FACULTY RESEARCH EDITION

of

The Savannah State College Bulletin

Published by

The Savannah State College

Volume 22, No. 2 Savannah, Georgia December, 1968

HOWARD JORDAN, JR., *President*

Articles are presented on the authority of their writers, and neither the Editorial Committee nor Savannah State College assumes responsibility for the views expressed by contributors.

Table Of Contents

Cottonseed Protein Structure I. Isolation of Protein, and Determination of N-Terminal Acids and Sulfhydryl Groups

by

Charles Pratt and Laura Grant

Introduction

This is the first of a series of papers which deal with work designed to determine the sequence of amino acids in cottonseed protein.

Attempts to isolate a large quantity of a single pure protein for study showed that at least three different proteins are present in cottonseed in large quantities. One of these was isolated, labelled Protein "A", and studied.

The following was found:

1. The protein is fibrous and consists of two strands.
2. The strands are held together by disulfide bridges.
3. There are six sulfhydryl groups in the protein, and these exist as disulfide bridges.
4. Arginine and Methionine were found to be the N-terminal amino acids of the two peptide strands of Protein "A".

Determination of N-terminal Acids

Of the protein isolated by the scheme on the next page, one gram was redisolved and streaked on large sheets (18¼ x 22½) of Whatman #3MM filter paper and developed in n-butanol-acetic acid-water (6:1:2 v/v). After developing 12-14 hours, the chromatograms were dried and a 2-inch vertical strip cut from each. The strips were sprayed with ninhydrin, 0.5% in alcohol, in order to locate the protein zone. There were 3 zones present, and the one which appeared to be in the largest quantity was selected. This was labelled Protein "A".

Each sprayed strip was placed back beside the paper from which it had been originally cut, and the zones marked off. The zone labelled Protein "A" was cut from each chromatogram and eluted. The "Protein A" eluants were combined and treated as follows to determine the N-terminal amino acid.

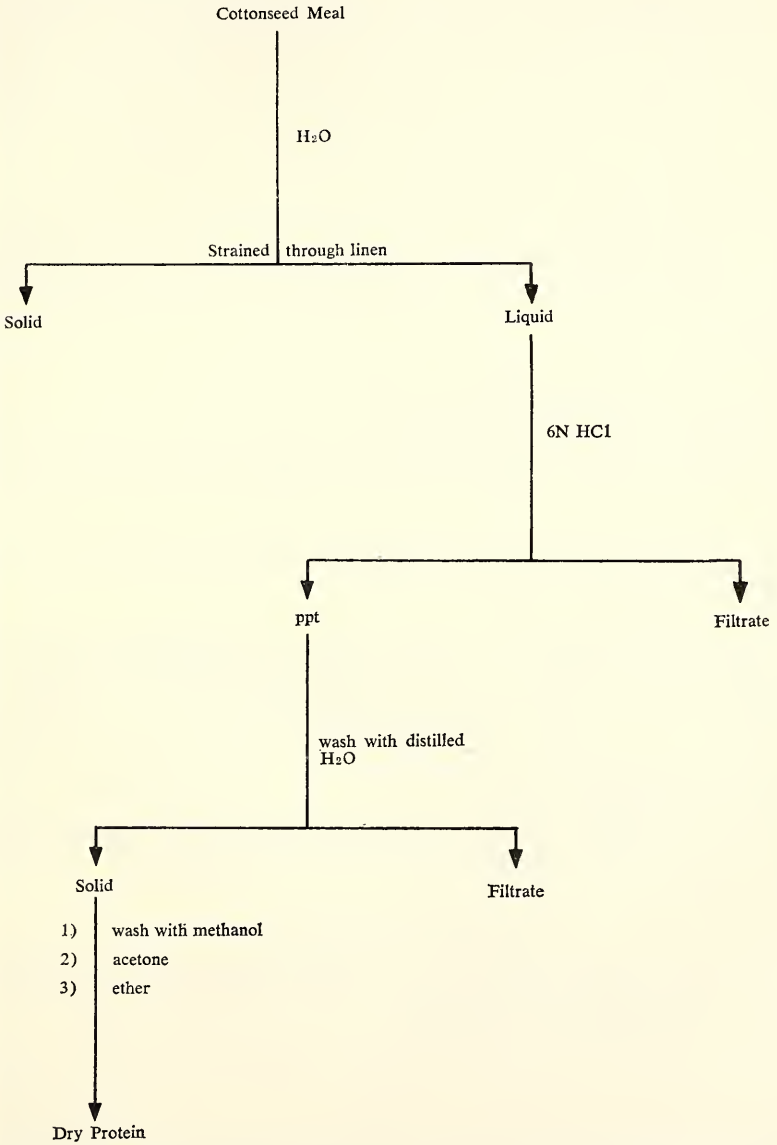
Twenty-five ml of the isolated solution were added to 25 ml of pyridine and brought to a pH of 9.5 using NaOH solution. To this was added 25 drops of phenylisothiocyanate and the mixture stirred at 37-40°C, keeping the pH 9-10 by occasional addition of NaOH

Experimental

Protein Isolation

The following scheme illustrates the general procedure used in the isolation of Cottonseed Protein.

General Procedure for Protein:



solution (as the reaction proceeded the pH tended to become acidic). When there was no longer a tendency for the pH to decrease (about 40 minutes), the reaction was assumed to be complete.

The mixture was cooled and washed with benzine to remove the pyridine and excess phenylisothiocyanate. The pH was then lowered to 3 and the phenylthiocarbamyl precipitated. The material was filtered.

A small portion of the phenylthiocarbamyl was dissolved, streaked on a small sheet (8″ x 8″) of chromatographic paper and developed in a solvent system of nitromethane saturated with dry HCL gas. The solvent brought about a formation of phenylthiohydantoin with the N-terminal amino acid. This hydantoin moved on the chromatogram, while the remaining peptide remained at the origin.

The hydantoins were identified by chromatographic comparison with hydantoins of known amino acids.

It was found that two hydantoins were present, that of methionine and arginine. This led to the conclusion that there must have been 2 N-terminal acids. Therefore, the protein must have contained two strands.

Determination of Sulfhydryl Groups

Following the conclusion that two strands were present in the protein under study, an attempt was made to determine how many disulfide bridges were involved. Briefly, the method used was as follows:

A sample of protein was dissolved in saturated guanidine hydrochloride and titrated with CH_3HgNO_3. Under these conditions all the sulfhydryl groups react.

Fifty mg of protein were dissolved in 1 ml. of saturated guanidine hydrochloride in M/15 phosphate buffer, pH 7 at O°C in a 15 ml. tube. The air in the tube was displaced with nitrogen, and 10 mg of solid indicator (sodium nitroprusside) added, to the solution of pH 9.

Three ml of 10^{-3}M CH_3HgNO_3 were titrated into the solution until the red color disappeared.

From experiment calculations, it was concluded that there are only six available sulfhydryl groups.

Acknowledgements

Financial support from the U.S. Army Research Office-Durham, N. C. is gratefully acknowledged.