linoleic acids. The boiling points of the methyl esters of the two fractions were in close agreement with those of methyl oleate and methyl linoleate.
The acids obtained by the author differed mostly from those obtained by Quadri-Khuda et al.

The method the latter adopted for the separation of the saturated and unsaturated acids was susceptible to serious drawbacks.

It is a well known fact that the separation of the saturated and the unsaturated acids is a difficult problem. The standard methods sometimes fail to separate them fully. The method adopted by Quadri-Khuda has been disproved already by a number of workers (Lewkowitsch and others).

There has been no mention in their work of the characteristic factors of acids — namely the iodine value and the neutralisation value. There is ample reason to believe that the unsaturated acids, they isolated, must have got some saturated acids with them. It may be mentioned here that though the acids — Neem acid C and Neem acid D (C_{18}H_{32}O_2 and C_{15}H_{28}O_2) obtained by them were unsaturated they were solids melting at 47-48° c and 31-32° c and the acids partially solidified on cooling. This indicated the presence of saturated acids, with them. The iodine value and the neutralization value of the acids had not been given for the support of the composition of the unsaturated acids they obtained. The most peculiar observation in their work was that they succeeded in isolating an unsaturated acid having a mol. formula C_{16}H_{28}O_2 which is an unusual observation in the case of fatty acids.
The fatty acids generally as a rule contain even number of carbon atoms. The claims so far made by different workers in obtaining acids with odd number of carbon atoms have been afterwards proved to be incorrect and the acids proved to be the mixture of different acids. The only substantial claim which was made by Gerard (Lewkowitz loc. cit) and others as regards the presence of butyric acid in vegetable oils having the molecular formula \( \text{C}_7\text{H}_{14}\text{O} \) has been recently disproved by S.L. Manjunathan and S.S. Siddappa (J. Indian Chem. Soc. 1935-12-400; J. Indian Chem. Soc. 1935-12-611). They have shown that it was a mixture of acids.

Here in this case also it was not found possible to isolate any acid having an odd number of carbon atoms by the method they adopted and by other methods. No support for the cyclic nature of the acid \( \text{C}_{17}\text{H}_{36}\text{O} \) obtained by them has been put forward by the workers.

The unsaponifiable matter, obtained from the sodium soaps of the mixed acids, was treated with charcoal twice to remove the colouring matter. When repeatedly crystallised from cold alcohol, it gave a product melting at 66.5° C. The substance was neutral; it burnt with a smoky flame. The preliminary experiments showed the absence of nitrogen, sulphur, the hydroxyl group, the carbonyl group and the aldehyde group. It was not soluble in cold concentrated sulphuric acid nor was it coloured by the contact of that
acid. It did not absorb bromine. Hence it was concluded that it was a saturated hydrocarbon.

Its molecular weight as found by the Rent method was 442.

The mother liquor after the removal of the hydrocarbon was concentrated to a small bulk and allowed to crystallise in cold when a product was obtained in plates (colourless) which gave a m.p. 119-121° C. On several recrystallisations from petroleum ether and finally from benzene and alcohol, the m.p. improved to 135-136° C. Its acetate was prepared by means of acetic anhydride—the m.p. found was 126-128° C. Hence it clearly showed the presence of the common phytosterol-sitosterol in the oil. It gave the usual tests for the sterol. For further verification of the observation the unsaponifiable matter was acetylated by acetic anhydride. Needlelike crystals were obtained—the m.p. of which was 126-128° C which definitely proved the presence of sitosterol (C_{27}H_{46}).

The unsaponifiable matter from neem oil thus contains a hydrocarbon of molecular weight 442 and a phytosterol—sitosterol C_{27}H_{46}.

As regards the deodourisation of the neem oil a number of experiments were undertaken, namely, (1) steam distillation for about 40 hours, (2) distillation by superheated steam for about 35 hours and the subsequent treatment of the residual oil by caustic soda and (3) the treatment of the oil with alcohols but the only method which proved successful to a large extent was the method of distillation by superheated steam. The oil was almost free from the odour by this treatment.
CONCLUSIONS

1. The physical and the chemical constants of a representative sample of the oil were determined.

2. The odoriferous and the bitter constituents of the oil were examined. The former was found to contain sulphur in the molecule while the latter was free from sulphur. It thus proved the observations of Khuda et al (loc. cit) as true and disproved the works of Watson et al (loc. cit) and Sen and Banerjee (loc. cit).

3. The saturated and the unsaturated acids of the oil were isolated and identified. The acids found were the following with the properties mentioned below:

<table>
<thead>
<tr>
<th>Acids</th>
<th>Melting point in °C</th>
<th>Molecular weight</th>
<th>Required molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>61-63</td>
<td>257</td>
<td>256</td>
</tr>
<tr>
<td>Arachidic</td>
<td>77-78</td>
<td>310.6</td>
<td>312</td>
</tr>
<tr>
<td>( \text{C}<em>{14} \text{H}</em>{28} \text{O}_2 )</td>
<td>66</td>
<td>229.8</td>
<td>228</td>
</tr>
</tbody>
</table>

Unsaturated:
- Oleic acid \( \text{C}_{18} \text{H}_{34} \text{O}_2 \)
- Linoleic acid \( \text{C}_{18} \text{H}_{32} \text{O}_2 \)
16.

The acids obtained by the author differed from those obtained by Khuda et al., namely, only one acid \( \text{C}_{14}\text{H}_{23}\text{O} \), m.p. 66°c had some resemblance with isotetradecic acid \( \text{C}_{14}\text{H}_{23}\text{O} \), m.p. 67°c obtained by them. The other three acids identified by them were iso-palmitic acid \( \text{C}_{16}\text{H}_{32}\text{O} \), m.p. 55°c, \( \text{C}_{15}\text{H}_{28}\text{O} \) (oleic acid series) m.p. 33-32°c and \( \text{C}_{16}\text{H}_{32}\text{O} \) (cyclic series) m.p. 47-48°c.

A phytosterol — sitosterol m.p. 135-136°c and m.p. acetate 126-128°c was isolated and identified, from the unsaponifiable matter of the neem oil.

A new saturated hydrocarbon having the molecular weight 442 was also isolated from the unsaponifiable matter.

Some experiments were undertaken to de-odorise the oil, but no practical method could be devised for the full removal of the odour of the oil.
EXPERIMENTAL