results of these determinations, arranged in the order of the solubility of each protein substance in ammonium sulphate solution.

Protein.	Lower limit. cc.	Most precipitated. cc.	cc.	Upper limit. cc.
Globulin, English walnut	2.8	2.8	4.6	6.6
Globulin, black walnut	2.8	2.8	4.6	6.6
Edestin	3.0	3.0	4.0	4.2
Edestin monochloride	3.0	3.0	3.9	3.9
Globulin flaxseed	3.1	3.3	4.6	4.7
Globulin castor bean	3.1	3.3	4.3	4.5
Globulin squash-seed	3.3	3.5	4.1	4.4
Amandin	3.5	3.5	5.0	5.3
Corylin	3.7	3.7	5.3	6.6
Excelsin	3.8	4.0	5.0	5.5
Conglutin (a)	4.2	4.3	6.0	7.3
Conglutin (b)	4.6	6.4	8.2	8.7
Globulin cottonseed	4.6	5.0	6.0	6.4
Legumin	5.4	5.5	6.5	7.5
Phaseolin	6.4	6.5	8.2	8.8

THE SPECIFIC ROTATION OF SOME VEGETABLE PROTEINS.

BY THOMAS B. OSBORNE AND ISAAC F. HARRIS.

THE specific rotation of very few of the vegetable proteins has been determined, the only observations, so far as we know, being those of Kjeldahl[1] on zein and gliadin, Alexander[2] on edestin, excelsin and the globulin of the flaxseed, and Chittenden and Mendel[3] on edestin. Having had an opportunity to make some observations on the proteins above named, as well as on a few others, we take this occasion to put then on record.

The determinations were made with a Schmidt and Haensch half-shade polariscope, provided with a sugar scale. The readings were calculated to degrees of circular polarization by multiplying the degrees observed on the sugar scale by 0.346. The results

[1] Kjeldahl : *Agricultur. Chem. Centrbl.*, **25**, 197 (1896).

[2] Alexander : *Jour. Expt. Med.*, I, No. 2 (1896).

[3] Chittenden and Mendel : *Jour. Physiol*, **17**, 40 (1894).

given were in all cases the average of a large number of observations, which in most cases agreed closely. The solutions of some of the proteins, although uncommonly clear and very nearly free from opalescence, were so opaque in long layers that satisfactory readings could be made only in comparatively dilute solutions and therefore the accuracy of these determinations was to a greater or less extent impaired.

The amount of dissolved protein was found by determining nitrogen in the contents of the polariscope tube, the capacity of which was accurately known. The amount of protein was obtained by multiplying the quantity of nitrogen found by a factor depending on the nitrogen content of the protein examined.

EDESTIN.

The preparation used had been repeatedly recrystallized and was perfectly neutral to phenolphthaleïn. A solution in 10 per cent. sodium chloride brine was filtered clear through a felt of paper pulp and examined with the following results:

I. $\alpha =$ observed rotation $-2.7°$.

$w =$ weight of protein per cubic centimeter 0.03385 gr.

$l =$ length of tube 2 dm.

$$(\alpha)_D^{20°} = -40°.$$

II. $\alpha = -2.07°$ $w = 0.0247$ gram $l = 2$ dm.

$$(\alpha)_D^{20°} = -41.9°.$$

III. $\alpha = -5.05°$ $w = 0.06095$ gram $l = 2$ dm.

$$(\alpha)_D^{20°} = -41.43°.$$

IV. $\alpha = -2.53°$ $w = 0.0610$ gram $l = 1$ dm.

$$(\alpha)_D^{20°} = -41.47°.$$

Another pure preparation of edestin gave the following results:

V. $\alpha = -1.73°$ $w = 0.0415$ gram $l = 1$ dm.

$$(\alpha)_D^{20°} = -41.7°.$$

The average of these results, $-41.3°$, agrees closely with that obtained by Alexander, namely, $-41.6°$, and does not differ greatly from that given by Chittenden and Mendel, $-43°$. There is no evidence that the degree of rotation is dependent on the concentration of the solution.

EXCELSIN.

The preparation of excelsin which we used was extracted from the brazil nut by sodium chloride brine and purified by recrystal-

lization. Although the solution in 10 per cent. sodium chloride brine used for polariscopic examination was almost free from every trace of opalescence, nevertheless, it was difficult to get satisfactory readings in strong solutions. Our results were as follows:

I. $\alpha = -3.38°$ $w = 0.0396$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -42.68°.$$

II. $\alpha = -3.95°$ $w = 0.0463$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -42.66°.$$

III. $\alpha = -2.00°$ $w = 0.0460$ gram $l = 1$ dm.
$$-43.48°.$$

The average of our figures, $-42.94°$, is considably higher than that of Alexander, $-40.3°$, but, as he appears to have been unable to get such clear solutions as we did and had great difficulty in making his readings, it is probable that our results are the more correct.

FLAXSEED GLOBULIN.

The carefully purified preparation of this globulin consisted of well-formed octahedral crystals, the solution of which, in 10 per cent. sodium chloride brine, when filtered, was so transparent that accurate readings were easily made.

I. $\alpha = -3.63°$ $w = 0.0415$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -43.73°.$$

II. $\alpha = -1.79°$ $w = 0.0413$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -43.34°.$$

The average of these determinations, $-43.53°$, is much higher than that found by Alexander, who gives $-38.7°$ as the mean of six observations on different solutions. This difference may be due to the much greater concentration of our solutions, as Alexander's results strongly indicate a considerable decrease in the specific rotation with decreasing percentage content of the solution in protein.

SQUASH-SEED GLOBULIN.

The squash-seed globulin was purified by repeatedly recrystallizing and found to have the following rotation when dissolved in 10 per cent. sodium chloride solution:

I. $\alpha = -4.70°$ $w = 0.0598$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -39.3°.$$

II. $\alpha = -4.12°$ $w = 0.0534$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -38.57°.$$

III. $\alpha = -2.05°$ $w = 0.0535$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -38.32°.$$

If we compare the average of these figures with those of the flaxseed globulin and of edestin, which were obtained with solutions of approximately the same strength, we find them as follows:

$$(\alpha)_D^{20°}.$$

Edestin.................................... 41.30°
Flaxseed globulin......................... 43.34°
Squash-seed globulin...................... 38.73°

These three globulins appear therefore to differ in rotatory power to such a degree that the differences cannot be attributed to experimental errors. Although alike in composition, reactions, solubility and crystalline form, we have recently shown[1] that these globulins yield such different proportions of the several classes of nitrogenous decomposition products that there can be no question but that they are different substances. To these differences must now be added those in specific rotatory power.

AMANDIN.

Amandin, the chief protein constituent of almonds and peach-seeds, when dissolved in 10 per cent. sodium chloride solution, was found to have the following specific rotation:

$$\alpha = -2.98° \quad w = 0.0264 \text{ gram} \quad l = 2 \text{ dm.}$$
$$(\alpha)_D^{20°} \doteq -56.44°.$$

CORYLIN.

Corylin is a globulin which is abundantly present in the hazel nut or filbert. Dissolved in 10 per cent. sodium chloride solution it showed the following rotatory power:

I. $\alpha = -2.87°$ $w = 0.0332$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -43.22°.$$

II. $\alpha = -1.40°$ $w = 0.0326$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -42.95°.$$

[1] Osborne and Harris: This Journal, **25**, 323 (1903).

The English walnut contains a globulin which so closely resembles corylin in its properties and composition that the writer[1] was led to regard it as probably identical with that substance. We have since found that it yields, on decomposition, a slightly, but distinctly, smaller amount of ammonia than does corylin and that a difference between the two is shown by the precipitation limits with ammonium sulphate. In harmony with this, we find a slight difference in the specific rotation of their solutions in 10 per cent. sodium chloride brine, as the following figures show :

I. $\alpha = -1.57°$ $w = 0.0357$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -44°.$$

II. $\alpha = -2.06°$ $w = 0.0227$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -45.37°.$$

III. $\alpha = -1.05°$ $w = 0.0227$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -46.25°.$$

The considerable differences between the above observations are chiefly due to the opacity of the solutions which, though filtered very clear, were, in a remarkable degree, impervious to light.

The specific rotation of the globulin of the English walnut is distinctly higher than that of the filbert, thereby showing another difference between these two very similar proteins.

The American black walnut is closely related to the English walnut and we have therefore made a close comparison of the globulins from these two nuts, but have discerned no notable difference between them. As the following figures show, the specific rotation of the globulin from American nut is practically the same as that of the one from English, the difference between the figures given being doubtless due to errors of observation caused by the opacity of the solutions. Dissolved in 10 per cent. sodium chloride solution, the following results were obtained :

I. $\alpha = -1.22°$ $w = 0.0272$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -44.85°.$$

II. $\alpha = -1.20°$ $w = 0.0273$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -44.0°.$$

[1] Osborne and Campbell : This Journal, 18, 609.

PHASEOLIN.

Phaseolin, which constitutes the greater part of the protein matter of the kidney bean (*Phaseolus vulgaris*), was found to have the following rotation when a crystallized preparation was dissolved in 10 per cent. sodium chloride solution:

I. $\alpha = -7.42°$ $w = 0.0900$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -41.22°.$$

II. $\alpha = -3.75$ $w = 0.0900$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -41.7°.$$

LEGUMIN.

A solution of a very pure preparation of legumin from the horse bean (*vicia faba*) was made with 10 per cent. of sodium chloride and found to have the following specific rotation:

I. $\alpha = -2.2°$ $w = 0.0494$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -44.53°.$$

II. $\alpha = -1.27°$ $w = 0.0291$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -43.64°.$$

ZEIN.

The principal protein of maize kernels is zein, which is soluble in strong alcohol. A solution of zein in alcohol of 90 per cent. by volume rotated as follows:

I. $\alpha = -3.03°$ $w = 0.0536$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -28.26°.$$

II. $\alpha = -1.45°$ $w = 0.0523$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -27.72°.$$

GLIADIN.

Gliadin, which forms about one-half of the protein of wheat gluten, when dissolved in alcohol, of 80 per cent. by volume, showed the following specific rotation:

I. $\alpha = -5.66°$ $w = 0.0308$ gram $l = 2$ dm.
$$(\alpha)_D^{20°} = -91.9°.$$

II. $\alpha = -2.86°$ $w = 0.0309$ gram $l = 1$ dm.
$$(\alpha)_D^{20°} = -92.55°.$$

The following table contains the specific rotation as determined for each of the preceding proteins:

Protein.	Source.	$(\alpha)_D^{20°}$.
Edestin	Hemp-seed	—41.3°
Globulin	Flaxseed	—43.53°
Globulin	Squash-seed	—38.73°
Excelsin	Brazil nut	—42.94°
Amandin	Almonds	—56.44°
Corylin	Filbert	—43.09°
Globulin	English walnut	--45.21°
Globulin	Black walnut	—44.43°
Phaseolin	Kidney bean	—41.46°
Legumin	Horse bean	—44.09°
Zein	Maize	—28.00°
Gliadin	Wheat	—92.28°

THE GLOBULIN OF THE ENGLISH WALNUT, THE AMER-ICAN BLACK WALNUT AND THE BUTTERNUT.

BY THOMAS B. OSBORNE AND ISAAC F. HARRIS.

PROTEINS, whose chemical identity is probable, have thus far been found only in seeds which are botanically closely related to each other.

Thus the chemical identity of gliadin from wheat or rye, of legumin from vetches, horse beans, lentils or peas, of vicilin from the three latter seeds, of phaseolin from the kidney or adzuki beans and of legumelin from the seeds of numerous legumes appears to be highly probable.

The strict chemical identity of carbon compounds of such high molecular weight can, of course, not be positively asserted, since the possibility of isomeric or homologous compounds of very similar properties is great. However, a rigid comparison of these supposedly identical proteins has as yet shown no difference whatever between them. Some proteins, which we had formerly regarded as identical, have recently been found to differ in the proportions of their various decomposition products, and it is certain that their molecules have a different structure. All these proteins were found in seeds which were *not* botanically closely related and should therefore, by analogy, *not* contain the same proteins. So marked is this difference in the protein constituents