A Study on the Glucosamine-Containing Constituents of the Seeds of Kidney Beans (*Phaseolus vulgaris*)

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1. The constituents of the seeds of kidney beans containing glucosamine that could be released by acid hydrolysis (0.5 N-hydrochloric acid at 100°) were extracted into the phenol-rich phase on partitioning between phenol and water at 70°. 2. These materials were also brought into solution on extracting the seeds with water (incomplete), or preferably with a slightly alkaline buffer in the absence of phenol. When these solutions were heated at 100° or treated with trichloroacetic acid, all the materials containing acid labile glucosamine were carried down with the precipitate. Treatment with pepsin rendered the materials containing glucosamine soluble again. 3. All proteins in these extracts of kidney bean were found to be associated with various amounts of neutral and amino sugars by high-voltage electrophoresis and chromatography on Sephadex G-200 columns. 4. The isolation and crystallization of the main sugar components, D-glucosamine and D-mannose, are described and their significance in plant glycoproteins is discussed.

In contrast with the large body of information available on the structure and occurrence of animal glycoproteins, similar materials from higher plants have not been studied extensively, although the isolation and partial characterization of a few plant proteins containing sugar residues have been reported (Melik-Sarkisyan, 1957; Melik-Sarkisyan, Sisakyan & Svetaflo, 1962; Henze, 1959; Mejbaum-Katzenellenbogen, Goriaczko-Morawiecka & Dobryszczyka, 1960; Waldschmidt-Leitz & Hochstrasser, 1961; Hochstrasser, 1961, 1963; Kündig, Neukom & Deuel, 1961). Certain of these proteins were associated with relatively large amounts of neutral sugars, but no data on their hexosamine content were published (Menke & Jordan, 1959; Krüpe & Eusgraber, 1960; Waldschmidt-Leitz, 1961; Neukom & Kündig, 1962; Jaffe, 1962; Rohrlisch, 1962). Wada, Pallansch & Liener (1958), on the other hand, reported some chromatographic evidence for the presence of glucosamine, but not for other sugars, in the acid hydrolysate of a purified haemagglutinin from soya bean. Some of these conjugated proteins were split easily into a protein and a polysaccharide part by changing the pH of the solution (Rigas & Osgood, 1955; Rohrlisch, 1962), and so were not, in a strict sense, glycoproteins; others were supposed to be proteins with covalently linked sugar residues (Kündig *et al.* 1961; Hochstrasser, 1961, 1963; Waldschmidt-Leitz, 1961; Waldschmidt-Leitz & Hochstrasser, 1961).

Pusztai (1964a,b) suggested that proteins containing amino and neutral sugars firmly bound to the peptide moiety are present in kidney beans. The amino sugar component of these materials was isolated, crystallized and identified as 2-amino-2-deoxy-D-glucose, and their main neutral sugar component was tentatively identified as D-mannose.

A critical examination of the extraction and the properties of the constituents of the seeds of kidney beans containing glucosamine under conditions known either to separate or affect differently polysaccharides and proteins is described below.

MATERIALS AND METHODS

Kidney beans. All seed samples were obtained locally from W. Smith and Sons (Aberdeen). The seeds were finely ground on an 8 in. hammer mill and stored in sealed containers in a cold room (0–4°).

Nitrogen. All samples were digested with H₂SO₄ containing K₂SO₄, CuSO₄ and Na₂SeO₄ according to the procedure of Chibnall, Rees & Williams (1943), and the resulting ammonia was estimated by the method of Markham (1942).

Amino sugars. The materials were hydrolysed with 0.5 N-HCl for 16 hr. or with 2 N-HCl for 2 hr. at 100°, and the free hexosamines were separated on an ion-exchange column and estimated with the modified Elson-Morgan reagent (Pusztai & Morgan, 1963; Pusztai, 1964a). Occasionally for qualitative tests 6 N-HCl was used to liberate hexosamines from the acid-stable polysaccharide fractions of the seed.

High-voltage electrophoresis. The extracts of the seed were subjected to high-voltage continuous electrophoresis in free-flowing buffer films in an apparatus (made by Dr Bender and Dr Hobein, Munich, Germany) described by Hannig (1961).

Chromatography on Sephadex G-200 columns. The Sephadex gel was equilibrated with 0.05 N-tris-acetate buffer, pH 8.3. The preparation and operation of the columns (55 cm. $\times$ 2.2 cm.) was similar to that described by Andrews (1962, 1964).

Paper chromatography. The individual sugars were separated on paper with, as solvent mixtures, the organic phase from ethyl acetate-pyridine-acetic acid-water (20:8:1:8, by vol.) and occasionally with butan-1-ol-acetic acid-water (6:1:3, by vol.), and developed with the *p*-anisidine reagent of Hough, Jones & Wadman (1950).

EXPERIMENTAL AND RESULTS

Extraction of kidney beans with phenol. The method used for partitioning the components of the seed was similar to that of Westphal, Lüderitz & Bister (1952). The ground seeds of kidney beans (25 g.) were stirred with 1 l. of phenol-water (1:1, w/v) mixture at 70° for 30 min., then allowed to cool; the phases were separated by centrifuging. The bottom layer was re-extracted with water (500 ml.), cooled and centrifuged. The aqueous phases and insoluble material were combined and extracted again with phenol (300 g.) at 70°, and, finally, the combined phenol phases were treated with water (150 ml.) under similar conditions. The aqueous phases were pooled, extracted with ether to remove phenol, concentrated *in vacuo* to 50 ml. and treated with 4 vol. of ethanol. The resulting precipitate was collected by centrifuging, washed with ethanol and then with ether, and finally dried. The supernatants were also collected, concentrated and dried *in vacuo*. The combined phenol phases were separated from some sedimented debris and filtered to remove the material precipitated between the two phases in the extraction step. This precipitate, after being washed with ethanol and ether, was also dried and recovered. The clear phenol-containing filtrate was fractionally precipitated by the addition of ethanol. The resulting precipitates were washed with ethanol and then ether, and dried. The final supernatant was dialysed free from phenol, concentrated and freeze-dried. All recovered materials were tested for hexosamines and the results are given in Table 1. The material precipitated at the interface of phenol and water was again extracted with phenol-water (1:1, w/v) at 70°. Practically all the residual hexosamine was recovered from the material soluble in phenol saturated with water.

Extraction with water and buffer solution of kidney beans. Finely ground seeds of kidney beans (50 g.) were stirred with distilled water (500 ml.)

Table 1. *Fractionation of materials of kidney beans with phenol-water (1:1, w/v)*

Materials	Hexosamine content	
	Amount (g.)	(g./100 g. of material)
Kidney beans	25	0.12
Material insoluble in phenol, soluble in water and precipitated with 80% (v/v) ethanol	2.1	None
Supernatant from above	0.2	None
Debris	4.1	Trace
Material precipitated at the interface of phenol and water	10.3	0.019
Material soluble in phenol and precipitated by:		
20% (v/v) ethanol	—	—
30% (v/v) ethanol	4.2	0.27
40% (v/v) ethanol	1.0	0.52
60% (v/v) ethanol	1.0	0.91
> 60% (v/v) ethanol	0.4	0.33
Total ...	23.3	

in a cold room for 30 min. and then centrifuged; the supernatant was concentrated and freeze-dried. The insoluble residue was stirred with fresh distilled water (500 ml.) and worked up as before. This process was repeated once more, after which the residue insoluble in water was also dried and re-ground before it was subjected to two further extractions with water (500 ml. in each case). The final water-insoluble portion was divided into two equal parts; the first part was extracted with 0.1 M-tris-acetate buffer, pH 8.3 (500 ml.), and the second part with phenol-water (1:1, w/v) mixture (300 ml.) as described above. All materials were tested for hexosamine after hydrolysis with 0.5 N-hydrochloric acid at 100° for 16 hr. and the results are given in Table 2.

In the next experiment kidney beans (50 g.) were extracted directly with 0.1 M-tris-acetate buffer, pH 8.3 (500 ml.), in a cold room. The insoluble residue was stirred up with another portion of tris buffer (500 ml.) and this process was repeated once more. The extracts were subjected to hydrolysis and hexosamine estimation. Hexosamine recoveries of 86.0, 8.5 and 3.2% of the original seed hexosamine were recorded for the first, second and third extract respectively. The extracts were pooled and a part of it (500 ml.) was heated at 100° for 10 min., allowed to cool to room temperature and adjusted to pH 5. The resulting precipitate

Table 2. *Results of various extraction experiments*

Experimental details are given in the text. Hexosamine estimations were performed on the materials after hydrolysis with 0.5 N-HCl at 100° for 16 hr.

Material	Amount (g.)	Hexosamine content of sample (mg.)
Kidney beans	50	60.0
First aqueous extract	7.0	8.2
Second aqueous extract	4.0	5.8
Third aqueous extract	2.2	3.3
Residue after third extraction	37.0	44.2
Fourth aqueous extract	2.8	3.6
Fifth aqueous extract	1.2	1.7
Residue after fifth extraction	32.8	39.2
Extraction with tris buffer (calc. for total amount of residue insoluble in water):		
Soluble part	8.8*	38.4
Insoluble residue	24.0	2.2
Extraction with phenol-water (calc. for total amount of residue insoluble in water):		
Material soluble and phenol	3.2	35.6
Material insoluble in phenol-water	29.2	2.9
Material soluble in water	0.9	1.4

* Calculated by difference.

was centrifuged, washed with water, ethanol, ether and dried.

A part of this material (1g.) suspended in 100 ml. of water was treated with pepsin (20 mg.) at 37° for 72 hr. The pH of the solution was kept at pH 2, and when all pH changes stopped the digest was dialysed against distilled water and the diffusates were collected. The indiffusible part after centrifuging was treated with 9 vol. of ethanol and centrifuged. The precipitated material was washed with ethanol and then ether, and dried. The supernatant was also recovered.

Another part (3 ml.) of the extract of kidney beans with tris buffer was treated with an equal volume of aq. 15% (w/v) trichloroacetic acid. The resulting precipitate was centrifuged, washed with ethanol and then ether, and dried. The precipitate obtained from another portion (3 ml.) of the extract with trichloroacetic acid under the conditions described above was, after being collected by centrifuging, washed thoroughly in the centrifuge tube by first adding water (1 ml.) to it and stirring it up with a glass rod. Any residue left on the rod was washed back into the centrifuge tube with an equal volume (1 ml.) of aq. 15% (w/v) trichloroacetic acid. The precipitate was then centrifuged and the whole process of washing was repeated

Table 3. *Effects of various treatments on water-soluble components of the seeds of kidney beans*

Experimental details are given in the text. Hexosamine estimations were performed on the materials after hydrolysis with 0.5 N-HCl at 100° for 16 hr.

Material	Amount (g.)	Hexosamine content of sample (mg.)
Kidney beans	50	60.0
Material precipitated on heating	14	58.4
Material precipitated with tri-chloroacetic acid	13	58.6
Material precipitated on heating, then extensively washed with tri-chloroacetic acid	—	58.2
Diffusate from pepsin digest	—	4.8
Materials brought into solution by treatment with pepsin:		
Material precipitated with 9 vol. of ethanol	10.1	45.0
Supernatant from above	—	5.6
Insoluble residue after treatment with pepsin	2.4	3.4

twice more. The material was recovered after being washed with ether and dried. The results of the hexosamine estimations performed on these materials after hydrolysis with 0.5 N-hydrochloric acid at 100° for 16 hr. are set out in Table 3.

Other examinations. Extracts of kidney beans (300 mg.) with 0.1 M-tris-acetate buffer, pH 8.3 (10 ml.), were subjected to high-voltage electrophoresis in the Hannig apparatus. The collected fractions were examined for neutral and amino sugars and their extinction values were read at 280 m μ (Fig. 1). In another test a similar extract was chromatographed on a Sephadex G-200 column. The effluents were collected and tested for neutral sugars and their extinction values read at 280 and 260 m μ (Fig. 2).

The tests indicated the presence of five partially resolved components in the extract of the seed, the first four of which, designated as α , β , γ and δ , were found to be proteins with E_{280}/E_{260} ratios between 1.3 and 1.5 (Warburg & Christian, 1941), whereas the fifth component containing the low-molecular-weight components of the seed had an absorption maximum at 260 m μ and no maximum at or near 280 m μ (E_{280}/E_{260} ratio about 0.7).

Isolation of sugar components. The isolation, crystallization and identification of the protein-bound hexosamine component of the seeds of kidney beans as 2-amino-2-deoxy-D-glucose has been described by Pusztai (1964a). An alternative method of isolating glucosamine together with the neutral sugar components is now given.

Fig. 1. Separation and examination of the constituents of the seeds of kidney bean on high-voltage electrophoresis (conditions: Hannig apparatus; 34 v/cm.; 220 mA, rate of buffer flow in the cuvette, 50 ml./hr.; sample introduction rate, 2 ml./hr.; passage time, 7 hr.; sample introduction point, as indicated by the arrow; 0.1M-tris-acetate buffer, pH 8.3). The separated fractions were tested for hexosamines ($\blacktriangle$) and neutral sugars ($\circ$) and their extinctions were read at 280 $m\mu$ ($\bullet$).

Fig. 2. Separation of the constituents of the seeds of kidney bean on Sephadex G-200 columns equilibrated with 0.05M-tris-acetate buffer, pH 8.3. The separated fractions (fraction size 5.5 ml.) were tested for neutral sugars ($\circ$) and their extinctions were read at 280 $m\mu$ ($\bullet$). The first four peaks, α , β , γ , δ , represent protein components; the fifth peak, X, represents non-protein diffusible material.

Material (10 g.) soluble in phenol saturated with water and precipitated by the addition of ethanol (final concn. 75%, v/v) was hydrolysed with 0.5N-hydrochloric acid (11.) at 100° for 16 hr. The hydrolysate was repeatedly evaporated on a rotary still below 40° until the solution was free from excess of hydrochloric acid. This solution (60 ml.) was applied on to a charcoal-Celite (1:1) column (4 cm. $\times$ 30 cm.) and eluted with water. The effluents were tested with the modified Elson-Morgan reagent (Rondle & Morgan, 1955), with the

ninhydrin reagent of Moore & Stein (1954) and with the phenol-sulphuric acid reagent of Dubois, Gilles, Hamilton, Rebers & Smith (1951). Two main broad fractions were collected, the first one containing material reacting with the Elson-Morgan reagent and ninhydrin, and the second containing neutral monosaccharides from the hydrolysate. The first fraction was concentrated to 5 ml. *in vacuo* and fed into the Hannig apparatus (position no. 1, about eight collecting tubes away from the anode side of the apparatus). The conditions of the electrophoresis were as follows: pyridine-acetate buffer (12 ml. of acetic acid and 4 ml. of pyridine/l.); pH 4.2; 32 v/cm.; 170 mA; sample application rate, 1.6 ml./hr.; buffer flow rate, 50 ml./hr. The collected fractions were tested by paper chromatography. The contents of tubes 27-33 were pooled, concentrated *in vacuo* and subjected to a second electrophoresis in the Hannig apparatus under the conditions described above. The collected fractions were again examined by paper chromatography. Tubes 27-33 contained glucosamine contaminated with some ornithine. Arginine and lysine under these conditions migrated to tubes 34-37, and histidine to tubes 35-39, and these were clearly separated from glucosamine. Ornithine was removed from the combined fractions containing glucosamine by partitioning them on a short kieselguhr column between the organic and the aqueous phases obtained on mixing ethyl acetate, pyridine, acetic acid and water (20:8:1:8, by vol.). The preparation and operation of the column was similar to that described by Matheson (1963) for the separation of DNP-amino acids. Glucosamine under these conditions was quantitatively eluted with the first 200 ml. of the mobile phase passing through the column, whereas ornithine was still firmly held by the kieselguhr. The glucosamine so obtained contained no ninhydrin-positive impurities, gave negative reaction with the phenol-sulphuric acid reagent (Dubois *et al.* 1951) and had no absorption maxima between 240 and 400 $m\mu$. It was crystallized and identified, as described by Pusztai (1964a), as 2-deoxy-2-amino-D-glucose.

The neutral sugars obtained from the second main fraction from the charcoal-Celite (1:1) column were subjected to a second chromatography on a similar charcoal-Celite column under the conditions described above. The eluted fractions were tested by the phenol-sulphuric acid procedure (Dubois *et al.* 1951) and by paper chromatography. Three distinct peaks were found, two minor and one major, and the materials associated with them were recovered and dried *in vacuo*. The first minor component (5% of total sugar) contained one sugar only and had R_{glc} values similar to those of arabinose in both solvent systems. A second minor component was also recovered containing galactose and traces

of glucose according to the results of examination by paper chromatography. The combined amount of these sugars did not exceed 5% of the total amount of neutral monosaccharides recovered.

The main sugar component (about 90% of total sugar) was obtained in a well-defined peak on chromatography on the charcoal-Celite column. Paper chromatography indicated it to be mannose. The pooled fractions containing this sugar were evaporated *in vacuo* to 12 ml. A part (6 ml.) of this concentrate was treated with acetic acid (0.4 ml.) and redistilled phenylhydrazine (0.6 ml.). The crystalline precipitate that formed was collected by filtering after standing overnight at 0–4° and washed on the filter with two 1 ml. portions of aq. 18% (w/v) acetic acid. The light-brown product had m.p. 175°. The colour was removed from the preparation on washing with small amounts of hot ethanol. The material after two recrystallizations from boiling water had m.p. 188° (corr.). The yield was 89 mg. Neuberger (1938) gave m.p. 189° (uncorr.) for the D-mannose phenylhydrazone isolated from crystalline egg albumin. A sample of commercial D-mannose was also converted into its crystalline phenylhydrazone, m.p. 188° (corr.). There was no depression of the melting point when the two samples were admixed. The crystalline phenylhydrazone had a rotation (1 hr.) $[\alpha]_D + 6.9 \pm 1.0^\circ$, which changed to $33.1 \pm 1.5^\circ$ (c 1 in pyridine) after 70 hr.

The remainder (6 ml.) of the concentrate from the charcoal-Celite column was treated with *p*-bromophenylhydrazine according to the method used by Neuberger (1938). The yield was 138 mg. of crystalline material, m.p. 202° (corr.). The mixed m.p. with an authentic sample of D-mannose *p*-bromophenylhydrazone (m.p. 203°), prepared according to the method of Neuberger (1938), was 202–204°.

The minor arabinose, galactose and glucose components were not investigated further owing to the small amounts recovered.

DISCUSSION

The experiments described above were done to find more evidence for or against the existence of glycoproteins in kidney bean. The problem needed a critical re-examination, as some of the proteins isolated from plant sources and claimed to be glycoproteins were proved to be proteins only loosely associated with carbohydrate material (Rigas & Osgood, 1955; Rohrich, 1962).

The two-phase system (phenol–water, 1:1, w/v; Westphal *et al.* 1952) or other phenol-containing solvents (Morgan & King, 1943; Synge, 1957; Rasmussen & Craig, 1961) separate the constituents of the cell into several more or less well-defined

groups of materials. This separation is the result of the strong hydrogen-bond-breaking and dissociating action of phenol (Craig, 1962; Bagdasarian, Matheson, Synge & Youngson, 1964). Proteins are irreversibly denatured by the action of phenol but become soluble in it. On the other hand, nucleic acids under certain conditions are insoluble in phenol (Kirby, 1957). Polysaccharides or even certain types of mucopolysaccharides with protein or peptide moieties up to about 20–25% will favour the aqueous phase on extraction with phenol–water mixtures (Morgan & King, 1943; Pusztai & Morgan, 1961), but proteins with small amounts of attached sugars should behave as simple proteins do.

The results in Table 1 show that nearly all the materials that yield their glucosamine in a free form on hydrolysis with 0.5 N-hydrochloric acid at 100° for 16 hr. were recovered from the phase rich in phenol. The small amount of glucosamine remaining in the material precipitated between the two phases was removed completely on a second extraction with phenol and recovered again from the phase rich in phenol.

The material recovered from the aqueous phase contained polysaccharides from the seed. This fraction also contained some unidentified material from which amino sugar was slowly released on strong acid hydrolysis (6 N-hydrochloric acid at 100°). This material may well be similar to that found in the 'phytoglycolipide' fraction of soya bean, corn, peanut etc. (Carter *et al.* 1958a), which contains amongst other things glucosamine, hexuronic acid and inositol. By acid hydrolysis an acid-resistant trisaccharide is obtained composed of these compounds (Carter, Gigg, Law, Nakayama & Weber, 1958b). Other polysaccharides containing glucosamine and resistant to acid may also be present in this fraction, like those described by Weissmann, Rapport, Linker & Meyer (1953) and Hirano, Hoffman & Meyer (1961).

The materials soluble in phenol saturated with water were fractionally precipitated at various ethanol concentrations. All recovered materials had a significantly higher glucosamine content than the original seed sample, and, further, this varied from fraction to fraction (Table 1), indicating the existence of several compounds in the seeds containing glucosamine. The N (Kjeldahl) values also show an increase from 4.50% (seed sample) to 12.6–15.4% for the various materials soluble in the phenol-rich phase. The material that was precipitated between 40 and 60% (v/v) ethanol concentrations, and contained 15.4% of N (Kjeldahl) and 0.91% of glucosamine, may be comprised of relatively large amounts of glycoproteins.

Some of these materials could be extracted with distilled water at the natural pH of the seeds in water (pH 5.5 for this variety of kidney beans), but,

when about 30–35% of the material containing glucosamine has been extracted, very little additional material could be brought into solution on further extraction with water (Table 2). The rest of the material containing glucosamine, however, could be extracted almost quantitatively with either tris-acetate buffer, pH 8.3, or with phenol in the usual way. Very little hexosamine-containing material resistant to hydrolysis with mild acids was extracted under these conditions. The tris buffer removed practically all the protein-bound glucosamine from the seed (Table 2), and it was found to be preferable to extraction with phenol if the protein molecules were to be studied in their natural state. This extract on heating at 100° and subsequent adjustment to pH 5 yielded a precipitate of denatured proteins. Practically all the glucosamine was found to be in this precipitate (Table 3). When this denatured protein was digested with pepsin at pH 2 about 95% of the total glucosamine could be recovered from the material soluble in water (Table 3). There was evidence of some extensive fragmentation of the original protein molecules, as 8% of the glucosamine was found to be associated with materials small enough to diffuse through a cellophane membrane, although no free glucosamine was found in the diffusates. Glucosamine was also found to be associated with the precipitate obtained from the extract of kidney bean on treatment with trichloroacetic acid, a frequently used precipitating agent for proteins (Table 3). This hexosamine could not be removed from the precipitates on thorough washing.

The cell constituents soluble in tris buffer were also examined by high-voltage electrophoresis and by chromatography on Sephadex G-200 columns (Figs. 1 and 2). The proteins resolved by these methods were all found to be associated with neutral and amino sugars. The sugar components were recovered after hydrolysis with acid and two of them crystallized and identified. The present procedure followed the accepted method of recovering mono- and oligo-saccharides by chromatography on charcoal-Celite columns. For the isolation of glucosamine the great resolving power of the Hannig apparatus was utilized. This avoided the time-consuming and laborious desalting of the fractions obtained from ion-exchange resin columns as described by Pusztai (1964a). The glucosamine obtained after the removal of the residual contaminations by partition chromatography was of high purity.

The main neutral sugar component was also recovered and converted into its crystalline phenyl- and *p*-bromophenyl-hydrazones. The optical rotation and m.p. of its phenylhydrazone after two recrystallizations became constant and agreed well with some of the values described for D-mannose

phenylhydrazone in the literature (Neuberger, 1938), although its m.p. was about 12° lower than that given by Hofmann (1909) and Bates and Associates (1942) and the optical rotation after 1 hr. was also lower than the nearest value given for D-mannose phenylhydrazone (26.3° at 0 hr. changing to 6.3° after 9 hr.). The final optical rotation measured after 70 hr. (33.1°), however, showed a good agreement with the accepted value (33.8°; Butler & Cretcher, 1931). The m.p. of the crystalline *p*-bromophenylhydrazone agreed well with the value given by Neuberger (1938) and also with that of the reference compound prepared from D-mannose.

It is not unreasonable to assume that the main neutral sugar component of the glycoproteins from kidney bean is identical with D-mannose in spite of the disagreement with some of the values described for these compounds in the literature. These deviations might be explained by differences in crystal form, size of the crystals etc., which could affect the melting point of crystals. The lack of depression of melting point when these compounds were mixed with the appropriate reference compounds prepared from D-mannose also points to D-mannose as the main neutral sugar component of the glycoproteins.

On the basis of the evidence given above it is likely that a large part of the proteins of kidney beans are strongly associated with sugar residues and that most of them may well contain only D-glucosamine, probably *N*-acetylated, and D-mannose as their sugar constituents. This type of glycoprotein may be similar to those from the animal kingdom, like egg albumin (Neuberger, 1938) or plasma glycoproteins (Winzler, 1960). There is, however, no direct evidence as yet that these sugar residues are covalently linked to amino acids as in egg albumin (Fletcher, Marshall & Neuberger, 1963) or in submaxillary mucoprotein (Graham, Murphy & Gottschalk, 1963). The results of the extraction, denaturation, precipitation and enzymic-digestion experiments nevertheless indicated a firm link between sugar and amino acid residues and not a loose connexion only, as for certain plant glycoproteins previously described (Rigas & Osgood, 1955; Rohrlisch, 1962). There is still a possibility that glycoproteins of other types may also exist in kidney beans. The small but definite amounts of arabinose and galactose may indicate the existence of such compounds, although the possibility that these sugars were contained in materials not removed completely from these glycoprotein preparations cannot be entirely ruled out. The use of phenol for their preparation, however, makes it unlikely that significant amounts of contaminations of polysaccharide nature were present.

Although the present paper deals with the seeds of kidney beans only, the occurrence of glyco-

proteins containing glucosamine is probably not confined to this particular example of the higher plants. All seed samples investigated previously (Pusztai, 1964a,b) contained various amounts of protein-bound glucosamine, and so these findings described for kidney beans may have general significance.

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