CCXVII. A PRELIMINARY NOTE ON A PROLAMIN ISOLATED FROM A LEGUME SEED.

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THE majority of the inhabitants of Egypt live on a variety of maize bread in the making of which a small proportion of the highly mucilaginous and bitter seeds of the legume known as fenugreek is added. Usually 2-5 % of fenugreek is used, but this small quantity has the advantage not only of conferring on the moist mixture better binding properties thus facilitating the production of the characteristic wafer-like loaf, but definitely makes the finished loaf more appetising.

While investigating the nutritional value of the protein mixture of this maize-fenugreek bread compared with that of ordinary wheaten bread, an alcohol-soluble protein was isolated from the dried powdered fenugreek seeds. This discovery appeared to be interesting since, according to Osborne [1924], alcohol-soluble proteins have so far been found only in the cereals.

Preparation.

After eliminating any cereal grains or other foreign materials, if present, the fenugreek seeds are ground in a clean strong laboratory mill, and the flour is subsequently extracted completely with ether. The ether extraction can be omitted, its only advantage being that it minimises loss in the later stages of the process as it gives a purer product.

The air-dried ether-extracted flour is now boiled with 70-72 % alcohol (1500 cc. to every 500 g.) on a water-bath. After 1 hour the hot contents of the flask are filtered rapidly through a Büchner funnel. The residue is extracted once more with the same strength of alcohol for another half hour, after which it is filtered hot as before.

On cooling, a yellowish-white precipitate begins to form and is allowed to settle in a covered vessel for 16 to 24 hours; the supernatant liquid is then carefully syphoned off and the moist precipitate centrifuged and subsequently washed with cold 70 % alcohol followed by 96 % until the washings are no longer yellow. The residue is then taken up in liquid phenol and again centrifuged. The greater part dissolves in the phenol giving a clear reddish-brown solution. The residue is treated two or three times more with phenol before it is discarded.

The phenol solutions are treated with 96 % alcohol until precipitation is maximum, and the precipitate is repeatedly washed with the same strength of alcohol until the last traces of phenol are removed. Many washings are required to effect this. Finally the precipitate is washed with absolute alcohol on a Büchner funnel and then with ether. The ether is allowed to evaporate and the dry material is powdered and kept in a desiccator over calcium chloride.

Properties.

The material thus obtained is an amorphous, tasteless and odourless white powder. It is very slightly soluble in distilled water, more soluble in dilute HCl but it dissolves freely in dilute alkali. Its solubility in alcohol is somewhat peculiar. It only dissolves in hot 70 % alcohol and separates on cooling; it is insoluble in 96 % alcohol. A solution of the material in dilute alkali gives the following reactions:

Biuret	Violet
Xanthoproteic	Positive
Millon's	"
Rosenheim's tryptophan test	Very faint, on standing
Sulphur test	Positive
Molisch's	Negative

It is precipitated by picric acid and also by hydroferrocyanic acid and by half saturation with ammonium sulphate. Half saturation with magnesium sulphate produces a definite turbidity, but complete precipitation is only brought about by full saturation with this salt. The isoelectric point lies approximately at p_H 5.3.

The material contains 17.18 % N and 1.115 % S.

The yield is very small. Roughly, 0.2–0.5 g. of material is obtained from 100 g. of flour. The major part comes out in the first extraction, and there is, therefore, no necessity to extract more than twice with 70 % alcohol.

CONCLUSIONS.

The above description shows that the material isolated from fenugreek by treating its dry powdered seeds with boiling 70 % alcohol is a protein. This protein, for which the name "helbin" is proposed (from "Helba," Arabic for fenugreek), might be classed with the gliadins for, like the gliadins it is soluble in boiling 70 % alcohol and in phenol and is precipitated from its phenolic solution by the addition of ethyl alcohol, acetone, amyl alcohol or ether. It differs, however, from the gliadins of wheat and maize, for example, in being insoluble in cold 70 % alcohol. It is therefore of interest to determine the exact relations of helbin to the known gliadins. The anaphylactic reaction should be of great help in this connection and it is intended to study the serological reactions of this protein and to compare them with those of the other gliadins.

SUMMARY.

1. A compound soluble in hot 70 % alcohol and possessing the characters of a protein has been isolated from the seeds of the legume known as fenugreek.
2. It differs from the known gliadins in being insoluble in cold, but soluble in hot 70 % alcohol.
3. Like the gliadins, this protein is soluble in phenol and is precipitated from its phenolic solution by the addition of ethyl alcohol, ether, *etc.*

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REFERENCE.

Osborne (1924). *The vegetable proteins.* (Longmans, London.)