LXXXVI. A NEW GLOBULIN FROM THE CASHEW NUT (*ANACARDIUM OCCIDENTALE*).

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As part of a scheme for the investigation of Indian food proteins not hitherto examined it was thought desirable to investigate the cashew nut which was found to contain in the raw state about 21.5 % of protein (%N $\times$ 6.25). The cashew crop is assuming great economic importance in India chiefly on account of the large exports to the U.S.A. where the nuts seem to be used, after expressing the oil, for the preparation of proprietary foods. In India it is eaten roasted or raw and is also used widely in confectionery. Its high nutritive value has been indicated by Chatterjee [1930], who claims to have found that young rats could attain normal growth on an exclusive diet of cashew nut.

Analysis of the whole kernels, obtained after removal of the shells and the brownish pericarp, gave the following values: moisture 7.0 to 7.5%; ash 2.5%; ether-extractable fat 52 to 53%; protein (%N $\times$ 6.25) 21.5%. Preliminary experiments seemed to indicate the presence of proteins of both albumin and globulin type, the former in preponderant amounts, as about 53.8 % of the total nitrogen of the fat-free meal could be removed by extraction with water and a further 35 % by subsequent extraction with sodium chloride solution. But further experiment showed that only one protein, a globulin, was concerned, that it was readily soluble in salt solutions of low concentrations and that aqueous extracts of cashew nut meal were sufficiently saline to dissolve out appreciable amounts of the globulin and also that the seed contained non-protein nitrogenous compounds extractable by water. The globulin was isolated in pure condition in yields amounting to 185 g. per kg. of the defatted seed material, representing about 50 % of the total N of the latter.

EXPERIMENTAL.

Preparation of meal for extraction.

The nuts, which were obtained from the Malabar coast, were shelled and the cream-coloured kernels passed three times through a meat mincer. The pasty material was then defatted by repeatedly shaking up with acetone (which also removed moisture and colouring matter) and finally with light petroleum. The dry granular material obtained was ground to a fine white powder and air-dried at room temperature. This meal, which contained on an average 4.0 to 6.2% moisture and 37 to 38% protein, was used for all subsequent extractions.

Preliminary extractions.

200 g. of the fat-free powder, containing 13.33 g. nitrogen, were exhaustively extracted with distilled water and 10 % sodium chloride solution in succession. Five extractions were found necessary with distilled water and three with salt

solution, the material being shaken up each time with 1 litre of the liquid. The combined aqueous extracts contained 7.17 g. or 53.8 % of the total nitrogen and the sodium chloride solution 4.31 g. or 32.4 % of the total nitrogen. This seemed to indicate the presence of unusual amounts of albumin in the seed, but further experiments proved this inference to be wrong. In the first place samples of protein prepared from the aqueous and the sodium chloride extracts as described later proved to be identical. Further, aqueous extracts were found to contain considerable amounts of phosphorus which could be removed completely by dialysis. On submitting such extracts to continuous dialysis till free from phosphorus and filtering off the precipitated material the filtrate contained only traces of protein.

Extraction with sodium chloride solutions of different concentrations. Comparative experiments gave the following results when 5 g. samples were extracted by shaking up twice for 1 hour each time with 25 ml. of solution at 45°: solutions containing 2.5 %, 5 %, 7.5 % and 10 % sodium chloride extracted respectively 73.1 %, 84.2 %, 86.3 % and 90.4 % of the total nitrogen.

Approximate determinations of the non-protein-N in the extracts were carried out by coagulating the protein by heat at p_H 4.8 and determining the nitrogen in the filtrate. It was thus found that about 24 % of the nitrogen in the aqueous extract and about 27 % in the sodium chloride extract represented non-protein nitrogenous material.

Preparation of the protein by different methods.

The property of the protein, isolated from the sodium chloride extract by the usual methods, of being soluble in aqueous solutions of low salinity and of peptising in water free from electrolytes, and its tendency to rapid denaturation first gave the impression that more than one type of protein was concerned. Preparations were therefore made by the following different methods to see if there was any difference in the products obtained.

(a) A warm 10 % sodium chloride extract was filtered through paper-pulp, the filtrate brought to p_H 4.8 (found by trial to be the region of maximum precipitation) by means of *N*/20 acetic acid and diluted with 10 to 15 volumes of distilled water at a temperature of 40°. The precipitate which formed on standing in the refrigerator overnight was separated by centrifuging, redissolved in a small quantity of salt solution, filtered again through paper-pulp and reprecipitated as before. The precipitate was separated by centrifuging, washed twice with ice-cold water, then with graded strengths of acetone ranging from 75 to 95 %. After a final washing with 95 % acetone at the pump the material was first dried in air and finally in the vacuum desiccator over sulphuric acid. The fine white powder so obtained represented when dry 18 to 19 % by weight of the fat-free meal used.

It was observed that washing with water if continued to the point at which the test for chloride was negative caused redispersion of some of the material; hence the last traces of chloride were removed by washing with dilute acetone. It was also found that in precipitating the protein from solution, if dilution preceded acidification, considerable denaturation occurred and only a small portion of the precipitate would redissolve in sodium chloride solution.

(b) The sodium chloride extract was brought to p_H 4.8 by means of sodium acetate-acetic acid buffer and then diluted with 10 volumes of water. The precipitate obtained after standing in the refrigerator overnight was worked up as before.

(c) The sodium chloride extract was diluted with 3 volumes of water, cooled to 0° and saturated with carbon dioxide. By using ice-cold water saturated with carbon dioxide the precipitate could be washed free from chloride without showing any tendency to disperse in the wash-water.

(d) The sodium chloride extract was dialysed against distilled water till the solution in the dialyser failed to give any test for chloride. The precipitate was washed and dried as before.

(e) The supernatant liquid from (d) had a p_H of about 7.2 and still contained a small amount of protein. This was precipitated by saturating with carbon dioxide as in (c). The filtrate from this precipitate contained only traces of protein.

For purposes of comparison three specimens were prepared from aqueous extracts of the meal also; (f) by acidifying to p_H 4.8, (g) by precipitating with carbon dioxide at 0° and (h) by dialysis till free from phosphorus. The supernatant liquids from all these precipitates gave no indication of the presence of more than traces of albumin.

The eight products obtained as above were identical in properties, being insoluble in water, readily soluble in sodium chloride solutions and precipitated from such solutions by dilution at p_H 4.8. As it was felt that the elementary composition would be of little significance in testing the identity of these preparations they were analysed for distribution of nitrogen in three groups—amide-N, amino-N and non-amino-N. The mean values obtained were: amide-N 11.9 % amino-N 63.5 %, non-amino-N 24.6 % (by difference), the variations with the different samples being well within the limits of experimental error.

It was not found possible to obtain the product crystalline by any of the methods tried, the protein always separating out as spheroids from the warm diluted solutions on cooling.

Preparation of the pure globulin.

The fat-free meal was extracted three times by shaking with warm 10 % sodium chloride solution (5 ml. solution for each g. of meal) the combined extracts filtered through paper-pulp, the clear filtrate brought to p_H 4.8 with $N/20$ acetic or hydrochloric acid and diluted with water at 40° till a faint turbidity appeared. The precipitate obtained on allowing to stand overnight in the refrigerator was separated by centrifuging, redissolved while still moist in a small amount of warm sodium chloride solution and after filtration through paper-pulp reprecipitated as before. This was repeated four times, a small portion of the precipitate obtained each time being washed and dehydrated with acetone as already described. After drying over phosphorus pentoxide *in vacuo* at 100° the different samples were analysed for their nitrogen content. There was no rise in the value of the latter after the fourth precipitation and the four-times precipitated samples were used for analysis. For preparation of the protein on a large scale two precipitations are however sufficient as the protein so obtained is practically pure, having a nitrogen content of 19.02 % compared with 19.30 % of the four- and five-times precipitated samples.

The protein gave the following positive reactions: xanthoproteic, Millon's, Ehrlich's and the Hopkin's-Cole tests. Tests for phosphorus and Molisch's reaction for carbohydrates were not given.

Analysis.

The four-times precipitated sample after air-drying had a moisture content of 11.37 % and gave 0.11 % ash (calculated on the dry weight of protein). After drying *in vacuo* over phosphorus pentoxide at 100° it analysed as follows: C, 50.41; H, 7.32; N, 19.30; S, 0.78; O, 22.19 %. Sulphur was determined by two methods, (1) by fusion with sodium peroxide and (2) by Pirie's [1932] method using a mixture of perchloric acid, nitric acid and copper nitrate as the oxidising agent, the values obtained being (1) 0.75, (2) 0.78 %.

Nitrogen distribution.

In determining nitrogen distribution by the Van Slyke method the following modifications were introduced in the usual procedure.

(1) In addition to the usual seven groups the dicarboxylic acid-N was determined according to Damodaran [1931].

(2) Arginine was determined separately, as recommended by Plimmer and Rosedale [1925], but by Kossel's method as adapted to the determination of arginine in small quantities of proteins by Kossel and Gross [1924]. For each determination 1 g. of protein was hydrolysed and the arginine precipitated and weighed as the flavianate.

(3) For determining the amino-N and non-amino-N in the basic fraction, the phosphotungstic acid precipitate obtained in the usual way was dissolved in about 30 to 50 ml. of Winterstein's acetone-water mixture (4:3 volumes), in which it was rapidly and completely soluble, and the phosphotungstic acid removed by means of a slight excess of baryta. The results are given in Table I.

Table I.

	% of total nitrogen
Humin-N	1.40
Amide-N	11.91
Dicarboxylic acid-N	18.79
Arginine-N	21.57
Histidine-N	4.78
Lysine-N	1.54
Monoamino-monocarboxylic acid-N	29.50
Non-amino-N	10.09
Total	99.58
<i>Other determinations</i>	
Cystine-N (colorimetric)	0.724
Tyrosine-N (bromination)	3.269

No value for cystine-N is included in the scheme of nitrogen distribution as it was found both by qualitative and quantitative tests that the solution of the bases was entirely free from sulphur. It was also ascertained that this was not due to the manipulations involved in the removal of phosphotungstic acid by means of barium hydroxide; the barium phosphotungstate was analysed for its sulphur content by the method of Plimmer and Lowndes [1927] and the amount of sulphur found to be no more than the blank value for the reagents. The filtrate from the bases however gave a strong nitroprusside reaction (after the preliminary addition of potassium cyanide) showing that cystine was present and had escaped precipitation. Cystine was therefore estimated colorimetrically [Folin and Marenzi, 1929] in a separate portion of the hydrolysate. Tyrosine was determined by bromination by the method of Plimmer and Eaves [1913].

SUMMARY.

1. A new globulin, for which the name *anacardein* is suggested, has been isolated from cashew nuts, *Anacardium occidentale*, in yields amounting to 17 to 18 % of the fat-free seed.

2. The protein has been analysed by the method of nitrogen distribution and shown to be a biologically complete protein. Cystine has been determined colorimetrically and tyrosine by bromination.

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