A STUDY OF THE CONSTITUENTS OF THE SEEDS OF *CROTON SPARSIFLORUS* (MORUNG)— PART I

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A—Preliminary Examination of the Seeds

Only two species of the genus *Croton* (family, Euphorbiaceæ), *Croton tiglium* and *Croton elliotianus* [*Meglocarpus* (Hutch)] appear to have been studied in any detail, the former being more thoroughly investigated on account of the physiological activity of the oil. The third member of the genus forms the subject of the present study. The study of this genus is highly interesting since the oils obtained from the seeds of the different species of this genus, vary considerably from one another in their properties.

Apart from a preliminary note by one of us,[2] there appears to have been no chemical investigation of the seed fat of *Croton sparsiflorus*. This plant (*Eliamanakku* and *Naimilakkai* in Tamil and *Kukka Mirapa* in Telugu) is a wild weed, almost becoming a menace to agriculturists and gardeners in South India. It appears to have spread from Bengal where the plant has been imported from South America about the year 1895. Mayuranathan[11] gives a detailed description of the plant. The leaves, the latex and the oil to some extent seem to be used locally as a dermaticide. The whole plant has been reported[1] to contain sufficient potash and nitrogen to be useful when composted as green manure.

The seeds are small and shiny and resemble castor beans. The ripening and the dispersal of the seeds normally take about 10–15 days. The seeds are so small that in the investigation the kernels are not separated, the whole seed being crushed before extraction. On an average a hundred seeds sun-dried for a week, weigh about 0·73 g. and analyse as in Table I.

On extraction successively with several solvents in a Soxhlet, the different amounts were as in Table II.

TABLE I

Analysis of the Whole Sun-dried Seeds

	%
Moisture	8·03
Solvent extractables	38·44
Cake (by difference)	53·53

TABLE II

Solvent	% on sun-dried seeds
Petroleum ether (50–60° C.)	32·67
Ether	1·37
Chloroform	1·19
Ethlyacetate	2·19
Alcohol	1·02

The petroleum ether extract gave a clear yellow oil, practically tasteless with a not disagreeble odour (the carbon tetrachloride extract in the earlier work seems to have given a different result). The oil visibly thickens on exposure to atmosphere and a drying time of 120 hours compares favourably with about 95 hours for raw linseed oil (Calcutta) and suggests its use as a drying oil.

The ether extract resulted in what appears to be three constituents, a white crystalline solid, a colourless amorphous body with a fibrous look and a brown oily liquid. The extract has a strong 'amylic' odour and was irritating to the mucous membrane.

The chloroform extract gave besides the amorphous substance obtained with ether, a darker brown oil somewhat greenish by reflected light. The irritating odour of this extract was characteristic and the substance re-dissolved in chloroform only with difficulty. Fractional precipitation with petroleum ether separated a dirty green amorphous powder, the filtrate from which left a fibrous looking product that could be recrystallised from dioxane to a high melting solid. This has not yet been further investigated.

Extraction with ethylacetate gave a waxy solid that did not re-dissolve in the solvent.

The final extraction with ethyl alcohol gave a dark viscous product which answered the common tests for alkaloids. The Prollius fluid extract also gave positive tests with alkaloidal reagents.

The neutral water extract showed evolution of carbon dioxide and frothing on warming. The extract did not answer tests for reducing substances. Steam distillation of the seeds gave no volatile products.

The analysis of the 'cake' left after the petroleum ether extraction of the oily constituents indicates a very high fibre content and a good percentage of proteins.

The ash content of the seeds was 3·62% and analysed as in Table IV. The high percentage of phosphorus, calcium, sodium and potassium in the ash and sufficiency of nitrogen in the ' cake ' suggests the utility of the meal as manure.

The meal from the oil extracted sample was tested for the hydrolysis of olive oil which established the presence of a lipolytic enzyme in the ' cake '. A qualitative comparison with the castor bean meal which is known to contain a lipase in a very active form showed that the lipase present in this seed is not as active as that in the castor bean.

TABLE III

Analysis of the ' Cake '

	%
Nitrogen (Kjeldahl)	4·97
Proteins (calculated)	31·05
Fibre	37·5

TABLE IV

Analysis of the Ash of the Seeds

	%
Iron as Fe_2O_3	Trace
Calcium as CaO	21·43
Sodium as Na_2O	16·70
Potassium as K_2O	14·33
Phosphorus as P_2O_5	29·88
Silica	13·88
Carbonate (by difference) ..	3·78

B—The Physical and Chemical Constants of the Oil

As indicated in the previous section, the seeds contain a high proportion of fixed oil most of which can be extracted with petroleum ether. The physical and chemical constants of this oil have been determined by usual methods and are collected in Tables V, VI and VII.

TABLE V

Physical Constants of the Oil

Colour (visual)	Light yellow
Odour	Resembles linseed oil
Specific gravity: $d_4^{30.5}$ ..	0·9270
Refractive Index (Abbe).	
$n_{29.5}$	1·4753
$n_{40.0}$	1·4709
Viscosity (in millipoises) ..	28·85

TABLE VI

Chemical Constants of the Oil

Acid Value	9·18
Saponification Value	189·5
Do. (in an atmosphere of nitrogen) ..	190·3
Saponification equivalent (calculated)	295·4
Ester Value (calculated) ..	180·72
% Glycerine	9·88
Acetyl Value	0·02
Iodine Value (Rosenmund-Kuhnhenn) (see also Table VII) ..	163·4
Thiocyanogen-Iodine Value ..	90·41
Hehner Value	91·96
Hexabromide Value (Steele and Washburn)	10·99
Reichert-Meissl Value	1·10
Polenske Value	4·18
Percentage unsaponifiable matter (Kerr-Sorber)	1·36
Do. (continuous extraction) ..	0·98
Maleic anhydride Value ..	3·3
Drying time of film	about 120 hours

TABLE VII

Iodine Value of the Oil by different methods

Method	Reagent	Value
Wijs	Iodine monochloride	172·0
Winkler	Sodium bromide, sodium bromate and hydrochloric acid	168·1
Hanus	Iodine monobromide	163·1
Kaufmann	Alcoholic bromine (with sodium bromide)	163·9
Rosenmund-Kuhnhenn ..	Pyridine sulphate-dibromide	163·4

The high iodine value comparable with that of raw linseed oil indicates a possible use of this oil as a substitute.

Hexabromide values have been usually determined by two methods but tropical temperatures preclude the adoption of the Eibner and Muggan-thaler method that involves filtration of ether solutions. This method did not yield in our hands pure hexabromo-stearic acid and also gave a very high value. Lewkowitsch[9] also considers that the results obtained by this method are unaccountably high. The method of Steele and Washburn[13] has therefore been adopted for the determination of this value.

The Kerr-Sorber method modified by Jamieson and co-workers[7a] for the determination of the unsaponifiable matter in oils involves the ether extraction of a dilute solution of the soap in a separatory funnel. The extraction of appreciable amounts of soap on account of the high laboratory temperature and the formation of stubborn emulsions render this method tedious and of doubtful value. Knapp[8] has shown that this method does not give an accurate estimate of the unsaponifiable matter and that the use of a continuous extractor in the determination has numerous advantages besides a more correct estimation of the percentage of unsaponifiables. The extractor used in the present study, following that designed by Kutsher and Steudd, was similar to that described by Knapp with this modification that the solvent distributor had a flat glass spiral on it, thus ensuring greater circulation of the soap solution. The design of the apparatus allows the maintenance of the extractor at any desired temperature.

The somewhat lower value obtained for the unsaponifiable matter by the continuous extractor method is to be attributed to the complete absence of dissolved soap in the final unsaponifiable matter collected, which is not excluded in the alternative method.

The maleic anhydride value of 3·3 (Ellis and Jones method[6]) may be taken to show the presence of some conjugation. However, it is difficult

to take this as conclusive evidence of any conjugation since it has been shown[4,5] that neither this nor the method of Kaufmann indicate accurately the extent of conjugation in oils of low diene numbers.

The drying time for a thin film of the oil is sufficient indication of the 'drying' properties of the oil. The drying time of 120 hours seems satisfactory for the purpose of a drying oil.

A deep blue colour with arsenophospho-tungstic acid is considered to indicate the presence of linolenic acid in the glyceride in the absence of acids of higher unsaturation.[10] The oil under investigation gave a positive test, though the intensity (visual) is much less than with raw linseed oil.

Experimental

Fully ripe seeds sun-dried for a week were used; and were taken to represent the initial sample in the analysis of seed constituents.

The moisture content was determined by the usual method of drying in an oven at 110° C.

Since the oil polymerises readily, only the sun-dried seeds were used for the determination of solvent extractables, the extraction being carried out in a Soxhlet.

The 'cake' was analysed for the protein, fibre and enzyme content. Nitrogen was estimated by Kjeldahl's method using 0·05 g. selenium as catalyst and 0·5–1·0 g. of cake.

The fibre content was estimated by usual methods.[3] The presence of a lipolytic enzyme was established by the method of Willstätter.[15]

The seeds were ashed at a dull red heat and the several components determined by standard methods of quantitative analysis.

For the determination of the physical and chemical constants of the oil, a petroleum ether extract of the seeds freed from solvent was used. Crushed sun-dried seeds were packed in an extractor of a modified form of Wester's apparatus[14] and the oil extracted with petroleum ether (50–60°). The extract was filtered off from any plant material that might have been mechanically carried through, dried over anhydrous sodium sulphate, and the major portion of the solvent was distilled off. The last traces of solvent were removed under reduced pressure (1–2 mm.). The oil was stored in a brown bottle in an atmosphere of carbon dioxide.

Refractive indices were determined at 29·5° and at 40° using an Abbe refractometer.

Viscosity measurements were made with an Ostwald viscometer, 'conductivity' water and anhydrous, thiophene free, crystallisable benzene of the correct melting point were used for comparison. The measurements were carried out at 30·2°. Taking the viscosity of water as standard from Landolt's tables and using the relation

$$\frac{\eta_1}{\eta_2} = \frac{S_1 t_1}{S_2 t_2}$$

the viscosity of the oil in millipoises was calculated.

Other constants were determined by standard methods.[7] In the determination of the saponification value thymolphthalein was found to be the best indicator both for macro- and semi-micro-determinations of the value.

In the determination of the thiocyanogen-iodine value the reagent was prepared by the method of Gardner and Weinberger. The acetic acid used was the Hopkin and Williams product, purified by the method of Orton and Bradfield.[12] This acid mixed with 10% acetic anhydride ANALAR was used as the solvent. The reagent was stored in a dry amber coloured bottle and tightly stoppered. The reagent was prepared fresh for each estimation. Polymerisation was noticeable on storage.

The method of Knapp[8] was followed in all details in the determination of the unsaponifiable percentage using a continuous extractor. Aliquots from the saponified solution, made up to a definite volume, gave closely comparable values.

The drying test was carried out by usual methods. Raw linseed oil (Calcutta) dried in 95 hours while the oil under investigation dried in 120 hours to the same consistency.

The oil gave a positive test for vitamin A with antimony trichloride.

Summary

The constituents of the seeds of *Croton sparsiflorus* have been examined and the physical and chemical constants of the petroleum ether extractable fixed oil have been determined.

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