LIPINS OF FENUGREEK (*TRIGONELLA FOENUM GRAECUM.*)

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Fenugreek, extensively cultivated throughout India and Egypt, forms an essential constituent of the Indian, Arab and Egyptian dietary. Data relating to the area under its cultivation in India are not available but statistics show that the seeds are exported to the extent of about 770 tons per annum valued at about 2 lakhs and a half—(1927), principally to Arabia, Egypt and Italy. Its nutritive value has long been recognised, aqueous extracts of the seed being commonly administered to anæmic and ricketty children. Dr. Blum (1927) states that fenugreek can be employed as a substitute for cod liver oil in cases of lymphatism, scorfula, rickets, anæmia and debility following infectious diseases or neurasthenia as well as in gout and diabetes in which it may be combined with insulin.

The seeds are valued for their tonic, astringent, emollient and aphrodosiac properties and recently their dietetic value is being more widely accepted. Andre (1932) reports an augmentation in the weight of adults (1·5 to 4 kg.) in 1–2 months by administering the powder to them in 2–3 gm. doses before food. Fenugreek is given to women after parturition in the form of powder or a decoction sweetened with sugar, for re-establishing original weight and improving lactation. It constitutes an essential ingredient of pills and powders administered for the treatment of diabetes mellitus ; it also acts as a safe nutrient in cases of diabetes.

Reutter (1927) has noted the presence of several bases such as choline, trigonelline, methylamine, neurine and betain, which possibly result during the hydrolysis of the lipins associated with the grain. The seed contains about 7 per cent. of a fixed oil (1919), bright yellow in colour, with a characteristic odour and taste. Wunschendorff (1919) has found that the oil possesses an iodine value of about 137, contains 6·25 per cent. of a lecithin and has a phosphorus content of (P_2O_5) 0·55 per cent. In a further communication (1919) he has reported the presence of a saponin, readily soluble in water and easily hydrolysed by dilute acids yielding dextrose.

Jahns (1885) isolated an akaloid, trigonelline, from the seeds by exhausting them with 70 per cent. alcohol. 0·14 per cent. of a light brown neutral volatile oil possessing the distinct odour of seeds has been described (1903). The preliminary study of the proteins of the seed has been conducted by Wunschendorff (1919) who has isolated 25 per cent. of a globulin, 20 per cent. of two albumins and 55 per cent. of an alkali soluble nucleoproteid having a specific lævo rotation of $[\alpha]_D = -97·7°$. More recently, the albumins and globulins have been examined in greater detail by Sreenivasa Rau and Sreenivasaya (1932) while a preliminary examination of the prolamin, "Helbin", has been conducted by Hassan and Basha (1932), a more complete study of which has been carried out by Sreenivasa Rau, Sastri and Narayana (1933).

The mucilage to which the emollient properties are attributed, is described to be a silico-phosphoric acid ester of mannogalactan (1932) in contradiction to the previous finding of Bourquelot and Herissey that it is a mannogalactan (1900). A critical examination of this question by Hariharan and Sastri (1933) has revealed that the mucilage is a simple mannogalactan.

It will be observed from the foregoing review of the work done on Fenugreek that there have been no systematic studies on the lipins of the seed to which the reputed tonic properties of the seed may largely be due. A detailed investigation of lipins has accordingly been undertaken in our laboratories and the present communication deals with the methods employed in the isolation, fractionation and chemical characterisation of the lipins.

Experimental.

The seeds, on crushing, separate roughly into two portions, a yellow flour which is easily powdered and a residue consisting of the snow-white gelatinous endosperms which cannot be ground. The yellow meal has a nitrogen value of 4·54 per cent. while the endosperm fraction, constituting the source of the mucilage, if carefully separated, is found to contain practically no nitrogen. Table I gives the proximate composition of the yellow meal in percentages of moisture-free material.

TABLE I.

Ether extract	98% alcohol extract	70% alcohol extract	Crude protein N × 6·3	Total Ash	Total P	Total S	Starch
8·1	4.9	11·2	29·9	3·5	0·62	0·003	2·1

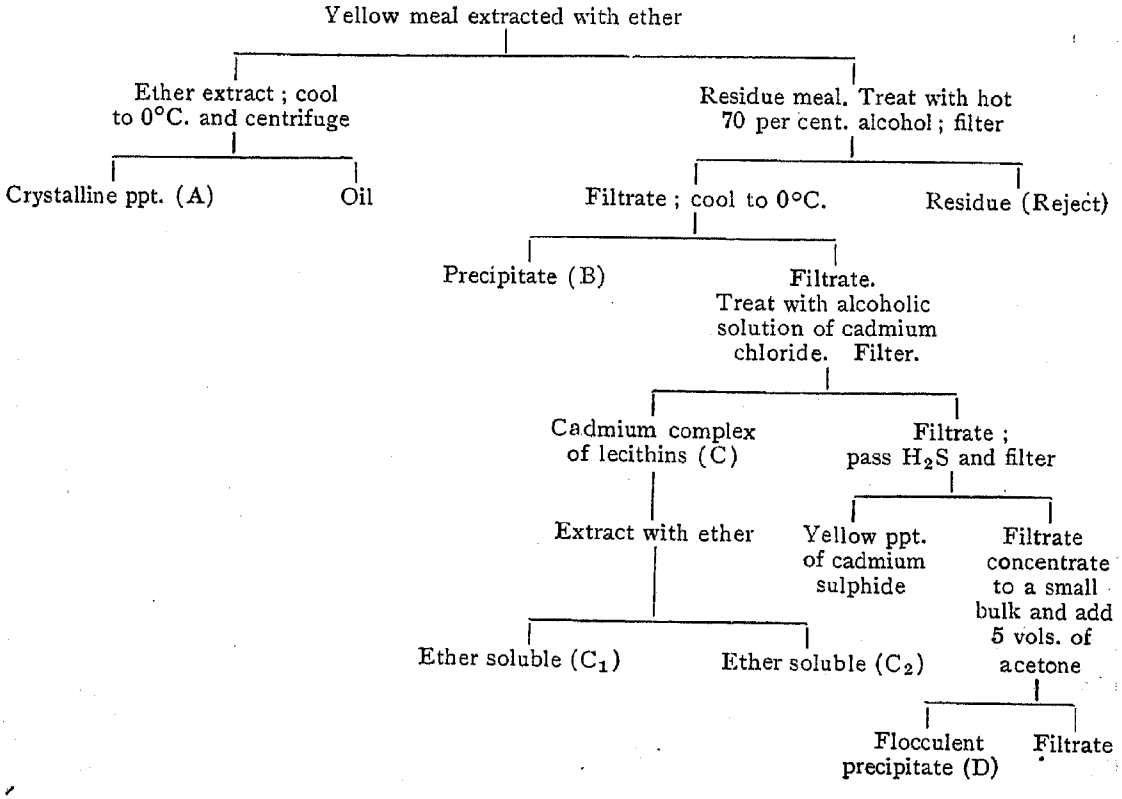
The nitrogen and phosphorus values of the ether extract are 0·38 and 0·52 per cent. respectively while the corresponding values for the alcohol

extract are (N) 1·10 and (P) 1·51 per cent., about thrice as high. About 3 per cent. of the total nitrogen and 30 of the total phosphorus of the yellow meal are extracted by successive treatments with ether and alcohol, while direct treatment with 70 per cent. alcohol, however, yields about 5·8 and 60 per cent. of the total nitrogen and phosphorus respectively.

The ether extract of the meal consists of a viscous semi-drying oil, golden yellow in colour. On cooling the oil deposits a white crystalline substance (A) which can be recovered by centrifuging and purified from the oil by washing with acetone in which the crystals are insoluble. The substance is sparingly soluble in ether and in absolute alcohol from which it could be recrystallised ; it is freely soluble in water and also in 70 per cent. alcohol from which it can be precipitated by adding 10 volumes of acetone. The purified product contains both nitrogen and phosphorus and represents the oil soluble phospholipin.

The ether extracted meal, on treatment with hot 70 per cent. alcohol, yields a clear light yellow solution which on cooling to 0° C. deposits a white precipitate (B) which can be filtered off and washed with cold alcohol. The filtrate is concentrated to a small bulk and treated with an alcoholic solution of cadmium chloride, to precipitate the true lecithins as double salt.

TABLE II.



The mixture is centrifuged to recover the cadmium complex (C) and the centrifugate treated with hydrogen sulphide to remove excess of cadmium as sulphide which is filtered off. The filtrate is concentrated to a small bulk and 5 volumes of acetone added when a flocculent precipitate (D) is obtained. Table II gives a schematic representation of the various treatments given to the material for isolating the different fractions.

The nitrogen and phosphorus contents of the five fractions in percentages of the material as also their N : P ratios are given in Table III. C_1 represents the ether soluble portion and represents the cadmium compound

TABLE III.

	A	B	C_1	C_2	D
Nitrogen ..	+	0·75	0·58	0·96	1·08
Phosphorus ..	+	0·78	1·32	0·68	0·06
N : P ..	..	2·5 : 1	1 : 1	3 : 1	..

of the true lecithin, a striking confirmation of which is obtained by the analytical data presented in Table III. B corresponds in composition to sphingomyelin. Fraction D contains nitrogen, is strongly hygroscopic, and freely soluble in water from which it can be reprecipitated by the addition of 10 volumes of absolute alcohol or acetone. The product has a very low content of phosphorus which can be eliminated by further purification. All attempts at obtaining crystalline substances from the crude product have so far been unsuccessful. The crude product is optically active, contains sulphur and on hydrolysis with dilute acids readily splits off a reducing sugar which has been identified as galactose both by its osazone and mucic acid tests.

A further fractionation of the crude galactolipins was carried out by dissolving the product in pyridine in which it was only partially soluble. The pyridine insoluble fraction was kept separate and the pyridine soluble portion was fractionally precipitated by successive additions of chloroform, yielding four fractions, *a*, *b*, *c*, *d*. These were purified by dissolving in dilute alcohol and precipitating with anhydrous acetone. An analysis of these fractions as also those of the crude product and the pyridine insoluble fraction is presented in Table IV.

The analytical data of the above four fractions, *a*, *b*, *c* and *d*, point to the conclusion that the pyridine soluble fraction consists mainly of two

TABLE IV.

	Crude product D	Pyridine insoluble fraction	Pyridine soluble			
			a	*b*	*c*	*d*
$[\alpha]_D^{25°C.}$ original product ..	31·58	91·22	67·1	28·73	—17·56	—22·22
,, after acid hydrolysis ..	21·05	28·01	22·0	10·85	—2·42	0·00
Nitrogen, N ..	1·08	1·67	1·38	0·34	0·15	0·26
Phosphorus, P ..	0·06	0·21	0·07	0·03	0·01	0·01
Sulphur, S ..	0·12	0·26	..	..	..	..
Galactose ..	32·89	38·42	43·01	37·55	26·33	31·14

substances, a dextro component predominent in fraction *a*, and a lævo component comprising the fraction *d*. Emulsin acts on both *a* and *d*, indicating thereby that the fractions contain glycosides.

The pyridine insoluble fraction is interesting because of its sulphur content and is being subjected to further investigation.

Summary.

1. A preliminary study of the lipins of Fenugreek seeds has been made. Methods for the isolation and fractionation of the lipins are described in detail.

2. A phospholipin allied to sphingomyelin, an oil soluble true lecithin which forms a complex with cadmium salts, a pyridine soluble galactolipin and a galactoside and a pyridine insoluble galactolipin containing sulphur are among the most interesting fractions that have been isolated from the seeds.

3. A detailed investigation of the cadmium complex and the pyridine insoluble galactolipin is under progress.

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