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SEED GLOBULINS OF VARIOUS SPECIES OF CUCURBITACEAE

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Key Word Index—Cucurbitaceae; seed globulins; polypeptides; amino acid composition; gel chromatography; disc-electrophoresis; ultracentrifugation analysis.

Abstract—Globulins isolated from 6 species of the Cucurbitaceae family (*C. maxima*, *C. pepo*, *C. moschata*, *Luffa cylindrica*, *Lagenaria vulgaris*, and *Momordica charantia*) were studied. Nitrogen content of the globulins varied from 18.3 to 18.8%, with a mean of 18.6%. Of the individual amino acids, the most abundant were arginine and glutamic acid. Content of histidine, proline, serine, and tyrosine showed relatively higher variability within the group of species compared. MWs of the globulins, determined with Sephadex G 200, were 241000 (*C. maxima*, *C. pepo*), 248000 (*C. moschata*, *L. cylindrica*), 256000 (*L. vulgaris*), and 218000 (*M. charantia*). By ultracentrifugal analysis of globulins in 2 M NaCl (pH 8), 3 fractions were identified in all the species except *M. charantia*, their sedimentation coefficients being in the range of (1) 5.2 S–7.2 S, (2) 15.3 S–17.2 S, and (3) 10.4–11.2 S. The latter fraction predominated in all the species, its amount was 94–96% of total globulins.

Differences in electrophoretic properties of the globulins and their subunits produced in the presence of 8 M urea and by oxidative splitting with performic acid, respectively, and results of electrophoresis in SDS-acrylamide gels are also discussed.

INTRODUCTION

So far, globulins have been prepared from seeds of several genera of cucurbits. Osborne [1] described a method of isolation of such proteins from squash seeds and subsequently other investigators used similar methods with application to a number of cucurbit seeds [2–4]. Many investigations have been carried out to determine the amino acid composition of the seed globulins from various genera and species of Cucurbitaceae [2–15]. A comparison of results from several studies suggests that these analyses have established the composition fairly definitely. Smith and Greene [13] have used such results to estimate minimum MW's. They give values of 58000 for globulins from pumpkin and squash seeds and 55000 for those from cucumber and watermelon seeds. The globulins from different genera are considered to be distinguishable on the basis of differences in the amino acid composition [12].

On the other hand, very little work has been done on the physical chemistry of these proteins. Byerrum *et al.* [16] studied the globulin from *C. pepo* electrophoretically and described it as essentially homogeneous at pH 4, the only value at which the electrophoresis was carried out. Kretovich *et al.* [17] found 2 electrophoretic fractions in the globulin from *C. maxima*. The results of Fuerst and his co-workers [18] have shown that the squash seed globulin in acetate buffers contained a single electrophoretic component at low pH but two sharply defined components at pH 4.7. Their sedimentation studies have shown that this globulin could be dissociated into 4 clearly defined fractions with mean s^0_{20} values of 3.0 S, 7.1 S, 9.4 S, and 12.1 S, respectively. The presence and proportion of these fractions were affected by concentration of the proteins, time and pH. Further information concerning the physico-chemical properties of the seed globulins of *C. maxima* have been provided by Mourgue, *et al.* [34]. They have demonstrated that the globulins consist of three individual components discernible by ultracentrifugation and chromatography on DEAE Sephadex columns. They have also determined the isoelectric point to be around pH 5.8 and MW of 340000.

Present work was carried out in order to examine the properties of storage globulins of several species of the Cucurbitaceae family from the point of view of physical chemistry and with respect to applicability in chemotaxonomy.

RESULTS

As results summarized in Table 1 show, no significant differences in the nitrogen content of the globulins from the species compared were found by chemical analysis. The mean value of the nitrogen content was 18.55%. Of the individual amino acids determined after acidic hydrolysis of the globulins from the individual species, the most abundant were arginine and glutamic acid. On average, the amount of arginine was about 3 times higher than that of glutamic acid. The content of some amino acids showed rather a high degree of variability within the framework of the group of species analyzed. They were histidine (coefficient variability, $v = 17.4\%$), proline ($v = 12.1\%$), serine ($v = 10.9\%$), and tyrosine ($v = 10.5\%$). Content of the other amino acids in the globulins was essentially the same in all the species studied, the respective coefficients of variability being lower than 10%.

Results of gel column chromatography on Sephadex G 200 showed that there is no substantial difference in elution properties of the globulins from the individual species of the Cucurbitaceae family. In all the six cases, the globulins were eluted as a single peak under given

Table 1. Chemical composition of globulins isolated from seeds of different species of Cucurbitaceae. (a) *Cucurbita maxima*, cv. Veltruska Giant; (b) *Cucurbita pepo*, cv. Kveta; (c) *Cucurbita moschata*, Bulgarian unknown variety; (d) *Luffa cylindrica*, var. pyriformis; (e) *Lagenaria vulgaris*, ssp. asiatica; (f) *Momordica charantia*, var. senegalensis

Species	a	b	c	d	e	f
Nitrogen (%)	18.57	18.72	18.29	18.79	18.56	18.36
Ash (%)†	0.98	1.50	1.98	1.35	0.58	0.36
Lysine	3.57	3.56	3.89	3.56	3.29	3.66
Histidine	1.29	1.26	1.38	1.51	1.57	1.98
Arginine	35.04	37.26	30.02	34.90	34.54	35.18
Aspartic	6.07	5.65	5.64	5.49	5.59	5.68
Threonine	1.90	2.07	2.15	2.08	2.30	1.82
Serine	4.47	4.23	4.46	3.83	3.63	3.45
Glutamic	11.17	9.59	10.03	10.70	9·80	9·81
Proline	2.82	2.87	2.81	3·02	3·21	3.79
Glycine	4.94	5.06	5.39	4.90	5.13	5.22
Alanine	4.21	4.06	4.21	4.72	4.66	4.61
Valine	3.31	3.18	3.64	3.15	3.03	3.63
Isoleucine	2.41	2.42	2.52	2.52	2.35	2.59
Leucine	4.56	4.86	5.09	4.61	4.17	4.77
Tyrosine	1.55	1.85	1.73	1.46	1.38	1.59
Phenylalanine	2.76	2.99	3.09	2.41	3.05	2.67

* Nitrogen content in 100% of ash-free dry matter of globulins. † Ash content in 100% of dry matter of globulins. Amino acid composition (g N of amino acid/100 g N), the values are the mean of duplicate analyses after hydrolysis in 6 N HCl at 105° (24 hr).

experimental conditions. Elution volume values (V_e) of the protein fractions varied in a narrow interval near the elution maximum of the catalase standard (Table 2). So did the determined MW's which varied within the range of 218000–256000. The lowest MW was found for the globulins isolated from *M. charantia* seeds (218000), the highest one for those from *L. vulgaris* seeds (256000). The MW of the globulins from *C. maxima* seeds was also determined by means of the sedimentation equilibrium method. In this case the MW value, calculated with the aid of an average value of partial specific volume (0.745 [19]), was 230400.

On the other hand, some specific differences offered in the electrophoretic behaviour of the globulin fractions (Fig. 1). Results of anion electrophoresis on 5% acrylamide gel columns showed that for globulins of all the species studied, two fractions are characteristic: one with a mean value of R_m 0.52 (coefficient of variability, $v = 2.7\%$) and the other with a mean R_m 0.32 ($v = 9.9\%$). As compared with the former, the latter fraction had higher scatter of the individual R_m values (Fig. 1, part 1). Densitometric measurements proved that the fraction with R_m 0.52 is the main component of the globulins of all the species analyzed. With the exceptions of *C. maxima* and *C. pepo*, other minor fractions with R_m 0.15–0.25 were identified in the globulins from all the species under examination. Evident differences were observed in electrophoretic properties of the subunits originating from the individual globulins in the presence of urea and after oxidative splitting with performic acid, respectively. Results of electrophoresis of the globulin subunits on 5% acrylamide gel columns with 8 M urea

Table 2. Results of gel column chromatography of Cucurbitaceae seed globulins on Sephadex G 200 (column 2.1 × 65 cm, markers were eluted with 0.2 M NaCl and the globulins with 2 M NaCl, both at pH 8)

Samples	V_e	V_e/V_t	V_e/V_0	K_{av}	MW
Jack bean urease	95	0.42	1.17	0.10	483000
Bovine liver catalase	115	0.51	1.42	0.24	230000
Human gamma globulin	128	0.57	1.58	0.33	140000
Bovine serum albumin	145	0.67	1.79	0.45	67000
Globulins from:					
Cucurbita maxima	114	0.51	1.41	0.23	241000
Cucurbita pepo	114	0.51	1.41	0.23	241000
Cucurbita moschata	113	0.50	1.40	0.22	248000
Luffa cylindrica	113	0.50	1.40	0.22	248000
Lagenaria vulgaris	112	0.49	1.38	0.21	256000
Momordica charantia	116	0.52	1.43	0.24	218000

V_e = elution vol (ml); V_0 = outer vol (81 ml, determined by blue dextran); V_t = total vol (224 ml, determined by K_2CrO_4); $K_{av} = (V_e - V_0)/(V_t - V_0)$. MW's of markers (with the exception of urease) were taken from ref [33], MW's of the globulins were calculated from a calibration graph, details see Experimental.

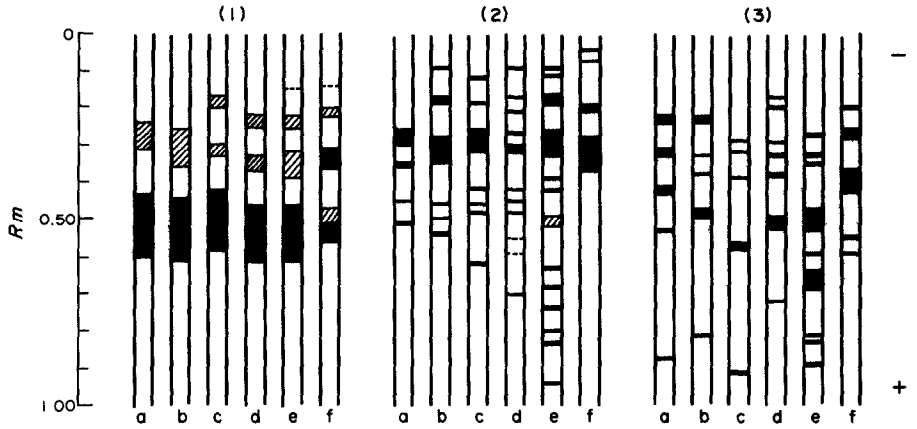


Fig. 1. Acrylamide gel electrophoresis of globulins isolated from seeds of different species of Cucurbitaceae. (a) *Cucurbita maxima*, cv. Veltruska Giant; (b) *Cucurbita pepo*, cv. Kveta; (c) *Cucurbita moschata*, Bulgarian unknown variety; (d) *Luffa cylindrica*, var. pyriformis; (e) *Lagenaria vulgaris*, ssp. *asiatica*; (f) *Momordica charantia*, var. senegalensis. (1) Electrophoresis of globulins on 5% gels dissolved in 2 M NaCl containing 3 mM 2-mercapto-ethanol, (2) Electrophoresis on 5% gels with 8 M urea, (3) Electrophoresis of polypeptides on 7.5% gels after oxidative splitting of the globulins with performic acid. All samples were run from the top of the figure towards the anode at the bottom. $R_m = 1.00$ corresponds to the bromophenol blue marker band.

demonstrated that the globulins of individual species differed from each other especially by the number of subunits formed (Fig. 1, part 2). The maximum number of the subunits (13) was detected in *L. vulgaris* samples, the minimum (4) in those from *C. maxima* and *M. charantia*. Mutual comparison of the respective R_m values of the individual subunits then showed relatively higher variability among the slower migrating fractions. Those with extremely low R_m values ($R_m < 0.10$) were identified in *C. pepo*, *L. cylindrica*, *L. vulgaris*, and *M. charantia*. Subunits with relatively high electrophoretic mobility were typical for *L. vulgaris* and they were also found in a small amount in *L. cylindrica*. On the contrary, the differences in electrophoretic properties of polypeptides formed by oxidative splitting the globulins were expressed by considerably high variability of the fractions with higher R_m values (Fig. 1, part 3). In this case, the differences in the number of the fractions were not so pronounced as in the case of electrophoresis in 8 M urea. The same number of fractions was detected in 4 of the six species compared. The exceptions were *L. cylindrica* and *L. vulgaris*. The differences in MW between

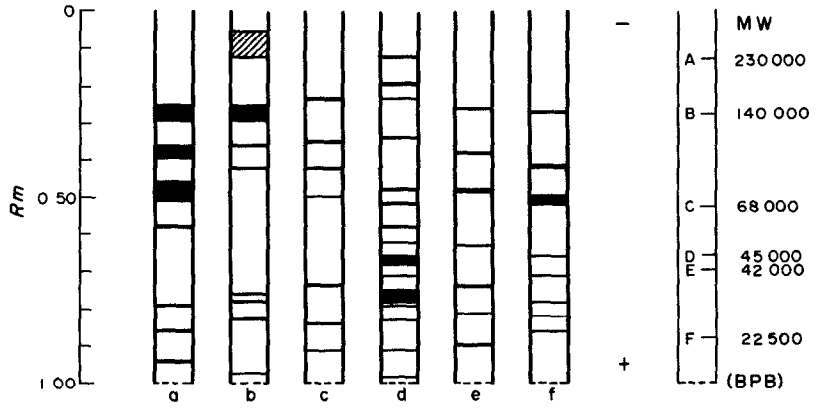


Fig. 2. SDS-electrophoresis on 7.5% acrylamide gels of polypeptides arising from the globulins after oxidative splitting with performic acid. (a–f see Fig. 1). The polypeptides were complexed with 0.5% SDS (sodium dodecyl sulphate) in tris–glycine buffer (pH 8.3), 12 hr before the electrophoresis. The proteins used as markers were: *catalase* (A), *human gamma globulin* (B), *bovine serum albumin* (C), *egg albumin* (D), *ovalbumin* (E), *chymotrypsin* (F); their relative positions as migrated in separate runs under standard conditions are schematically represented by the respective capitals. All the samples were run from the top of the figure towards the anode at the bottom. BPB = bromophenol blue marker band.

Table 3. MW's of subunits of the polypeptides obtained from globulins splitted with performic acid as determined by SDS–PAA gel electrophoresis.

Range of MW's	a	b	c	d	e	f	Variability coefficient (%)
$>2.00 \times 10^5$	–	–	–	+	–	–	
$1.50–2.00 \times 10^5$	1.50	1.50	1.70	1.90 1.70	1.55	1.50	5.1*
$1.00–1.50 \times 10^5$	1.08	1.15	1.18	1.20	1.08	–	4.5
$8.00–10.0 \times 10^4$	–	9.50	9.50	–	–	9.50	
$7.00–8.00 \times 10^4$	7.60	–	7.40	7.80	7.80	7.20	3.5
$6.00–7.00 \times 10^4$	–	–	–	6.90	–	–	
$5.00–6.00 \times 10^4$	5.80	–	–	5.50 5.10	–	–	
$4.00–5.00 \times 10^4$	–	–	–	4.35 3.80	4.90	4.35	
$3.00–4.00 \times 10^4$	3.00	3.30 3.10	3.50	3.20 3.00	3.45	3.80 3.10	7.8
$2.20–3.00 \times 10^4$	2.40	2.60	2.57	2.65	2.80	2.70 2.40	10.0
$<2.20 \times 10^4$	+	+	+	++	+	–	

(a–f) see Table 1. * Calculated with omission of the value 1.90×10^5; – not identified; +, ++ 1 or 2 components identified, respectively.

the polypeptides of the individual species are demonstrated by results of electrophoresis in SDS-acrylamide gels (Figs. 2). In this case *L. cylindrica* behaved quite anomalously, differing from all the other species especially by the presence of subunits with MW's in the range of 50000–70000 and of those with relatively high MW's (Table 3). Behaviour of the polypeptides of the other 5 species was similar to each other, the small differences observed were in the occurrence and number of low MW subunits.

The globulins of the individual species were also analysed by an ultracentrifugal technique under conditions corresponding to those in gel chromatography (i.e. NaCl concentration, pH). Except those from *M. charantia*, all the globulins revealed the presence of 3 fractions (Table 4). The areas under the peaks recorded during ultracentrifugation of the individual globulin samples were integrated and relative concentrations of the fractions were calculated. The fraction appearing to have sedimentation coefficient value within the range of 10.4 S–11.2 S predominated (94–96%) in the globulins of all species tested. The two minor fractions had sedimentation coefficient values within the range of 5.2 S–7.2 S and 15.3 S–17.2 S, respectively. They were present in approximately equal proportion and their aggregate percentage was not higher than 5% of the total globulins.

Table 4. Results of ultracentrifugal analyses of Cucurbitaceae seed globulins

Species	$S_{w,20}$ (in Svedbergs)		
Cucurbita maxima	6.6	10.7	16.2
Cucurbita pepo	7.2	10.9	16.1
Cucurbita moschata	6.0	11.0	16.1
Luffa cylindrica	5.2	11.1	16.2
Lagenaria vulgaris	6.8	11.2	17.2
Momordica charantia	–	10.4	15.3

Samples were centrifuged in 2 M NaCl (pH 8) at 20° in an AN–D rotor at 59780 rpm. Bar angle 60°.

DISCUSSION

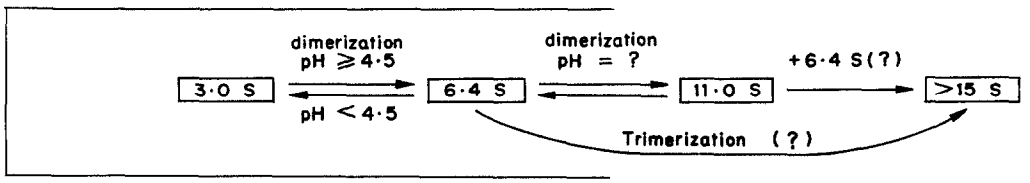
The results obtained by nitrogen content analysis of the Cucurbitaceae seed globulins confirmed previous results published by various authors. Jones and Gersdorff [2] present the value of 18.51% N for *C. maxima*, Vickery *et al.* [4] 18.52% N, and Kretovich *et al.* [17] the values of 17.92, 18.25, and 18.84% N. Similar values of the nitrogen content are also published for globulins from other species of the Cucurbitaceae. For globulins isolated from *C. pepo* seeds, Vickery and Winternitz [11] found the value of 18.55% N, Vickery *et al.* measured 18.42% N for *C. pepo* and 18.55% N for *C. moschata* globulins, Hirohata [6] 18.33% N for *L. cylindrica* and 18.38% N for *L. vulgaris* seed globulins.

It has been ascertained by comparison of the results of the amino acid composition that the globulins of all the species examined were noted for their relatively high content of arginine and glutamic acid. This finding is in accord not only with the results published for species of the Cucurbitaceae [2, 4, 6, 13, 14] but also with commonly accepted notions of amino acid composition of all dicotyledon seed globulins so far studied [20–22]. Although the content of some amino acids (His, Pro, Ser, and Tyr) was found to vary among the individual globulin preparations, it was not possible to detect significant differences in the total amino acid composition of the globulins from the plants sources compared. It corresponds to the earlier opinion that the globulins of the individual Cucurbitaceae species cannot be distinguished on the basis of amino acid composition analysis [4]. Also Smith *et al.* [12] consider such differences to be sufficiently significant for distinguishing the species only at the level of higher taxonomic units.

Little information is available on physico-chemical properties of the seed globulins of Cucurbitaceae species. Smith and Greene [13] used the amino acid composition values for a theoretical estimation of minimum MWs of the globulins. They calculated a value of 58000 for the globulins from *C. pepo* and *C. maxima* seeds and

55000 for those from cucumber and watermelon seeds. MW's of the globulins from the compared species, as determined by gel chromatography on a Sephadex G 200 column, are within the limits of 218000–256000. Variability of approximate MW values of the globulins isolated from the individual species was in a very good agreement with the variability of sedimentation coefficients of the globulin main fraction (i.e. 10.4 S–11.2 S). Because, under certain conditions, these values may be assumed to be in a linear relation, the differences in MW's of the globulins from the individual species seem to be real. To verify the determined MW of *C. maxima* globulins, a comparison of the results of gel chromatography with those of sedimentation equilibrium method was made. The approximate value (241000) determined by gel chromatography was very near the value determined by the sedimentation equilibrium method (230400). In fact, the difference between the two values was only 5%. However, these values are not consistent with the estimated MW (340000) of *C. maxima* globulins by Mourgue, *et al.* [34].

The results of sedimentation analyses showed that, except for *M. charantia* globulins, there are 3 fractions in globulins of the other species examined under the chosen experimental conditions. Sedimentation coefficients of these fractions are 5.2 S–7.2 S (a mean $\bar{x} = 6.4$ S), 10.4 S–11.2 S ($\bar{x} = 10.9$), and 15.3 S–17.2 S ($\bar{x} = 16{\cdot}2$ S). The one with the mean of 6.4 S was not detected in globulin preparations from *M. charantia*. This agrees with the 3 fractions of globulins isolated from *C. maxima* seeds by Mourgue *et al.* [34], however, these authors have not indicated the respective sedimentation coefficients. Fuerst *et al.* [18] demonstrated under various pH conditions that there are 4 well defined fractions in disperse solutions of globulins from Golden Hubbard squash (I, 3.0 S; II, 7.1 S; III, 9.4 S; IV, 12.1 S). The presence and proportion of these fractions were affected by concentration of the proteins, pH and time. While fraction II (7.1 S) was detectable within rather a wide interval of pH (from 3.8 to 11.4) fraction I (3.0 S) was present only from pH 3.8 to 4.5. This fraction predominated in the dispersion at pH 3.8. Its amount dropped with increasing pH and there were no traces detectable at pH $\geq$4.5. On the other hand, fraction IV was identified only in the alkaline region (pH 8.2–10.9). The existence of fraction III (9.4 S) was evidenced only under high concentration of globulins in the dispersion. Fuerst and his co-workers drew a conclusion on the basis of these results that fraction II (7.1 S) was likely to come into existence by an association of fraction I (3.0 S) components at pH 4.1–4.8 and fraction IV (12.1 S) by a subsequent association (dimerization) of two II (7.1 S) components. It seems to be likely, considering the conditions under which the presented sedimentation analyses were carried out, that fraction II (7.1 S) as identified by Fuerst is probably equivalent to the fraction with sedimentation coefficient of 5.2 S–7.2 S ($\bar{x} = 6.4$ S) as presented here, and Fuerst's fraction IV (12.1 S) to the one with sedimentation coefficient of 10.4 S–11.2 S ($\bar{x} = 10.9$ S). However, the results remain questionable regarding the occurrence of fraction with sedimentation coefficients higher than 15 S. A tentative scheme of reversible dissociation–association reactions of the globulin fraction may be suggested from so far available results of sedimentation analyses, at least for *C. maxima* globulins:



As for electrophoretic properties of the globulins, only results obtained by means of Tisselius' classical electrophoresis are published [16–18]. The globulins of *C. maxima* and *pepo* are described in this way as a system with a single fraction at pH < 4 [16, 17] and 2 fractions at pH > 5, respectively [17, 18]. The evidence for the occurrence of 2 electrophoretic fractions in alkaline medium is supported by the present results obtained by electrophoresis of *C. maxima* and *C. pepo* on 5% acrylamide gel columns at pH 8.3. Three electrophoretic fractions, however, were identified in the globulins from *C. moschata*, *L. cylindrica*, *L. vulgaris*, and *M. charantia*. Nevertheless, no substantial differences between R_m values of the individual fractions were found when comparing the individual species examined. Likewise electrophoretic patterns of the globulins from the individual species showed a degree of similarity. Strikingly large differences, on the other hand, were observed in electrophoretic properties of subunits arising from the globulins of the individual species in the presence of urea and after oxidative splitting with performic acid. This leads to an assumption of potential differences in tertiary structure of these globulins. At the same time it may offer a prospective tool in employing the above mode of analysis as one of chemotaxonomic criteria for determination of at least generic pertinence within a given family or tribus.

EXPERIMENTAL

Plant material. Seed samples of *Cucurbita maxima* (cv. Veltruska Giant), *Cucurbita moschata* (Bulgarian unknown variety), *Luffa cylindrica* var. pyriformis, *Lagenaria vulgaris* ssp. *asiatica*, and *Momordica charantia* var. senegalensis were kindly supplied by Institute of Tropical and Subtropical Agriculture of Agricultural University in Prague. Seed samples of *Cucurbita pepo* (cv. Kveta) were supplied by the Dept of Plant Physiology and Soil Biology of Charles University in Prague. All seeds used for experiments were obtained from plants grown from the above seed samples in the botanical gardens of Agricultural or Charles Universities during summer of 1971.

Preparation of globulins. Preparation of globulins was carried out as described in ref [18] with certain modifications. *Ca* 25 g of seeds from each of the examined species were homogenized with 300 ml of Me_2CO cooled to $-25°$ before homogenization. The homogenate was filtered and rinsed with Me_2CO ($-25°$) until the filtrate was colourless. The defatted material was dried in the air at laboratory temp, then gently milled and stored in stoppered glass containers at $-20°$. The meal (Me_2CO powder) was extracted for 2 hr with stirring in 2 M NaCl added in the ratio 1:10 at 40°. After extraction, the suspension was heated to 65° for denaturation of albumens for 15 min. The slurry was allowed to settle and the supernatant decanted into 100 ml centrifuge tubes and spun at 5000 *g*

for 30 min. The supernatant was recentrifuged at 30000 *g* for 1 hr at 2°. The final supernatant was clear except for some light flaky particles which were removed by filtration through coarse filter paper with a layer of cellulose powder. The filtrate was dialysed for 3 days, at first against running H_2O (5 hr) and then against glass-dist. H_2O at 4° (× 5 changes). The resultant ppt. (globulin fraction) was collected by centrifugation at 5000 *g* for 15 min at 2°. The supernatant was discarded and the ppt. of globulins washed with H_2O and collected again by centrifugation under the same conditions as above (× 3 repeated). The globulins were finally suspended in a minimum of H_2O, lyophilized and stored at −20°.

Nitrogen and amino acid determination. N was determined after conversion into NH_3 [23]. Amino acid determinations were carried out by ion exchange chromatography [24]. Protein was hydrolysed at 105° with an azeotropic mixture of 6 N HCl for 24 hr. No corrections were made for loss during hydrolysis.

Oxidation with performic acid. Was according to a method described in ref [25].

Gel chromatography. This was carried out on a Sephadex G 200 column (2.1 × 65 cm). Samples (2 ml) were applied containing about 5 mg of proteins in 2 M NaCl and the column was eluted with 2 M NaCl adjusted with tris to pH 8 at operation pressure 15 cm/m. Fractions were continuously monitored for *A* at 254 nm. In order to determine approximate MW's, the column was calibrated with samples (20 mg/ml) of jack bean urease, catalase, human gamma globulin, and bovine serum albumin. The approximate MW's of the Cucurbitaceae seed globulin components were determined by reference to a plot of elution vol. vs log MW.

Disc electrophoresis. Analyses were made on gels containing 5% acrylamide with or without 8 M urea, respectively [26, 27]. Samples were dissolved in either 2 M NaCl or 2 M NaCl containing 8 M urea, made viscous with 20% sucrose and vols containing about 200 μg of proteins were applied to the top of each gel column. Polypeptides arising after oxidation of globulins with performic acid were usually run on 7.5% small-pore gels with the usual large-pore stacking gel [28]. Polypeptides were disolved in Tris–glycine buffer (pH 8.3) containing 3 mM 2-mercaptoethanol, centrifuged at 10000 *g* for 15 min at 2° to remove the insoluble remainders. Supernatants were made viscous with 20% sucrose and vols containing about 200 μg of proteins were applied to the top of each column. SDS-electrophoresis of polypeptides was carried out as described by Stoklosa and Latz [29]. MW's of subunits were determined from a calibration plot of the values of standard proteins. In all cases, electrophoresis was carried out at room temp with a current of 4 mA per gel and in Tris–glycine buffer cooled to 5° prior to electrophoresis. Bromophenol blue was used as the marker and R_m values were calculated in relation to its band. After electrophoresis, gels were at first fixed in 5% TCA for 30 min, then stained in 0.2% Amido black 10 B in a mixture of MeOH–HOAc–H_2O (5:1:5) for 1 hr, and destained in a mixture of MeOH–HOAc–H_2O (4:1:10).

Ultracentrifugation analysis. These studies were performed on an analytical ultracentrifuge. Samples for analysis were dissolved in 2 M NaCl, adjusted with tris to pH 8 and then dialyzed against the same soln for 3 days at room temp. Velocity sedimentation measurements were performed with double sector cells and with schlieren optics at 20°. The rotor speed was 59780 rpm and the protein concn was 10 mg per ml. Sedimentation coefficients and $s_{20,w}$ values were determined by the method of ref [30]. For the determination of MW, the sedimentation equilibrium method of ref [31] was employed using a rotor speed 12590 rpm at 20°. Calculation of MW [32] was carried out using the partial specific vol. value of 0.745 (for 20°) which is characteristic for most plant globulins [19].

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