The amount of reaction which most of the secondary and tertiary bromides in Table II show is relatively small. Table III gives a better view of the behavior of these compounds. At this higher reaction temperature it is seen that isopropyl and secondary butyl bromide definitely react to form a tertiary amine, but that cyclohexyl bromide, which appeared to be showing the same type of reaction in Table II, when forced into a more complete reaction shows practically the same tendency as the tertiary bromides to lose hydrobromic acid and forms only a relatively small amount of a tertiary amine. This fact would seem to lend further support to the suggestion made above that the secondary bromides which react with piperidine to form a tertiary amine probably rearrange to primary bromides before reacting and react as such. This possibility will be tested experimentally in this Laboratory and the results reported later. The tertiary bromides in Table III follow more completely the course which they started in Table II.

Summary

1. The rate and course of the reaction between piperidine and eighteen alkyl bromides has been determined.

2. It has been found that the primary and secondary bromides, with the exception of cyclohexyl bromide, react with piperidine to form tertiary amines. Cyclohexyl bromide and the tertiary bromides which were studied react, in the main, to lose hydrobromic acid and form, presumably, an unsaturated compound.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF CHICAGO]

STUDIES IN PROTEINS. II. CONCERNING THE UNIFORMITY OF THE PROTEIN FRACTION EXTRACTED FROM ORANGE SEED MEAL BY SALT SOLUTIONS

BY FELIX SAUNDERS

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Introduction

In a series of recent papers, Gortner and his co-workers[1] have reported the results of studies on the "peptization" of wheat flour proteins. Their figures show a great variation in the fraction of the total nitrogen extracted by various salts. From their data, the authors draw the following conclusions: (1) both anions and cations show a lyotropic series, (2) "protein 'solubility' in neutral salt solutions is, in reality, protein peptization, and

[1] Gortner, Hoffman and Sinclair, *Cereal Chemistry*, **6**, 1 (1928); "Colloid Symposium Monograph," The Chemical Catalog Co., Inc., New York, **1928**, Vol. V, p. 179.

as such it is governed as to rate and extent by the nature of the particular anions or cations present in the salt solution," (3) "we do not believe that any salt used, in any one of the concentrations, extracts a chemical entity which should be designated by the term 'globulin'."[2] While Gortner's conclusions seem entirely justified with respect to the systems with which he worked, they do not seem to apply in the case of a system containing a representative crystalline globulin. In the present paper, studies are reported similar to Gortner's except that they were made on material in which most or all of the nitrogenous substance was crystalline globulin. Furthermore, a crystalline globulin was isolated using the different salts as extracting agents and the percentage of nitrogen in the protein determined in each case.

Material.—The material used in this study was orange seeds,[3] which yield a crystalline globulin, pomelin.[4] The whole seeds were ground in a Wiley mill. The oil was removed by repeated extractions with benzol. The meal which was left after filtering off the benzol was air-dried and then sifted. The fraction which passed through the 40-mesh sieve but not through the 60-mesh was used.

Method.—In order to have the results comparable, Gortner's procedure was followed as closely as possible in the extraction studies. Six grams of the orange seed meal was placed in a centrifuge bottle and covered with 50 cc. of the solution of the salt being investigated. The suspension was shaken mechanically for thirty minutes, centrifuged, and the supernatant liquid was poured into a Kjeldahl flask. Two more extractions were carried out in the same way. The nitrogen in the combined extractions was determined by the usual Kjeldahl procedure.

The pure protein was isolated in the following way. One liter of the salt solution was warmed to 55° and added to 100 g. of the seed meal. The mixture was allowed to stand for about two hours without any further warming. After as much of the liquid as possible had been recovered by centrifuging, solid ammonium sulfate was added to saturation. The globulin which precipitated was separated in the centrifuge and then redissolved by adding distilled water. Any undissolved material was removed by centrifuging and the solution was dialyzed in viscose bags[5] against cold, running, distilled water for about sixty-four hours. The supernatant liquid was separated from the protein precipitate by centrifuging. With-

[2] These statements were intended to apply only to wheat flour.

[3] These seeds were supplied by the California Fruit Growers Exchange. It is a pleasure to be able to acknowledge their courtesy in collecting and sending this material.

[4] Pomelin is the name which has been given to the crystalline globulin isolated from citrus seeds. It is easily obtained as octahedral crystals by the dialysis of a salt solution of the protein. A more detailed report on this protein will be published soon.

[5] Grateful acknowledgment is made to the Visking Corporation for furnishing the viscose tubing used in this work.

out being removed from the centrifuge cup, the protein was washed by suspending it in successive portions of alcohol of gradually increasing concentration starting with 40% and running up to absolute. The protein was then filtered off and washed with absolute ether. Finally it was dried to constant weight in a vacuum oven at 50°. The protein prepared in this way is a snow-white powder which easily passes through a 100-mesh sieve without any grinding. Each preparation of protein was examined under the microscope during dialysis to be sure that crystallization was proceeding properly.

As a further check, the residue from the protein extraction was dried on the steam-bath, ground until it all passed through a 60-mesh sieve and then analyzed for nitrogen. Of course, a correction for residual salt had to be made. At first an attempt was made to determine salt directly by ashing but this method proved unsatisfactory. An indirect calculation of the salt content on the basis of the amount of liquid left in the meal after centrifuging was much more trustworthy.

Experimental.—The experimental data are summarized in the following table. The variation in the yield of globulin can be explained by technical difficulties. In the case of the sodium bromide and potassium bromide some of the material was accidentally lost. The discrepancy in the values of the nitrogen in the residue from the extraction with sodium iodide and potassium iodide has been accounted for by difficulties in ash determination.

TABLE I

SOLUTION OF THE PROTEIN OF ORANGE SEED BY NORMAL HALIDES

Salt	% of total N extracted	Grams of N in 100 g. of residue	Yield of globulin, grams	N in protein, %
LiCl	72.5	3.1	7.02	16.9
NaCl	72.5	3.0	5.36	17.1
KCl	73.4	3.0	5.62	17.1
LiBr	73.9	3.0	6.80	16.9
NaBr	73.4	2.8	3.42	16.8
KBr	74.2	2.8	3.56	17.0
NaI	73.9	2.4	5.52	16.8
KI	73.4	3.4	6.90	17.0
Average	73.4	2.9	5.53	16.9

Discussion

The experimental data show very clearly that the alkali halides are alike in their ability to extract the protein of orange seeds. This finding is confirmed by the analysis of the residue. Considered by themselves, the analytical figures would not prove that the protein isolated was always the same. However, when taken in conjunction with the extraction figures, they probably warrant the conclusion that the protein is the same in every

case. Furthermore, the crystal form and general behavior were the same in every preparation.

According to Gortner, a normal solution of potassium chloride extracts from wheat flour 23% of the total nitrogen, potassium bromide extracts 37% and potassium iodide extracts 64%. Using the same technique on orange seed meal, the figures obtained were: potassium chloride, 73.4; potassium bromide, 74.2; and potassium iodide, 73.4. The difference between the figures of the two series is of no significance since they were obtained on different materials, but the variation between results within each series is important. The first set shows a definite lyotropic series but the variation within the second series is no greater than the experimental error.

The discrepancy may be the result of differences in the type of materials used. Wheat flour contains a complex mixture of proteins in which gliadin and glutenin predominate. It is, therefore, hardly a suitable substance for the study of the action of salt solutions on globulins, even if we assume that flour contains a globulin fraction. Gortner's results justify the statement that all the material extracted by salt solutions from wheat flour is not necessarily globulin, but they do not force the conclusion that there is no such thing as a chemical entity which we may designate "globulin." In fact, of all the proteins there are probably none better entitled to consideration as chemical entities than the crystalline globulins. The real need is a definition which will distinguish between the crystalline or semicrystalline globulins and the nitrogenous material which can be extracted from almost any biological material.

In a private communication, Dr. Gortner asserts that he had no intention of denying the existence of globulins but was merely questioning the definition of the globulins and the methods for isolating them. Unfortunately many persons have construed his remarks to mean a complete denial of the existence of globulins as a class of proteins. It should be noted that Gortner's statements were made only in connection with the wheat flour system which he studied and it is not intended in this paper to imply that he wished at any time to extend his conclusions to other systems.

The question remains whether the existence of a lyotropic series in the extracting power of various salts may not still be demonstrated even in representative seed materials which are known to yield crystalline globulins. Dr. Gortner has very kindly sent the author a private communication in which he gives some figures from recent work in his laboratory. His results show very conclusively that there may be a series even with seeds which give a crystalline globulin. However, the existence or nonexistence of such a lyotropic series need not affect the other considerations regarding the nature of the protein extracted. In other words, different salt solutions may show a variation in their peptizing power toward a given system and yet be extracting the same protein only in varying amounts.

Unpublished results from this Laboratory show that the foregoing statement is probably true in some cases at least. Of course, the converse may also be true, that is, different salts may extract the same amount of nitrogen from a meal and yet may not be extracting a chemical entity.

This study is being extended to include other seeds known to yield crystalline globulins, and different salts at varying concentrations are also being used.

Conclusions

1. The alkali halide salts in normal concentration all extract the same amount of nitrogen from orange seed meal. There is no evidence for the existence of a lyotropic series in the extracting power of different salts on orange seed meal.

2. The protein isolated from orange seed meal by extraction with different salts is probably the same in every case.

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A NEW TYPE OF OXIDATION PRODUCT DERIVED FROM QUINONES[1]

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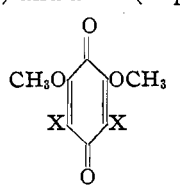
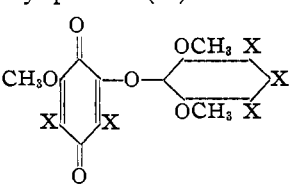
During a study of the oxidation of tribromo- and trichloropyrogallol-2, 6-dimethyl ether, Hunter and Levine[2] showed that when treated with any of a number of oxidants, these phenols gave both a yellow mononuclear quinone (I) and a red (or purple) phenoxy quinone (II).



I (X = Br or Cl)



II

However, when the oxidations were carried out with chromium trioxide in 50% acetic acid[3] there were obtained, in addition to these expected products, small amounts of colorless compounds. These compounds seemed to be of a nature different from any of the oxidation products previously isolated during this series of investigations. Their further study therefore

[1] The work described in this paper formed part of a thesis submitted to the Graduate Faculty of the University of Minnesota by Murray M. Sprung in partial fulfilment of the requirements for the degree of Doctor of Philosophy, September, 1928.

[2] Hunter and Levine, THIS JOURNAL, **48**, 1608 (1926); cf. Hunter and Morse, *ibid.*, **48**, 1615 (1926).

[3] Hunter and Levine, Ref. 2, p. 1612.