

CHEMICAL EXAMINATION OF THE SEEDS OF *CLEOME*
PENTAPHYLLA LINN. PART I: ISOLATION OF
CLEOMIN.

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Cleome pentaphylla Linn. or *Gynandropsis pentaphylla* DC. is a plant belonging to the natural order Capparidæ and is called *Hurhur* in Hindustani, *Hurhuriya* in Bengali, and *Surjāvarta* or *Arkapushpika* in Sanskrit. It is a common weed throughout the warm parts of India and is very common in Ceylon in waste and in cultivated land.

As regards its medicinal properties it has been used in Indian medicine for a very long time and the seeds, leaf, and the roots are all made use of. Sir W. Jones thinks that it promises antispasmodic virtues and according to Dr. Wright the bruised leaves are rubefacient and vasicant. The expressed juice is popularly known as an application for otalgia, both in India and in the West Indies, where also the plant is indigenous. The seeds are anthelmintic and rubefacient and are internally used for expelling round-worms and externally as a counter-irritant. The juice of the leaves is used in otalgia and the leaves are put on boils to prevent formation of pus (Kirtikar and Basu, *Ind. Med. Plants*, 1918, I, 101). A decoction of the roots is said to be a mild febrifuge.

Regarding the chemical properties of this highly interesting drug practically nothing is known. Hooper found that 'the seeds yield to ether about 25% of a thick, greenish, drying oil, having an Acid Value of 6.4, Saponification Value 194.6, Iodine Value 119.5' (*Ann. Rept. Indian Museum, Industrial Section*, 1908-09). The present authors made a systematic examination of the seeds, as a result of which the active principle has been isolated in a state of purity (0.25%); a fixed oil (22.0%), which will be described in a subsequent paper; tannins (1%) and reducing sugars. The active principle has been named *cleomin* after the generic name of the plant from which it has been derived.

Cleomin has a molecular formula $C_{17}H_{14}O_7$. It gives a dull reddish-yellow colour with alcoholic ferric chloride, but no precipitate with lead acetate, basic lead acetate, silver nitrate or mercuric chloride. It forms a mono-acetyl and a mono-benzoyl derivative. The mono-methyl derivative could not be obtained in a crystalline condition. Thus there must be one oxygen atom as an hydroxyl group. Cleomin did not show the presence of any aldehydic or ketonic groups, since it does not react with hydroxylamine, phenylhydrazine, or semi-carbazide; nor does it reduce Fehling's solution.

Tollen's reagent was gradually reduced by it. It dissolves in alcoholic caustic potash on warming to a beautiful yellow coloured solution, and is reprecipitated on acidification. It gives no colour with alcoholic potassium nitroprusside. These reactions, which are shared by some of the natural lactones, at once place cleomin in the class of $\Delta^{\alpha:\beta}$ unsaturated lactones, which were first of all studied by Thiele (*Ann.* 1901, 319, 155), and more exhaustively by Jacobs and collaborators (cf. also Späth and collaborators, *Ber.* 1931, 2203; 1933, 749, 914, 751). Thus out of seven oxygen atoms three have been accounted for, one in the hydroxyl group and two in the lactone group.

In this connection it is very interesting to note that from a series of Indian medicinal plants which are reputed to possess anthelmintic properties substances of the nature of a lactone have been isolated. Thus, Sen isolated corchoritin from jute seeds (*J.I.C.S.* 1931, 8, 651); Dixit and Dutt, marmelosin from *Aegle Marmelos* (*J.I.C.S.* 1930, 7, 759); Agarwal and Dutt, α -elaterin from *Citrullus Colocynthis* (*Proc. Acad. Sc. U.P.*, 1935, 3, 250); and Agarwal and Dutt, cuscutalin from *Cuscuta Reflexa* (*J.I.C.S.* 1935, 12, 384, 586). However, the physiological examination of cuscutalin and cleomin which is in progress in our laboratories will probably throw more light on this aspect of the problem. Further work to elucidate the constitution of cleomin is in progress and will be communicated shortly.

EXPERIMENTAL.

2 kg. of a genuine sample of *Cleome pentaphylla* Linn. was obtained from the neighbourhood and dried completely in shade. It was then crushed finely. A sample when burnt completely in a porcelain dish left 8.2% of a brownish white ash consisting of 18.2% water soluble and 81.8% water insoluble portions. The following inorganic elements and radicles were detected in the ash :—

Na, K, Mg (traces), Ca, NO₃, PO₄, and silica.

In order to obtain an idea of the soluble portions of the drug a sample of the dried and crushed material was exhaustively extracted in a Soxhlet's apparatus using various solvents successively, when the following amounts of extracts dried at 100°C. were obtained :—

Benzene extract : 22%, consisting of a light green oil.

Chloroform extract : 1.35% consisting of a greenish syrupy gummy material.

Ethyl acetate extract : 3.0%. A dirty brown extract was obtained giving a bluish colour with ferric chloride and a copious precipitate with lead acetate.

Acetone extract : 2.2%, a brownish sticky extract having properties similar to the alcoholic extract.

Alcohol extract : 4.8%, consisting of a brown semi-solid material which on keeping deposited minute crystals of a characteristic lens shape. The extract was found to contain tannins, and reducing sugars besides, and gave

a green colour with alcoholic ferric chloride, reduced Fehling's solution, gave a flocculent precipitate with alcoholic lead acetate and basic lead acetate.

The main bulk of the powdered material was then extracted in a large extraction flask with benzene repeatedly, till a portion of it was found to leave no oily residue on complete removal of the solvent by evaporation. The oil thus obtained amounted to 445 gms. and was worked up separately. The defatted mass was freed completely from benzene and then extracted exhaustively with boiling ethyl alcohol.

Isolation of Cleomin.—The various alcoholic extracts of a light brown colour were combined together and concentrated whereby a syrupy residue was obtained. This on standing for about three weeks deposited cleomin as a pale yellow crystalline powder melting with decomposition irregularly between 220–227°C. This was filtered from the syrupy mother liquor over a pump with a good suction. The crude stuff thus obtained was then crystallized from a very large quantity of alcohol. The final purification was effected by recrystallizing it several times from aqueous pyridine, from which it was obtained in clusters of needles. These under a powerful microscope revealed plates with hexagonal ends. Cleomin can also be crystallized from alcohol in characteristic lens-shaped crystals. In this manner 2.8 gm. of the finally purified material of a very light yellow colour was obtained. On heating it changes colour between 230–240° to dark grey, shrinks at 243°C., and melts completely with decomposition at 245–246°C.

Properties of Cleomin.—Cleomin is a light yellow crystalline substance soluble in pyridine, very little soluble in ethyl and methyl alcohols, acetone, ethyl acetate, and glacial acetic acid; totally insoluble in benzene, ligroin, petroleum ether, chloroform, carbon tetrachloride, carbon disulphide, and water. It dissolves in hot alcoholic caustic potash and caustic soda solutions giving a beautiful yellow coloration from which a white precipitate is thrown down on acidification. It decolorizes bromine water and a dilute solution of potassium permanganate. It gives a positive Salkowsky's reaction, i.e. a solution of cleomin in pyridine and conc. sulphuric acid gives a red and finally a green coloration. In conc. sulphuric acid it dissolves giving a light pale yellow colour, which on warming gradually turns from various shades of reddish-yellow to green and on heating further changes to a deep bluish-violet colour with greenish fluorescence and finally a deep violet; from the solution a flocculent precipitate separates on addition of excess of water. This reaction is very sensitive and can be utilized for detecting minute quantities of cleomin. Whereas a neutral solution of potassium permanganate is decolorized only slowly, it is immediately decolorized in the alkaline state. Cleomin is not acted upon by hydrochloric acid while cold, hot, or even boiling. In nitric acid it is soluble to a deep red coloured solution with some effervescence. From the solution a white flocculent deposit is thrown down on dilution with water. With Tollen's reagent colour develops on warming and passes gradually through orange-yellow, orange, grey, to finally black with separation of metallic

silver. It does not give a positive reaction with sodium nitroprusside, nor a positive Keller-Killiani reaction. It gives no precipitates with lead acetate, basic lead acetate, silver nitrate, or mercuric chloride. (Found: C, 61.55, 61.38, 61.42; H, 4.91, 4.70, 4.53; M.W. (Rast's camphor method) 353, 358, 320; $C_{17}H_{14}O_7$ requires C, 61.8; H, 4.2; M.W. 330.)

For the determination of the molecular weight we were confronted with unforeseen difficulties. The ordinary cryoscopic and ebullioscopic methods were rendered useless on account of the exceedingly small solubility of the substance in almost all organic solvents and its total insolubility in most of them. It was only the Rast's camphor method which gave a series of concordant values.

Mono-acetyl Cleomin.—Cleomin (1.0 gm.) was suspended in acetic anhydride (5 cc.) along with fused sodium acetate (2 gm.), and the mixture refluxed over a sand bath for three hours. The resulting mass was then poured into water when a thick oily layer deposited at the bottom. The oil solidified on repeated washing with water and standing. The colourless amorphous mass thus obtained was filtered and recrystallized from 80% acetic acid. In this manner it was obtained in soft colourless needles of m.p. 175-76°C. It was found to be easily soluble in methyl alcohol, ethyl alcohol, glacial acetic acid, acetone, and ethyl acetate, and was insoluble in benzene, petroleum ether, chloroform and water. It gave no colour with alcoholic ferric chloride. (Found: C, 61.11, 61.23; H, 5.14, 5.01; $C_{19}H_{16}O_8$ requires, C, 61.3, H, 4.30.)

Mono-benzoyl Cleomin.—Cleomin (.8 gm.) was dissolved in pyridine (10 cc.) and benzoyl chloride added drop by drop with continuous shaking, till in sufficient excess. The red coloured mass was then poured into water and treated with sodium bicarbonate to remove the benzoic acid formed. The resulting oily layer solidified on long standing and crumbled to a fine powder of a brick-red colour. On crystallization from methyl alcohol it deposited colourless clusters of nodules. These on heating shrink at 179° and melt completely at 182-83°C. (Found: C, 66.1, 66.16; H, 4.8, 4.92; $C_{23}H_{18}O_8$ requires C, 66.36, H, 4.15.)

Mono-methyl Cleomin.—Cleomin (.64 gm.) was dissolved in 10% aqueous caustic soda (50 cc.) and treated with excess of dimethyl sulphate in instalments keeping the solution always alkaline. On shaking the mixture a greyish black oil separated which failed to solidify. It was repeatedly extracted with chloroform but no crystalline derivative could be obtained.

Saponification of Cleomin.—Cleomin (.2730 gm.) was dissolved in 30 cc. of $\frac{N}{1.92}$ alcoholic caustic potash and refluxed for 2 hours. The resulting bright yellow coloured solution was then titrated with standard hydrochloric acid in order to estimate the amount of unreacted alkali. A blank experiment was done in order to minimize the error. The neutralized solution on further addition of acid deposited a white crystalline powder which on recrystallization from aqueous methyl alcohol gave microscopic needles of M.P. 240°C. This

was identified to be cleomin. A mixed melting point with the authentic sample remained undepressed at 240°C. (Found, Neutralization Value 103, calc. M.W. 309 ; $C_{17}H_{14}O_7$ requires, Neut. Value 110, M.W. 330.)

The mother liquor after separation of cleomin as described above was then diluted with ethyl alcohol and treated with excess of an alcoholic solution of lead acetate. The resulting yellow precipitate was filtered and washed with alcohol and decomposed in alcoholic suspension by hydrogen sulphide. The filtrate from the precipitated lead sulphide gave a tarry dark coloured mass which answered to all reactions of tannins.

The filtrate from the lead salt was concentrated after freeing it from excess of lead, and on standing yielded a further crop of cleomin crystals. This was purified in the usual manner. The mother liquor from this gave all reactions of reducing sugars and an osazone was prepared. This melted at 203°C., showing the presence of glucose in the seeds.

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