# CHEMICAL EXAMINATION OF *CLEOME PENTAPHYLLA* LINN. PART II. CONSTITUENTS OF THE OIL FROM THE SEEDS.

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In a previous communication on the subject Misra and Dutt,[1] in the course of their investigations on the chemical constituents of *Cleome pentaphylla* Linn. (N. O. Capparideae), isolated from it a substance of the nature of a lactone called by them cleomin, and reported that the drug also contains an oil to the extent of 22%. A few constants of this oil were previously determined by Hooper (*Ann. Rept., Indian Museum, Industrial Section*, 1908-09). Hooper found that it had Acid Value 6·4 ; Saponification Value 194·6 ; and Iodine Value 119·5. This appears to be all the work done on the fatty constituents of this highly medicinal drug. A systematic examination of the oil was undertaken by the present authors and forms the subject of this communication.

The three constants found by Hooper appear to be in fairly good agreement except for the acid value. These, as determined by the present authors, were found to be Acid Value 36·5 ; Saponification Value 194 ; and Iodine Value 122·6. As these represent the mean of several determinations conducted with all precautions, they are more reliable.

It was not found practicable to determine the proportion of unsaturated acids in a quantitative manner, as even on repeated attempts at bromination no crystalline bromo-derivatives could be separated. Qualitatively, however, the presence of oleic and linolic acids has been proved and is described in detail in the experimental part.

## EXPERIMENTAL.

*Extraction of the oil.*—2 kg. of the seeds were obtained from the neighbourhood and finely crushed in an iron mortar, having previously been dried in the shade. The crushed mass was then extracted exhaustively with benzene in a large extraction flask. The benzene extracts were collected together and the solvent removed by distillation when an oil of a light green colour was left (446 gms.), having a faint odour of mustard. The oil was then purified with animal charcoal and Fuller's earth. Even on prolonged keeping no sediment or crystalline matter was deposited.

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[1] *Proc. Nat. Inst. Sci. India*, Vol. III, p. 45, (1937).

*Examination of the oil.*—The oil contained no nitrogen or sulphur and was optically inactive. It burned with a partially sooty and colourless flame and a thin film exposed to the air in the laboratory resinified in a few days, indicating that it belongs to the class of semi-drying oils. Table I gives its physical and chemical constants.

TABLE I.

| | |
|---|---|
| Specific Gravity | 0·9268 at 20°C. |
| Refractive Index | 1·4653 at 25°C. |
| Solidification Point | −12°C. |
| Acid Value | 36·5. |
| Saponification Value | 194. |
| Iodine Value | 122·6. |
| Acetyl Value | 33·5. |
| Hehner's Value | 91·5. |
| Unsaponifiable Matter | 2·08%. |

150 gms. of the oil were saponified with alcoholic caustic potash and the soap extracted with ether to remove the unsaponifiable matter. It was then dissolved in excess of water and decomposed with dilute sulphuric acid in presence of petroleum ether. The petroleum ether solution of the fatty acids was washed free from traces of sulphuric acid in a separating funnel and left in contact with fused calcium chloride to remove the moisture. It was then filtered and the mixed fatty acids obtained by removal of the solvent by distillation. Table II gives the constants of the mixed fatty acids.

TABLE II.

| | |
|---|---|
| Consistency | Semi-solid. |
| Liquifying Point | 33–35°C. |
| Specific Gravity | 0·8873 at 40°C. |
| Neutralization Value | 188. |
| Mean Molecular Weight | 298. |
| Iodine Value | 126·5. |

The mixed fatty acids (50 gms.) were then separated into saturated (solid) and unsaturated (liquid) acids by Twitchell's lead-salt-alcohol method (Twitchell, *J. Ind. Eng. Chem.*, 1921, *13*, 806). Table III gives the percentage, mean molecular weight, and the iodine values of the saturated and unsaturated acids.

TABLE III.

| Acid. | Per cent in mixed acids. | Iodine Value. | Mean Molar Weight. |
|---|---|---|---|
| Saturated | 22·4 | 1·7 | 259·8 |
| Unsaturated | 77·6 | 139·5 | 280·3 |

*Examination of Unsaturated Acids.*—The quantitative separation of bromo-derivatives according to the method of Eibner and Muggenthalor (*Chem.*

*Tech. of Oils*, etc., 5th Ed., I, 573) perfected by Jamieson and Boughman (*J. Amer. Chem. Soc.*, 1920, *42*, 1197) was unsuccessful even on repeated attempts. No crystalline products could be obtained. The following qualitative method was therefore adopted.

*Oxidation with potassium permanganate.*—10 gms. of the unsaturated acids were dissolved in aqueous caustic potash and a 2% solution of potassium permanganate added in a thin stream with constant stirring, till the pink colour became persistent indicating the end of the oxidation process. A current of sulphur dioxide was next passed into the mixture to dissolve the precipitated manganese dioxide. The products of oxidation were thus left in the form of soft white flocculent flakes that settled down. The precipitate was filtered, washed with water, and extracted with ether. The ethereal extract on removal of the solvent by evaporation deposited a white crystalline solid which on further purification and recrystallization from alcohol melted at 131-32°C. and was identified as dihydroxy-stearic acid. This established the presence of oleic acid in the mixture of unsaturated acids. The ether insoluble product of oxidation was next extracted with a large volume of boiling water repeatedly and on cooling deposited white minute crystals which on recrystallization from water melted at 164-65°C. These were found to be tetra-hydroxy-stearic acid as the melting point with an authentic sample remained undiminished at 164-65°C. The formation of this acid on oxidation proves conclusively the presence of linolic acid in the mixture.

The filtrate from the oxidation products was reduced to about 1/15th its volume by evaporation, made alkaline with dilute caustic soda, and neutralized with dilute sulphuric acid. It was then extracted with ether. The ethereal extract was washed repeatedly with small quantities of water and then dried by keeping it in contact with anhydrous calcium chloride. On removal of ether by evaporation a white crystalline deposit was left behind. This was purified by recrystallization from water and ethyl alcohol successively and identified as azelaic acid of m.p. 106°C. This acid is always formed as a bye-product of such oxidation. The presence of hexahydroxy-stearic acid could not be proved conclusively which shows that linolinic acid is either absent or present only in minute traces.

The iodine value of the mixed fatty acids was 139·5. Since linolinic acid can only be present in very minute traces, if at all, it may be regarded for all practical purposes that the mixture of the unsaturated acids consists of oleic and linolic acids alone. The proportion of these acids can be calculated with the help of the following two equations :

$$X+Y = 100 \ldots\ldots\text{(i)}.$$

$$90{\cdot}07X+181{\cdot}14Y = 100\times 139{\cdot}5 \ldots\ldots\text{(ii)}.$$

where X represents percentage of oleic acid :
and Y represents percentage of linolic acid :

Table IV gives the percentages of these acids calculated by this method.

TABLE IV.

| Acids. | Per cent in unsat. acids. | Per cent in mixed acids. | Per cent in oil. |
|---|---|---|---|
| Oleic .. .. .. | 45·2 | 35·07 | 32·02 |
| Linolic .. .. .. | 54·8 | 42·53 | 38·97 |

*Examination of Saturated Acids.*—These were freed from traces of unsaturated acids by pressing over a porous plate and obtained in a white solid form melting at 52–58°C. The mixture of saturated acids was converted into methyl esters by dissolving in absolute methyl alcohol and passing a current of dry hydrogen chloride till the solution became saturated. It was then refluxed over a water bath for 18 hours. The mixed esters were treated with sodium bicarbonate and washed with water and extracted with ether. The ether extract was washed and dried and the esters recovered by removal of ether by evaporation.

The methyl esters thus obtained were subjected to fractional distillation under highly reduced pressure and the boiling points and the pressure of various fractions was noted. Their iodine and saponification values were determined and the mean molecular weights calculated. These were found to range between 270·3 and 298·4 which are the molecular weights of methyl palmitate and stearate respectively, except the residue whose mean molecular weight was found to be 300·2. This indicates the presence of a higher acid namely arachidic acid. The percentages of the acids were calculated in various fractions by means of their iodine and saponification values.

Table V gives the results of distillation and Table VI those of analysis.

TABLE V.

| Fraction. | B.P. | Pressure. | Weight in gms. |
|---|---|---|---|
| I .. | 150–55°C. rose to 165° | 5 mm. | 0·71 |
| II .. | 165–68°C. rose to 175° | Do. | 3·27 |
| III .. | 175-76°C. rose quickly to 183° | Do. | 3·28 |
| IV .. | 183–90°C. rose to 200° and above. | Do. | 2·28 |
| Residue .. | above 200°C. | Do. | 2·35 |

TABLE VI.

| Fraction. | Iodine value. | Saponification value. | Mean M.W. | Acids. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Palmitic gm. per cent. | | Stearic gm. per cent. | | Arachidic gm. per cent. | | Unsaturated gm. per cent. | |
| I | 1·25 | 203·5 | 275·7 | 0·47 | 66·2 | 0·19 | 26·7 | .. | .. | 0·006 | 0·89 |
| II .. | 1·37 | 200·6 | 279·7 | 2·01 | 64·8 | 1·05 | 33·9 | .. | .. | 0·032 | 0·98 |
| III .. | 1·87 | 198·2 | 283·1 | 1·65 | 50·3 | 1·33 | 40·5 | .. | .. | 0·045 | 1·34 |
| IV .. | 3·28 | 193·5 | 189·9 | 0·60 | 26·3 | 1·19 | 52·2 | .. | .. | 0·053 | 2·35 |
| Residue | 20·28 | 186·9 | 300·2 | .. | .. | 0·95 | 40·4 | 0·22 | 9·7 | ·341 | 14·54 |
| | | | | 4·73 | | 4·71 | | 0·22 | | 0·477 | |

The percentages of various acids in saturated acids and the oil is given below, Table VII.

TABLE VII.

| Acids. | Per cent in saturated acids. | Per cent in original oil. |
|---|---|---|
| Palmitic .. .. .. | 46·69 | 9·57 |
| Stearic .. .. .. | 46·47 | 9·53 |
| Arachidic .. .. .. | 2·15 | 0·44 |
| Unsaturated .. .. .. | 4·69 | 69·45 |

*Examination of unsaponifiable matter.*—This was extracted from the oil by ether extraction after saponification, in the form of a white waxy material. It was purified by repeatedly crystallizing from ethyl alcohol and finally gave a phytosterol of m.p. 131-32°C.

## SUMMARY.

The oil from the seeds of *Cleome pentaphylla* Linn. has been analysed and found to consist of glycerides of the following :—

| | |
|---|---|
| Palmitic Acid .. .. .. | 9·57%. |
| Stearic .. .. .. .. | 9·53%. |
| Arachidic Acid .. .. .. | 0·44%. |
| Oleic Acid .. .. .. | 32·02%. |
| Linolic Acid .. .. .. | 38·97%. |
| Unsaponifiable matter, a phytosterol .. | 2·08%. |

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