THE ROTARY PROPERTIES OF SOME VEGETABLE PROTEIDS

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I. Introductory.

So far as the writer has been able to ascertain, no reliable determinations have ever been made of the rotary properties of any of the vegetable proteids, although some of them can now be obtained in a pure state and in considerable quantities. The object of this investigation was to determine accurately the rotary power of some of the crystallized vegetable proteids. The globulin found in hemp seed was chosen as the main subject of study, because it could be obtained in a perfectly crystalline form with comparative ease. For purposes of comparison, the rotary powers of the crystallized globulins obtained from flaxseed and the Brazil nut were also determined under similar conditions. The identity of each of these three proteids has been completely established by the researches of Osborne,* Chittenden and Mendel,† and others, and a guarantee of chemical purity is furnished in their crystalline form. Their individual compositions and properties will be described later in connection with the results of the polarimeter observations.

A complete determination of the rotary properties of any substance should include a study of the effect on its rotary power of three factors—(1) the solvent used; (2) the concentration of the solution; (3) its temperature. The proteids investigated by the writer were imperfectly soluble in most solvents at the temperature of the room, and their solutions were more or less coloured. On account of their opacity, only dilute solutions containing about one or two

* *American Chemical Journal*, vol. xiv, pp. 629, 662.

† *Journal of Physiology*, vol. xvii, p. 50.

per cent of proteid matter could be used, so that the angles measured had to be multiplied by a large reduction factor to find the rotation for the standard length and density. The errors of observation were thus multiplied many times. For this reason the results were not sufficiently exact, nor were the variations sufficiently great, to determine the manner in which the rotary power varied either with the temperature or the concentration of the solutions. The maximum range in concentration was from 0·50 per cent of proteid to about 2·50 per cent, and in temperature from 20° C. (below which precipitation was likely to occur) to 70° C., above which coagulation soon took place. The specific rotation, as will be seen later, appeared to decrease with the percentage of proteid in solution. The variation with the temperature was apparently slight, and it seemed useless to try to determine it exactly. In fact, the variation of the specific rotation with the solvent was the only factor that could be determined with any exactness.

Preparation of Solutions.

Salt Solutions.—The salt solutions used as solvents were all prepared in the same manner. A given quantity of the chemically pure salt, dried in a desiccator, was weighed out, dissolved in distilled water, and made up with distilled water to the required volume. The solution was then filtered. The residue of salt, when dried at 110° C., was next carefully determined, several portions of the solution of 20 or 30 cubic centimetres each being dried to constant weight at 110° C., and the results, which never differed by more than 2 or 3 milligrammes, averaged. None of the vegetable globulins experimented upon being completely soluble in these solutions, as concentrated a solution of globulin as possible was made and then filtered clear of the undissolved proteid. The percentage of proteid matter in solution was afterward determined by drying down 20 cubic centimetres or so to constant weight at 110° C., and subtracting from the residue the weight of the included salt. The percentage of ash in the dried proteid having been previously determined by ignition, the weight of ash- and water-free proteid in 100 cubic centi-

metres could easily be calculated. The concentration of the proteid solution was varied by diluting it in a graduated flask with the original solvent.

Acid and Alkaline Solutions.—The globulins studied, except when very dry, can be completely dissolved in dilute acid or alkaline solutions with comparative ease. This simplified very much the preparation of such solutions. The percentage of moisture in the globulin, which was kept in a bottle under fairly constant conditions of temperature and humidity, was carefully determined by drying it at 110° C. Thus, the percentage of moisture and of ash being known, the amount of water- and ash-free matter in a given weight could be readily found. In making the solutions a few grammes of the globulin were weighed out and completely dissolved in the acid or alkali, and the solutions were then made up to the required strength in a graduated flask.

A few alkaline solutions were also prepared by the addition of a definite volume of eight-per-cent potassium hydroxide to a sodium-chloride solution of the globulin. If the alkali is not too weak it can be added to a salt solution in this way without precipitating the globulin. Acids, on the contrary, produce precipitation immediately when added to such a globulin solution.

The 0·2-per-cent sodium-carbonate solution used as a solvent was made in a similar manner to the neutral salt solutions, the sodium carbonate being weighed out and dissolved in distilled water. The other alkalis and acids used as solvents were made up approximately to the requisite strength with distilled water, and their exact strength was afterward determined by titration.

Apparatus and Methods of Observation.

The polarimeter observations were made in a large dark room partitioned off in the centre of the building, and a "half-shadow" polarimeter of the Laurent type,* constructed by J. Dubose, of Paris, was employed to measure the rotary power of the proteid so-

* *Dingl. Polyt. Journ.*, vol. ccxxiii, p. 608.

lutions. This was a large instrument designed for use as a saccharimeter and capable of holding tubes 400 and 500 millimetres long, with a 20-centimetre circle divided to half-degrees, and a vernier with magnifying glass reading to 0·05°. Sodium light was alone used, as no other monochromatic light could be found of sufficient intensity. The exact position of the analyzer when the two halves of the field became of the same shade could not be determined by a single setting closer than 0·05° to 0·10°. However, by taking a large number of observations and averaging them, the error of the mean could be reduced to less than 0·01°. In general, from twenty to forty settings were made and readings taken, the position of uniform shade being approached alternately from the right and the left hand. The temperature of the solution in the tube was found by removing the cover and inserting a thermometer immediately after the rotation had been measured. Instead of depending on the result of one such measurement of the rotation, the tube was always emptied and refilled with a fresh portion of the solution and a new series of readings were taken. This was repeated a number of times, and the weighted mean ot the results obtained was adopted as the best value.

The specific rotation was calculated by the formula

$$(a)_D = \frac{100}{l\,c}\,a,$$

where a is the angle of rotation measured, l the length of the tube in decimetres, and c the weight of ash- and water-free substance in 100 cubic centimetres. The factor $\frac{100}{l\,c}$ in this investigation varied, according to the percentage of proteid in the solution, from 50 to as large a value as 200. Thus an error of 0·01° in measuring the angle of rotation, a, meant an error of from 0·5° to 2·0° in the value of the specific rotation, $(a)_D$.

Sources of Error.

A careful investigation of all possible sources of error, both in determining the strength of the solutions and in measuring their ro-

tary power, led to the conclusion that by far the most important source of error lay in the observer himself. The values found at different times for a single setting of the polarimeter, each the mean of thirty or forty readings and having a probable error of less than 0·01°, would frequently differ among themselves by 0·05° or more, especially when the intensity of the sodium flame varied or the observer's eyes became fatigued. The error thus introduced into the value of the specific rotation was from two to five per cent, according to the size of the angle measured, while the errors introduced by the methods of determining the percentage of proteid matter in solution, which ranked next in magnitude, never exceeded 0·3 or 0·4 per cent.

To find the probable error of the measured angle of rotation, the probable error of the initial setting with an empty tube was calculated from a large number of readings covering an interval of some days, and the probable error of the mean rotation reading was calculated in each case from the results of a number of independent series of readings. The square root of the sum of the squares of these gives the probable error in the value of the total rotation, and the probable error of the specific rotation is easily calculated from this. In calculating the probable error of a number of measurements of the same angle the abbreviated formula *

$$r_0 = \frac{0{\cdot}8453\ \Sigma\ v}{n\sqrt{n-1}}$$

was used, where r_0 is the probable error of the mean, v the deviation of each observation from the mean, Σ the usual sign of summation, and n the number of observations.

II. Hemp-seed Globulin.

Preparation and Composition.

The globulin from hemp seed can be easily obtained in a crystalline form in comparatively large quantities. The following method of preparation was employed by the writer:

* See Merriam's Text of Least Squares, 2d ed., p. 93.

The raw, ground hemp seed was treated with a five-per-cent sodium-chloride solution at 60° C. for an hour which was then strained and filtered while hot. The filtrate obtained was rich in globulin, which was precipitated by cooling in the form of perfect but minute crystals. After decanting the supernatant liquid, the precipitate was thrown on a filter and washed successively with distilled water, dilute alcohol, absolute alcohol, and ether, thus removing all salts, fats, and other impurities soluble in water, alcohol, or ether. The ether was evaporated by exposure to the air, and the globulin was finally dried in a desiccator over sulphuric acid. In extracting the globulin in this way, about 50 grammes were obtained on an average from a kilogramme of the ground hemp seed. The crystals examined under a microscope and by polarized light were found to be perfect isometric octahedra with perhaps a few hexagonal plates.

The reactions of this proteid are given in full by Osborne, Chittenden and Mendel, and others.* Its composition has also been carefully determined. Chittenden and Mendel give the following analysis :

Carbon	51·63	Sulphur	0·90
Hydrogen	6·90	Oxygen	21·79
Nitrogen	18·78		100·00

0·8189 gramme of the crystallized globulin prepared by the writer, dried to constant weight at 110° C., yielded, after ignition, a residue of 0·0016 gramme of ash. This gives: Percentage ash =0·20 per cent.

Sodium-chloride Solutions.

The mode of preparing these solutions has already been described. Hemp-seed globulin is fairly soluble in a ten-per-cent sodium-chloride solution at temperatures above 20° C. At 15° C. it is much less soluble, and at 10° C. it is only very slightly soluble. The solutions of hemp-seed globulin are of a brownish-yellow colour. Although somewhat opaque, the writer was able to use solutions containing as much as two or three per cent of the proteid in a 100-millimetre tube.

* Osborne, *American Chemical Journal*, vol. xiv, p. 673. Chittenden and Mendel, *Journal of Physiology*, vol. xvii, p. 50.

Four independent solutions of hemp-seed globulin were made in a ten-per-cent sodium-chloride solution, and the percentage of globulin in solution was determined by drying at 110° C. The results of the polarimeter observations with these solutions are shown in the following table:

No. of solution.	Ash-free glob. in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.	Remarks.
	Grms.	Deg. C.		Mm.	Deg.	Deg.	Deg.	
1	3·40	25·0	2	100	−1·47	−43·2	±0·5	Solution quite opaque.
2	2·91	26·5	3	100	1·26	43·2	1·0	" " "
4	2·30	24·8	4	100	0·995	43·2	0·5	Less opaque.
1 (diluted)	1·70	24·8	6	100 and 200	0·730	42·9	0·8	
2 "	1·45	26·4	2	100	0·596	41·0	2·0	Eyes fatigued.
2 "	0·97	26·2	4	100 and 200	0·389	40·0	1·1	
4 "	0·92	20·3	6	200	0·365	39·7	0·9	
3	0·80	22·3	9	200 and 300	0·342	43·0	0·9	

With the exception of those for solutions containing 0·97 and 0·92 per cent of globulin, the values obtained for $(a)_D$ agree well within the given limits of error. In general they seem to indicate a decrease in the rotary power of the globulin with the percentage in solution. Assuming such a decrease in the rotary power, by interpolating and extrapolating for each separate solution and averaging the results we obtain $(a)_D = -41{\cdot}6° \pm 0{\cdot}5°$, as the specific rotation of hemp-seed globulin dissolved in a ten-per-cent sodium-chloride solution, each 100 cubic centimetres of the resultant solution containing 1 gramme of the proteid. For purposes of comparison the value

$$(a)_D = -41{\cdot}5°$$

probably represents the specific rotation of hemp-seed globulin in such a solution within at least 1°.

Sodium-sulphate Solutions.

The sodium-sulphate solution used as a solvent was made up to a strength of ten per cent with sodium sulphate dried in a desiccator, but of only five per cent of the anhydrous salt (dried at 110° C.). The hemp-seed globulin was only slightly soluble in this solution at the temperature of the room, and so the globulin solutions were

made at a temperature of about 35° C. To avoid precipitation during the polarimeter observations, the tubes were warmed to about 50° C. and the solution to 35° or 40° C. before filling. Despite the care taken, several sets of observations were spoiled by the precipitation of the globulin.

Only two independent solutions of hemp-seed globulin were made in the sodium-sulphate solution. The results of the polarimeter observations and from drying down at 110° C. were as follows:

No. of solution.	Ash-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(\alpha)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
5	1·015	31·8	7	100 and 200	−0·393	−38·7	±0·8
6	0·864	30·0	5	100	0·333	38·6	1·4

These results are quite concordant, and show the specific rotation of hemp-seed globulin in a ten-per-cent (five-per-cent solution of the anhydrous salt) sodium-sulphate solution to be some three degrees less than in a ten-per-cent sodium-chloride solution.

Ammonium-sulphate Solutions.

The hemp-seed globulin was more soluble in a ten-per-cent ammonium-sulphate solution than in the preceding sodium-sulphate solution, but less soluble than in a ten-per-cent sodium-chloride solution.

Two independent solutions of the globulin in a ten-per-cent ammonium-sulphate solution were made, and the following results obtained from them:

No. of solution.	Ash-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(\alpha)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
7	1·10	27·5	4	100 and 200	−0·442	−40·0	±0·8
7 (diluted)	0·48	26·5	3	200 " 220	0·205	42·8	5·6
8	1·18	30·4	6	100 " 200	0·456	38·6	1·3

These results, although not very concordant or reliable, are sufficiently so to show that the specific rotation of hemp-seed globulin in an ammonium-sulphate solution lies between the values found respectively for sodium-sulphate and sodium-chloride solutions.

The weighted mean of the above gives for the specific rotation of hemp-seed globulin in a ten-per-cent ammonium-sulphate solution

$$(a)_D = -39.8°.$$

Sodium-chloride-potassium-hydroxide Solution.

The remnants of Solutions 3 and 4 (hemp-seed globulin in a ten-per-cent sodium-chloride solution) were used to form an alkaline solution by adding to them a definite volume of an 8·2-per-cent potassium-hydroxide solution; 100 cubic centimetres of the solution thus formed contained 0·766 gramme of hemp-seed globulin, 9 grammes of sodium chloride, and 0·82 gramme of potassium hydroxide.

Five measurements of the rotation due to this solution gave a mean angle of $-0·371° \pm 0·011°$ for a 100-millimetre tube, the average temperature of the solution being 22·1° C. The specific rotation of the hemp-seed globulin calculated from this is

$$(a)_D = -57·6° \pm 1·4°.$$

After these measurements the solution was heated to 40° C. for fifteen or twenty minutes to insure the complete conversion of the proteid into an alkali-albumin, and, after cooling, its rotary power was again determined.

Four sets of observations with a 100-millimetre tube gave a mean rotation of $-0·412° \pm 0·011°$, the average temperature of the solution being 21·2° C. The specific rotation of the globulin calculated from this is

$$(a)_D = -61·6° \pm 1·4°.$$

This is only 4° greater than the value obtained before warming the solution, which would seemingly indicate that the proteid must have been almost entirely converted into an alkali-albumin before the first determination of the specific rotation was made.

Dilute Potassium-hydroxide Solution.

Two determinations of the amount of moisture in the air-dry hemp-seed globulin were made at different times by drying it at 110° C. The first determination gave a loss of weight of 0·0654 gramme for each gramme of globulin, and the second of 0·0659

gramme, showing that the percentage of moisture remained practically unchanged.

3·3720 grammes of this air-dry globulin were dissolved in an 0·18-per-cent KOH solution, and made up with the same solution to 200 cubic centimetres. The rotary power of this solution of hemp-seed globulin was determined immediately after making it, and again after heating it at 40° or 50° C. for some thirty to forty minutes, with the following results:

	Ash- and water-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tube.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
Before heating.	1·57	23	4	100	−1·017	−64·7	±1·0
After " .	1·57	25	5	100	0·999	63·6	0·7

The values found for $(a)_D$ agree within the limit of error of observation, and are slightly larger than those obtained for hemp-seed globulin in the last solution. Their agreement would indicate that the action of the alkali in raising the rotary power of the globulin is exerted at once and without the aid of heat, the proteid being presumably changed more or less completely into an alkali-albumin when dissolved in the alkaline solution.

Dilute Sodium-carbonate Solution.

5·6987 grammes of the air-dry hemp-seed globulin were dissolved in a 0·2-per-cent sodium-carbonate solution and then made up with the same sodium-carbonate solution to 240 cubic centimetres. The rotary power of this globulin solution was determined, (1) immediately after making up the solution, (2) six hours after, and (3) after heating it at 40° to 50° C. thirty or forty minutes and cooling. The results of the polarimeter observations were as follows:

	Ash- and water-free globulins in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
1	2·21	22·0	4	100	−1·072	−48·4	±0·5
2	2·21	22·5	4	100	1·105	49·9	0·4
3	2·21	23·0	4	100	1·200	54·2	0·7

These show a slight increase in the specific rotation during the first six hours, and a marked increase after heating the solution of over 4°. The globulin is evidently not so readily nor so completely transformed by the sodium carbonate as by the stronger caustic alkali.

Dilute Hydrochloric-acid Solution.

3·6906 grammes of the air-dry hemp-seed globulin were dissolved in a 0·26-per-cent hydrochloric-acid solution and made up to 262 cubic centimetres with the same solution. This solution was exceptionally transparent and the most satisfactory for use in the polarimeter of any proteid solution tried by the writer. Its rotary power was determined, (1) two hours after making the solution up, (2) twenty hours after making it up, (3) after heating it at about 40° C. for twenty minutes, and (4) after letting it stand for thirty-four days. The results of the polarimeter observations were as follows:

	Ash- and water-free globulins in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	Mean value $(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
1	1·31	24	6	100	−1·120	−85·3	±0·7
2	1·31	20	4	200	1·066	81·2	0·4
3	1·31	21	4	300	1·079	81·9	0·2
4	1·31	24	4	200	1·097	83·6	0·3

The largest value obtained for the specific rotation was within two hours after making the solution up and before heating it. That obtained twenty hours after was some 4° lower, and there was only a slight increase in the specific rotation after the solution had been heated. After the solution had stood for over a month the specific rotation was found to have increased about 1·7°.

III. Flaxseed Globulin.

Preparation and Composition.

On account of the gummy character of the extracts from flaxseed and the consequent difficulty in filtering them, it was not easy to obtain this proteid in large quantities. That used in this investigation was prepared by the following method:

Ordinary flaxseed meal was treated with benzine (petroleum ether) to extract the fat, and then, after drying, was passed through a fine sieve to remove the husk as far as possible. The globulin was extracted from this prepared meal by means of a ten-per-cent sodium-chloride solution and the extract filtered clear. When extracted at 60° C. the extract obtained was richer in globulin but more difficult to filter than when extracted at 20° C. Extracts were obtained at both temperatures. The precipitation of the globulin by cooling was found to be impracticable, even after excessive dilution of the filtrate with water. The extracts were therefore submitted to dialysis for a few days, yielding in every case a fair percentage of well-crystallized globulin. The crystallized precipitate was collected on a filter and washed successively with dilute alcohol, absolute alcohol, and ether, and dried, after the ether had evaporated, in a desiccator over sulphuric acid. The minute crystals thus obtained were perfect isometric octahedra—somewhat smaller than those obtained from the hemp seed.

The reactions of this globulin from flaxseed have been fully given by Osborne,* and differ but little from those found for the hemp-seed globulin. As the result of a number of independent analyses, he found the flaxseed globulin to have the following composition:

Carbon	51·48	Sulphur	0·81
Hydrogen	6·94	Oxygen	22·17
Nitrogen	18·60		100·00

It will be seen by comparison that the composition of this proteid is almost identical with that of the globulin from hemp seed, which it also resembles, as stated, in its reactions, and in the form of its crystals, except that none of the crystals of the flaxseed globulin are of a hexagonal form. Osborne, because of the similarity in their composition and behaviour toward reagents, considers these two globulins—and also those from the castor bean, cotton seed, squash seed, and a number of cereals—as one and the same proteid body.†

* *American Chemical Journal*, vol. xiv, pp. 629, 681.

† *Ibid.*, vol. xiv, p. 687, and vol. xv, p. 24. Also *Rep. Conn. Agric. Exp. Sta. for 1893*, pp. 179, 216, and same for 1895, p. 172.

This body he has named *edestin*, from its occurrence in so many food stuffs.

0·7778 gramme of the flaxseed globulin prepared by the writer, dried at 110° C., yielded, after ignition, a residue of 0·0075 gramme of ash. This would give a percentage of ash of 0·96 per cent.

As the quantity of crystallized flaxseed globulin prepared was not sufficient for an extended investigation of its rotary properties, the specific rotation was determined for only a single solvent, a ten-per-cent sodium-chloride solution being chosen as the most available. A good basis was thus obtained for comparing its rotary power with that of the other proteids studied.

Sodium-chloride Solutions.

These solutions of flaxseed globulin had a rather strong yellow colour. They proved, however, to be fairly transparent to sodium light, so that the writer was able to use in the polarimeter solutions that appeared quite opaque when in a beaker.

Three independent solutions of flaxseed globulin in ten-per-cent sodium chloride were made, and used in the polarimeter. The crystals of the globulin used in the first solution (Solution 13) were somewhat imperfect, having been rounded and broken during the processes of filtering and drying. They were separated by dialysis from a ten-per-cent sodium-chloride extract obtained from the fat-free flaxseed meal by a second extraction at 20° C.* The globulin used in the other two solutions contained none but perfect crystals. Part of it was extracted from the flaxseed meal by a ten-per-cent sodium-chloride solution at 20° C., and the rest was similarly extracted at 60° C. In both cases the globulin was precipitated from the extract by dialysis.

The results of the polarimeter observations and from drying down are tabulated below. With the exception of the first and last, the values found for the specific rotation agree well within the limits of error, although they apparently decrease with the percentage of globu-

* With these were mixed some perfect crystals obtained by cooling from a five-per-cent sodium-chloride extract at 60° C.

lin in solution. The table shows clearly how the error in the mean value of $(a)_D$ is increased when a weak solution is used :

No. of solution.	Ash-free globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tubes.	Mean rot. per 100 mm.	$(a)_D$.	Probable error.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.
15	1·51	23·7	8	100	−0·572	−38·0	±0·5
13	1·19	25·5	10	100 and 200	0·472	39·7	0·6
14	1·08	24·0	7	100	0·426	39·4	0·9
15 (diluted)	0·94	22·5	8	200 and 300	0·364	38·8	1·2
13 "	0·60	23·0	5	200 and 300	0·225	37·8	1·8
14 "	0·47	23·5	10	200 and 300	0·172	36·7	1·3

The weighted mean of the last column but one gives

$$(a)_D = -38·7°,$$

with a probable error of less than half a degree.

Sodium-chloride-potassium-hydroxide Solution.

A remnant of Solution 15 (diluted), containing 90 cubic centimetres, was made up in a graduated flask to 100 cubic centimetres with an eight-per-cent potassium-hydroxide solution. The solution thus formed contained, in 100 cubic centimetres, 0·85 gramme of flaxseed globulin, 9 grammes of sodium chloride, and 0·8 gramme of potassium hydroxide.

The colour of the solution was changed by the potassium hydroxide from a bright-yellow to an orange tint, which made it more difficult to measure the rotation, even with a 100-millimetre tube. As a result of four measurements with a 100-millimetre tube at the temperature of 23° C., the rotation of the plane of polarization was found to be $-0·464° \pm 0·013$. This would give as the specific rotation of flaxseed globulin in such an alkaline solution,

$$(a)_D = -54·5° \pm 1·5°$$

IV. Brazil-nut Globulin.

Preparation and Composition.

The globulin obtained from the Brazil nut was chosen for investigation because it differed somewhat in its chemical composition and reactions from the two previously studied. These differences are regarded by Osborne as sufficient to warrant its being classed as

a distinct proteid body.* The preparations used in this investigation were obtained by the following methods:

A fine white meal was prepared from the meat of the Brazil nut by grating it, extracting the oil with benzine, and then sifting it through a fine sieve. Two methods were employed to obtain the globulin. By the first it was extracted from the prepared meal with distilled water at 60° C. and then precipitated by cooling. This precipitate was collected on a filter and washed successively with dilute alcohol, absolute alcohol, and ether, and dried, after the evaporation of the ether, over sulphuric acid. By the second method the globulin was extracted from the prepared meal with a ten-per-cent sodium-chloride solution at 20° C., and the extract submitted to dialysis for a few days. The resulting precipitate was collected on a filter and washed and dried in the same manner as that obtained by cooling from the water extract. The second method gave the largest yield of globulin, and was on the whole found to be the more satisfactory, although the preparation was not as well crystallized as that from the water extract. In neither case were the globulin crystals as perfect as those obtained from the flaxseed and hemp seed. The Brazil-nut globulin crystals varied in form from a thin hexagonal plate, through more or less rounded forms, to a perfect spheroid. The most common form, and in fact the only one found in the globulin precipitated by cooling, was a thick hexagonal plate with every other angle rounded off. In bulk the crystallized globulin appeared as a very fine, pure-white powder.

This globulin from the Brazil nut has been analyzed by Sacchse Ritthausen, and others.† The following analyses are given by Osborne:

	Crystals.	Spheroids.	Spheroids.
Carbon	52·18	52·35	52·16
Hydrogen	6·92	6·96	6·98
Nitrogen	18·30	18·16	18·32
Sulphur	1·06	1·12	1·07
Oxygen	21·54	21·41	21·47
Totals	100·00	100·00	100·00

* *American Chemical Journal*, vol. xiv, p. 687. † *Ibid.*, p. 669.

The writer found the percentage of ash to be 0·42 per cent in the globulin preparation obtained by cooling a hot aqueous extract of the Brazil-nut meal.

Sodium-chloride Solutions.

The globulin from the Brazil nut was quite soluble in a ten-per-cent sodium-chloride solution, forming a rather dense opaque solution—so opaque to sodium light that it was difficult to measure its specific rotation, even with solutions containing from 0·5 to 1·0 per cent of the proteid. The opacity of its solutions and the limited quantities in which this globulin could be prepared prevented an extensive investigation of its rotary properties. As with the flaxseed globulin, the specific rotation of the Brazil-nut globulin was only determined for solutions with sodium chloride.

Three solutions of this globulin in a ten-per-cent sodium-chloride solution were prepared. The crystallized globulin used in solutions numbered 17 and 19 was obtained by cooling a hot (60° C.) water extract of fat-free Brazil-nut meal. That used in Solution 18 was obtained by dialysis from a cold (20° C.) ten-per-cent sodium-chloride extract of the Brazil-nut meal. This last solution was of a whitish colour and much more opaque to sodium light than the others. After repeated dilution a measurement of its rotary power was finally effected, but the result was so poor that no attempt was made to determine the small correction for the ash present.

The results of drying the solutions down at 110° C. and of the polarimeter observations are shown in the following table:

No. of solution.	Globulin in 100 c. c.	Temperature.	No. of measurements.	Length of tube.	Mean rot. per 100 mm.	$(\alpha)_D$.	Probable error.	Remarks.
	Grm.	Deg. C.		Mm.	Deg.	Deg.	Deg.	
17	2·13	22·3	6	100	−0·866	−40·75	±0·4	
17 (diluted)	0·92	20·8	12	100 and 200	0·366	39·9	1·9	
19	0·92	23·3	6	100	0·390	42·5	1·3	
18	0·77	22·3	2	100	0·285	37·1	2·4	Not corrected for ash.
17 (rediluted)	0·55	20·3	7	200 and 300	0·184	33·5	1·5	Unreliable; trace of ether.
19 (diluted)	0·45	22·7	9	200	0·173	38·1	0·9	

The values found for $(a)_D$ are not very concordant or accurate, but are the best that the writer could obtain with such opaque solutions. Omitting the value $-33{\cdot}5°$, which was somewhat doubtful, and the value $37{\cdot}1°$, which was not corrected for ash, the weighted mean of these is

$$(a)_D = -40{\cdot}3°.$$

Sodium-chloride-potassium-hydroxide Solution.—When potassium hydroxide was added to a sodium-chloride solution of Brazil-nut globulin the resulting solution had to be made much more strongly alkaline to prevent precipitation than with solutions of either flaxseed or hemp-seed globulin.

Sixty-nine cubic centimetres of Solution 17, diluted, were made up with an 8·2-per-cent potassium-hydroxide solution to 100 cubic centimetres. The resulting solution contained, in 100 cubic centimetres, 0·633 gramme of ash-free globulin, 6·90 grammes of sodium chloride, and 2·55 grammes of potassium hydroxide.

The rotary power of this solution was determined within three hours after making up, and again after heating to 50° C. for twenty minutes, with the following results:

	Globulin in 100 c. c.	No. of measurements.	Length of tube.	Mean rot. per 100 mm.	$(a)_D$.	Probable error.	
	Grm.		Mm.	Deg.	Deg.	Deg.	
Before heating...	0·63	5	100	−0·370	−58·5	±1·2	Temperature about 20° C.
After " ...	0·63	5	100	0·364	57·5	1·3	

The value of $(a)_D$ obtained after warming the solution agrees, within the limits of error, with that obtained before warming it. It is therefore obvious that the full action of the alkali is exerted without the aid of heat.*

V. Summary and Conclusions.

The specific rotation obtained from ten-per-cent sodium-chloride solutions, containing about one per cent of proteid matter, were as follows:

* In summarizing, the values of $(a)_D$ are given to the nearest 0·5°.

Hemp-seed globulin ..... $(a)_D = -41{\cdot}5°$
Brazil-nut globulin ..... $(a)_D = -40{\cdot}5°$
Flaxseed globulin ..... $(a)_D = -38{\cdot}5°$

The differences in the rotary powers of these substances are not very large or unexpected when we consider the slight differences in their chemical composition and behaviour, and in their content of ash.

Effect of Dilution.—The observations made with sodium-chloride solutions of these globulins showed in general a decrease in the rotary power with the percentage of proteid in solution, although the observations were not sufficiently accurate to determine the rate of this decrease.

Effect of Temperature.—As already stated, the changes in the specific rotation with the temperature were apparently very slight, and no attempt was made to determine them.

Effect of Solvent.—The following results obtained with the globulin from hemp seed show that the salt solution used as a solvent was not without influence on its rotary power:

Solvent.	Value of $(a)_D$.
10-per-cent sodium-chloride solution	$-41{\cdot}5°$
" ammonium-sulphate solution	$-40{\cdot}0°$
" sodium-sulphate solution. Equals 5-per-cent anhydrous salt	$-38{\cdot}5°$

Alkali-albumins.—The differences in the specific rotations of the globulins studied have already been noted. Their alkali-albumins also differ in their rotary powers in the same order, as the following table shows:

Proteid.	Per cent proteid.	Per cent NaCl.	Per cent KOH.	Value $(a)_D$.
Hemp-seed globulin	0·75	9·0	0·8	$-61{\cdot}7°$ *
Brazil-nut "	0·65	6·9	2·55	$-58{\cdot}0°$ †
Flaxseed "	0·85	9·0	0·8	$-54{\cdot}5°$

Alkaline and Acid Solutions.—The globulin from hemp seed was the only one whose rotary power was investigated in dilute alkaline and acid solutions. In general, as with other proteids, the effect of

* This was the value of $(a)_D$ after heating the solution. Heating increased it.
† Heating this solution did not materially affect the value of (a).

the alkali or acid was to increase the specific rotation from fifty to one hundred per cent, this increase being greatest in the acid solutions. Except in the sodium-carbonate solution, the increase in the rotary power took place almost immediately, and heating the solution did not materially alter its value. When dissolved in 0·2-per-cent hydrochloric acid there was at first a large increase and then a slight decrease in the rotary power of the proteid. The following are the results obtained with alkali and acid solutions:

Solvent.	Per cent proteid.	$(\alpha)_D$.
0·18 per cent potassium hydroxide	1·5	−64·5° within an hour. −63·5° after heating.
0·20 per cent sodium carbonate	2·2	−48·5° within an hour. −50·0° after 6 hours. −54·0° after heating.
0·26 per cent hydrochloric acid	1·3	−85·5° within 2 hours. −81·0° after 20 hours. −82·0° after heating. −83·5° after 34 days.

It is worth noting that the specific rotations of the animal and vegetable globulins, so far as known, lie between −38° and −48°, or perhaps within still closer limits. Besides those studied by the writer, the specific rotation of serum globulin is −48°, that of fibrinogen lies between −45° and −50°, and we have good reason for believing that that of egg globulin is about −40°.

In conclusion, the writer desires to acknowledge his indebtedness to Prof. R. H. Chittenden and Dr. L. B. Mendel, of the Sheffield Scientific School, for assistance and suggestions in the preparation of the proteids and their solutions; the success of this investigation is due in a large measure to their able and hearty co-operation. And also to Prof. C. S. Hastings, of the same institution, in whose laboratory the polarimeter observations were all made.