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ISOLATION AND CHARACTERIZATION OF THE LECTINS FROM THE SEEDS OF *PSOPHOCARPUS SCANDENS*

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Key Word Index—*Psophocarpus scandens*; Leguminosae; lectin purification; acidic and basic lectins; chemical characterization; sugar specificity.

Abstract—The seeds of *Psophocarpus scandens* contain two distinct groups of lectins. The lectins adsorbed by melibiose Bio-Gel P150 yield two distinct components (lectins B1 and B2) on gel filtration on Ultrogel AcA 44. Both are glycoproteins (~9% carbohydrate) M_r ~62 000, composed of two non-covalently bound subunits with isoelectric points (pI range 5.9–6.7) much lower than the corresponding melibiose adsorbed lectins from *Psophocarpus tetragonolobus* (winged bean). Isoelectric focusing revealed the presence of subunit charge heterogeneity resulting in several isolectin forms. The *N*-terminal sequences of the lectins were unique and showed extensive homology with other *Psophocarpus* and legume lectins. The two lectins were distinguished by different carbohydrate and erythrocyte specificities. Lectin B1 was inhibited by α-D-galactosides, such as melibiose, but not β-D-galactosides, such as lactose, and it preferentially agglutinated trypsinized rabbit erythrocytes while trypsinized human type 0 erythrocytes were very weakly agglutinated. Lectin B2 was equally well inhibited by both α-D-galactosides and β-D-galactosides, and agglutinated trypsinized rabbit and human type (A, B, O) erythrocytes to the same extent.

The lectins not adsorbed by the melibiose Bio-Gel P150 were purified by chromatgraphy on Ultrogel AcA 44 and SP-Sephadex C-25 to yield three major components. These lectins are also glycoproteins (~6% carbohydrate), M_r ~60 000, composed of two subunits. SDS-PAGE gave subunits of varying sizes (M_r 32 500–35 500) which showed little charge heterogeneity. The amino acid compositions and *N*-terminal sequences showed that these lectins are closely related to the acidic lectins of *P. tetragonolobus*. Furthermore, their erythrocyte haemagglutinating and sugar specificities are very similar to those of the acidic lectins of *P. tetragonolobus*.

INTRODUCTION

The genus *Psophocarpus* contains some nine species [1] of which *P. tetragonolobus* (the winged bean) is used as a subsistence crop in a number of tropical countries [2]. The potential of the winged bean as a protein rich food source for the humid tropics has stimulated a wide range of research projects aimed at its development as a field crop [3, 4].

Two other species of *Psophocarpus*, *P. palustris* and *P. scandens*, are used as foods [5] and *P. scandens* has been used as a cover crop [5, 6]. Both *P. palustris* and *P. scandens* have edible pods and appear to be resistant to fungal diseases which attack *P. tetragonolobus*. At present, there is some confusion over the taxonomy of *Psophocarpus* in the literature with regard to the identity of the small leafed species [1, 5], it appears that species previously referred to as *P. palustris* were in fact *P. scandens*. *Psophocarpus scandens*, grown in Zaire, has been found to have the same food value as *P. tetragonolobus*; however, nothing is known about the seed proteins of this species of *Psophocarpus*.

The mature seeds of *P. tetragonolobus* have been shown to contain significant quantities of proteinase inhibitors [7, 8] and haemagglutinins [8]. The seed lectins of *P. tetragonolobus* have been isolated by affinity chromatography [9, 10] and their properties characterized. Recently, it was established that the seeds of *P. tetragonolobus* contain two distinct groups of lectins characterized by different isoelectric points (pI ~5.5 and >9.5), erythrocyte specificities and carbohydrate binding specificities [11–15].

Psophocarpus scandens seed extracts were found to contain a potent haemagglutinin activity. The present work describes the isolation, resolution and properties of the two types of lectin found in the mature seeds of *P. scandens* and compares their properties with the recently described *P. tetragonolobus* lectins.

RESULTS

Purification of the α-D-galactopyranosyl-specific lectins

Affinity chromatography, as described for the isolation of winged bean lectins [16], showed that the *P. scandens* seed extracts contained two types of lectins. Chromatography of *P. scandens* seed extracts on immobilized melibiose adsorbed quantitatively the α-D-galactopyranosyl-specific lectins (Fig. 1). The yield of lectin was 4.2 mg/g of defatted seed meal. Alkaline and SDS-PAGE analysis indicated that this lectin preparation was composed of two components. Gel filtration on Ultrogel AcA 44 (Fig. 2) showed that one of these components was retarded significantly by the agarose-matrix and two peaks, with different haemagglutinin activities (see Table 1), were resolved. Although the isoelectric points of these *P. scandens* lectins adsorbed by the immobilized melibiose were lower than the corresponding lectins from *P.*

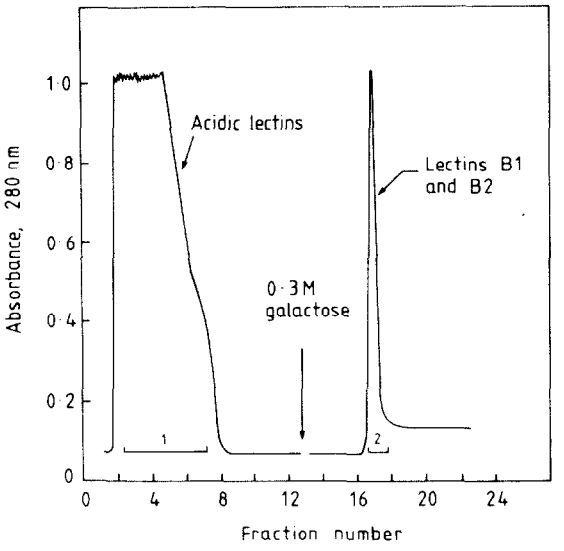

Fig. 1. Affinity chromatography of *P. scandens* seed extract on melibiose Bio-Gel P150. The column (6.0 × 1.5 cm) was equilibrated with PBS and 5.0 ml fractions were collected at a flow rate of 20 ml/hr. The adsorbed lectins were eluted with 0.3 M galactose in PBS applied as indicated by the arrow. Peaks 1 (fractions 2–7) and 2 (fractions 17, 18) were pooled as indicated by the bars.

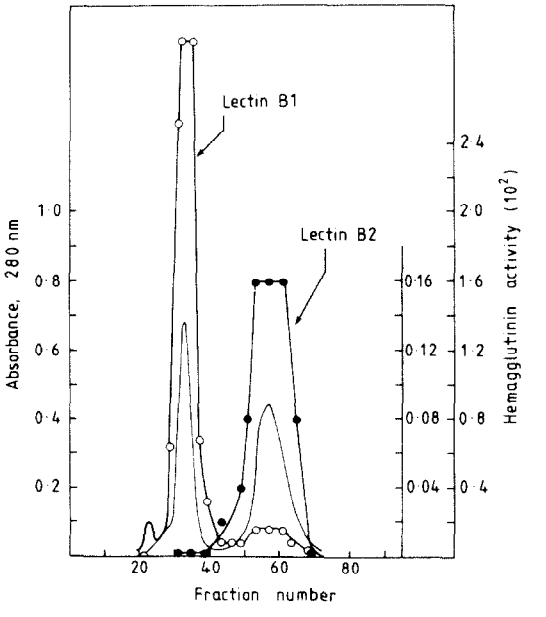

Fig. 2. Chromatography of the adsorbed lectin peak from Fig. 1 on Ultrogel AcA 44. The column (150 × 1 cm) was equilibrated with PBS and 1.6 ml fractions were collected at a flow rate of 6.4 ml/hr. ——, absorbance at 280 nm; o—o, haemagglutinin activity with trypsinized rabbit erythrocytes (outer right scale); ●—●, haemagglutinin activity with trypsinized human type O erythrocytes (inner right scale).

tetragonolobus, they were designated as (basic) lectins B1 and B2 (Fig. 2), respectively, according to the nomenclature used for the *P. tetragonolobus* α-D-galactopyranosyl-specific lectins.

Table 1. Minimum concentration of *P. scandens* lectins to give a positive haemagglutination reaction*

Erythrocyte type	Minimum concentration of lectin (μg/ml)		
	Lectin B1	Lectin B2	Acidic lectins
A	1.6	1.6	4.2
B	0.8	0.8	1.8
O(H)	25.0	0.8	0.5
Rabbit	0.1	3.1	>

*Trypsinized erythrocytes used.

On alkaline PAGE, lectin B1 migrated as a diffuse protein band while lectin B2 migrated as a single sharp protein band (Fig. 3a, lanes 1 and 2). On SDS-PAGE, lectin B1 migrated as a single protein band ($M_r \sim 32\,000$), in the absence and presence (not shown) of 2-mercaptoethanol (Fig. 3b, lane 1). Lectin B2 also yielded a major protein band of M_r 32 000, with a minor component of $M_r \sim 64\,000$, in the absence and presence of 2-mercaptoethanol (Fig. 3b, lanes 2 and 4). However, treatment of lectin B2 with SDS at room temperature prior to loading yielded a protein band of $M_r \sim 60\,000$ with no evidence of the higher M_r minor component (Fig. 3b, lane 3). This suggests that on denaturation by heating in SDS some of the lectin B2 subunits interact to form a new dimeric species which is different from the species observed (presumably undissociated dimer) when the sample is just dissolved in the SDS buffer. For comparison, lanes 5 and 6 (Fig. 3b) show the behaviour of the *P. tetragonolobus* lectins on SDS-PAGE.

Purification of the β-D-galactopyranosyl specific lectins

The haemagglutinin activity in the *P. scandens* extracts not adsorbed to the melibiose Bio-Gel P150 (peak 1, Fig. 1) was not adsorbed by lactose-Sepharose 6B. Gel filtration of this material (peak 1, Fig. 1) on Ultrogel AcA 44 resolved several components and the haemagglutinin activity was associated with only a single protein peak (Fig. 4). Ion-exchange chromatography on SP-Sephadex C-25 of this active material separated a broad protein peak with haemagglutinin activity as shown in Fig. 5 from several inactive components. Alkaline PAGE of fractions across this peak revealed the presence of three distinct protein components. By selective pooling of fractions containing haemagglutinin activity, as indicated by the gel results (Fig. 5), two of the three proteins were isolated essentially as single components (Fig. 6a, lanes 1 and 6). The third protein, however, contained minor amounts (< 10%) of the other two proteins (Fig. 6a, lane 3). These were designated as *P. scandens* (acidic) lectins A1, A2 and A3, respectively, as indicated in Fig. 5.

SDS-PAGE (Fig. 6b) showed that these three lectins were composed of four types of subunits with apparent M_r's of 32 500 33 500, 34 500 and 35 500 respectively. Reduction with 2-mercaptoethanol did not alter this subunit composition. Lectin A1 was composed of the two lower M_r subunits while lectin A3 contained the two higher M_r subunits; lectin A2 contained all four subunits (Fig. 6b) suggesting that it is a mixture of heterodimers composed of the subunits observed for lectins A1 and A2.

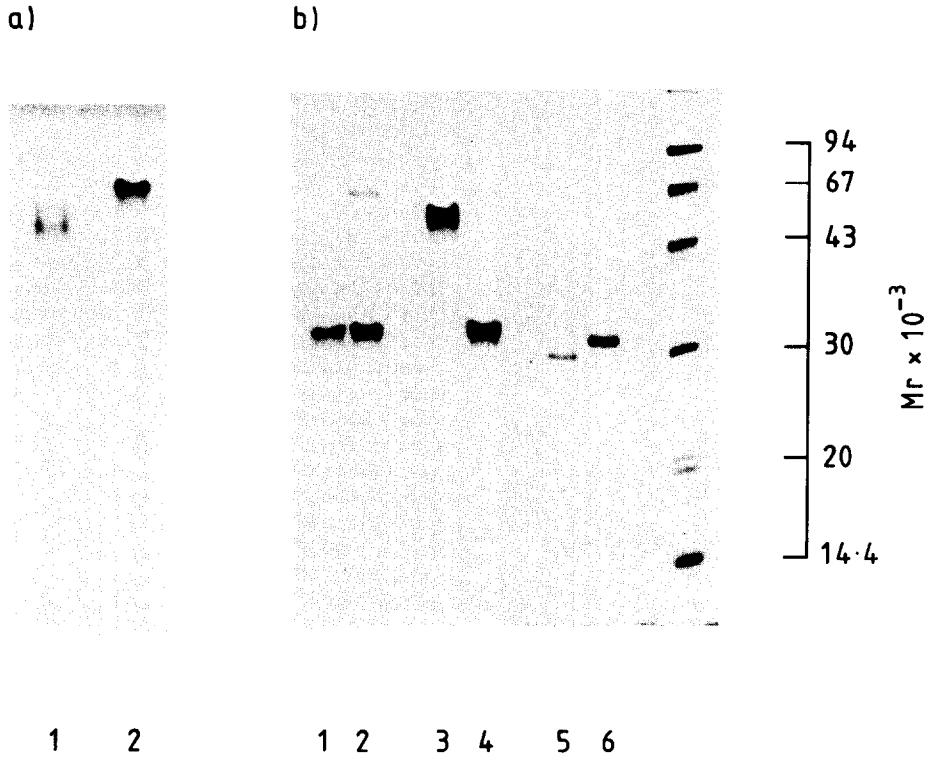

Fig. 3. (a) PAGE of *P. scandens* lectins B1 and B2 at pH 8.8. (1) lectin B1 (peak 1,Fig. 2); (2) lectin B2 (peak 2, Fig. 2); (b) SDS-PAGE of *P. scandens* lectins. (1) lectin B1; (2) lectin B2; both in the absence of 2-mercaptoethanol; (3) lectin B2 dissolved in 1% (w/v) SDS–0.05 M Tris–HCl, pH 8.0, immediately prior to loading sample; (4) lectin B2 reduced with 2-mercaptoethanol; (5) *P. tetragonolobus* basic lectins; (6) *P. tetragonolobus* acidic lectins both unreduced.

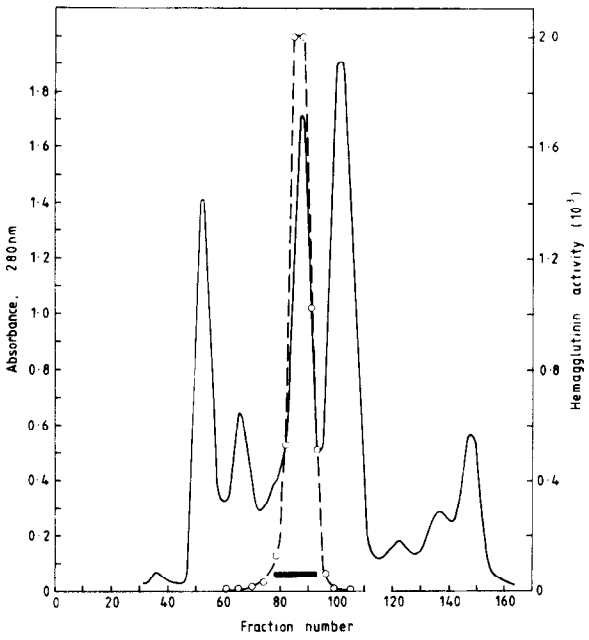

Fig. 4. Chromatography of fraction 1 (Fig. 1) on Ultrogel AcA 44. The column (150 × 2 cm) was equilibrated with PBS and 4.5 ml fractions were collected at a flow rate of 18 ml/hr. The active fractions, as indicated by the bar, were pooled. ——, *A* at 280 nm; o—o, haemagglutinin activity with trypsinized human type O erythrocytes.

Isoelectric focusing of P. scandens *lectins*

On isoelectric focusing *P. scandens* lectins A1 and A3 each showed the presence of a single major component with a pI of ~4.7 while lectin A2 appeared to be a mixture of these components (Fig. 7a), consistent with the suggestion that lectin A2 is a heterodimer of the subunits observed in lectins A1 and A3. Lectin B1 showed 3–4 protein bands (isolectins) with pI's in the range 5.9–6.2 while lectin B2 showed three to four bands with pI's in the range 6.2–6.7 (Fig. 7a). On isoelectric focusing in 8 M urea lectin A1 and A3 protomers each yielded a single subunit polypeptide (data not shown) indicating that the subunits observed on SDS-PAGE show essentially no charge heterogeneity; in contrast the *P. scandens* lectin B1 and B2 protomers yielded two major and one to two minor polypeptide chains (Fig. 7b) indicating considerable subunit charge heterogeneity. The difference in isoelectric points between the *P. scandens* α-D-galactopyranosyl-specific, lectins B1 and B2, and the *P. tetragonolobus* α-D-galactopyranosyl-specific (basic) lectins is illustrated in Fig. 7b.

Estimation of relative molecular mass

Gel filtration of the affinity isolated fraction (lectins B1 and B2) on a calibrated Sephadex G-100 (150 × 1 cm) column gave an apparent $M_r \sim 60000$ while on an Ultrogel AcA 44 (150 × 1 cm) column lectin B2 was markedly retarded by the agarose gel matrix; when the Ultrogel AcA 44 column was run in PBS/0.3 M galactose

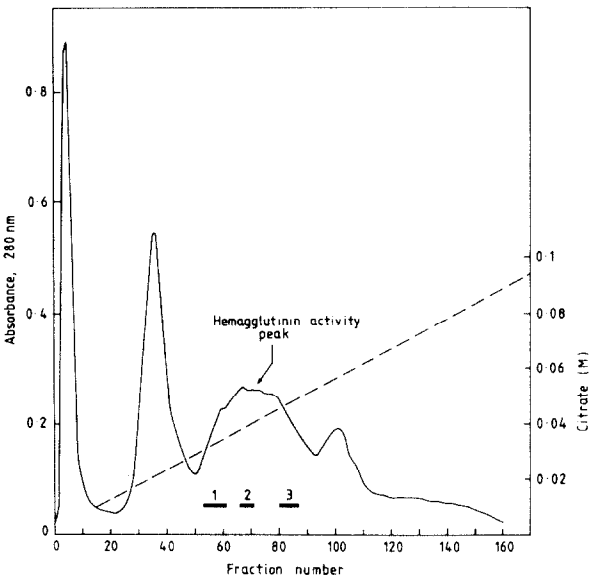

Fig. 5. Chromatography of the protein peak with haemagglutinin activity from Fig. 4 on SP-Sephadex C-25. The column (20 × 1 cm) was equilibrated with 0.01 M citrate buffer, pH 5.1. The active fraction (150A280 units) was dialysed against the starting buffer and applied to the column. A linear gradient of 0.01–0.1 M citrate, pH 5.1, over 800 ml was applied at fraction 15 and 4.5 ml fractions were collected at a flow rate of 18 ml/hr. Fractions 50–85, eluting between 0.02 and 0.045 M citrate, contained the haemagglutinin activity and the active fractions were pooled, after electrophoretic analysis, as follows: 53–61; 62–65; 66–71; 72–75; 76–79 and 80–87. The labelled bars refer to lectin A1, A2 and A3 respectively. , *A* at 280 nm.

both lectins B1 and B2 co-eluted with a $M_r \sim 60\,000$. The M_r of lectin B2 was also determined in the ultracentrifuge. The protein was homogeneous and a M_r of $62\,000 \pm 2000$ was found. The M_r's of 32 000 estimated by SDS-PAGE indicated that both lectins are dimers of non-covalently bound subunits.

Gel filtration of *P. scandens* acidic lectins on the calibrated Ultrogel AcA 44 column yielded a single peak of apparent $M_r \sim 60\,000$; SDS-PAGE showed three to four subunits of different size in the range of M_r 32 500–35 500. These results indicate that the acidic lectins are dimers of non-covalently bound subunits and that several isolectin forms may arise from the association of the different subunits to form the dimer.

Amino acid and carbohydrate compositions of P. scandens *lectins*

The amino acid compositions of the three *P. scandens* acidic lectins showed no significant differences (Table 2). They contain no half-cystine and have two methionine residues per subunit. Comparison of the composition of the *P. scandens* acidic lectins (expressed as residues per 100 residues) with that of affinity isolated *P. tetragonolobus* acidic lectins [16] shows that these lectins from the two species are similar in composition (Table 2).

In contrast, lectins B1 and B2 show some differences in their compositions, especially in the basic residues (Table 3). These lectins contain no half-cystine; lectin B1 has no methionine while lectin B2 contains two residues of methionine per subunit ($M_r \sim 32\,000$). A comparison of the compositions of *P. scandens* lectins B1 and B2 with the affinity isolated *P. tetragonolobus* basic lectins (Table 3) shows that they are similar.

Carbohydrate analyses showed that the *P. scandens* lectins were glycoproteins. The acidic lectins had a total carbohydrate content of 5–6% while lectins B1 and B2 had a total carbohydrate content of ~9%. The analyses showed that lectins A1 and A2 contain two GlcNAc residues and seven to eight mannose residues per subunit chain with only traces of fucose and xylose; lectin A3, however, contains two GlcNAc residues and only ~4 mannose residues per subunit with a relatively high content of glucose (Table 4). Lectins A1 and A2 also contain some glucose, a sugar found only in trace amounts in *P. scandens* lectins B1 and B2. Whether the glucose is part of the normal carbohydrate structure or bound to the protein independently via an ε-amino group of lysine is not known. The *P. tetragonolobus* acidic lectins also contain comparable amounts of glucose [16].

In contrast, *P. scandens* lectins B1 and B2 contain four GlcNAc and eight to nine mannose residues per subunit chain with several residues of fucose and xylose (Table 4). If the carbohydrate is linked to the protein via GlcNAc-GlcNAc-Asn, as for other plant lectins [17], then the acidic lectins probably contain one carbohydrate chain per subunit ($M_r \sim 32\,000$) while lectins B1 and B2 probably contain two carbohydrate chains per subunit ($M_r \sim 32\,000$). A similar distribution of carbohydrate chains per subunit was found for the acidic and basic lectins of *P. tetragonolobus* [11, 12, 16].

Amino-terminal sequence of P. scandens *lectins*

The amino-terminal sequences of the *P. scandens* lectins are summarized in Fig. 8. Lectins A1 and A3 contain only three changes in the first 15 residues suggesting that these proteins may be highly homologous. The amino-terminal sequences of lectins B1 and B2 show more variation but are still clearly homologous with the *P. scandens* acidic lectins and the previously characterized *P. tetragonolobus* lectins (Fig. 8). Residues 5–8 and residue 11 have been conserved in this family of *Psophocarpus* lectins. As noted before, this homology in the amino-terminal region of the lectins of the *Psophocarpus* genus and the other legume lectins is in agreement with the proposal that all Leguminosae lectins have evolved from a common ancestral precursor [18].

Agglutination specificity

The *P. scandens* lectins have different erythrocyte specificities (Table 1). Lectin B1 strongly agglutinated rabbit erythrocytes and human type A and B erythrocytes to a lesser extent, while human type O erythrocytes were only weakly agglutinated. In contrast, lectin B2 agglutinated rabbit and human type A, B and O erythrocytes with about the same efficiency. The *P. scandens* acidic lectins agglutinated human type A, B and O erythrocytes but not rabbit erythrocytes (Table 1). Trypsin treatment of the erythrocytes increased the haemagglutinin titre four-fold.

The inhibition of the haemagglutinating activity of *P. scandens* lectins by a number of sugars is given in Table 5. Lectin B1 agglutination was inhibited by D-galactose and related monosaccharides with a clear preference for the α-

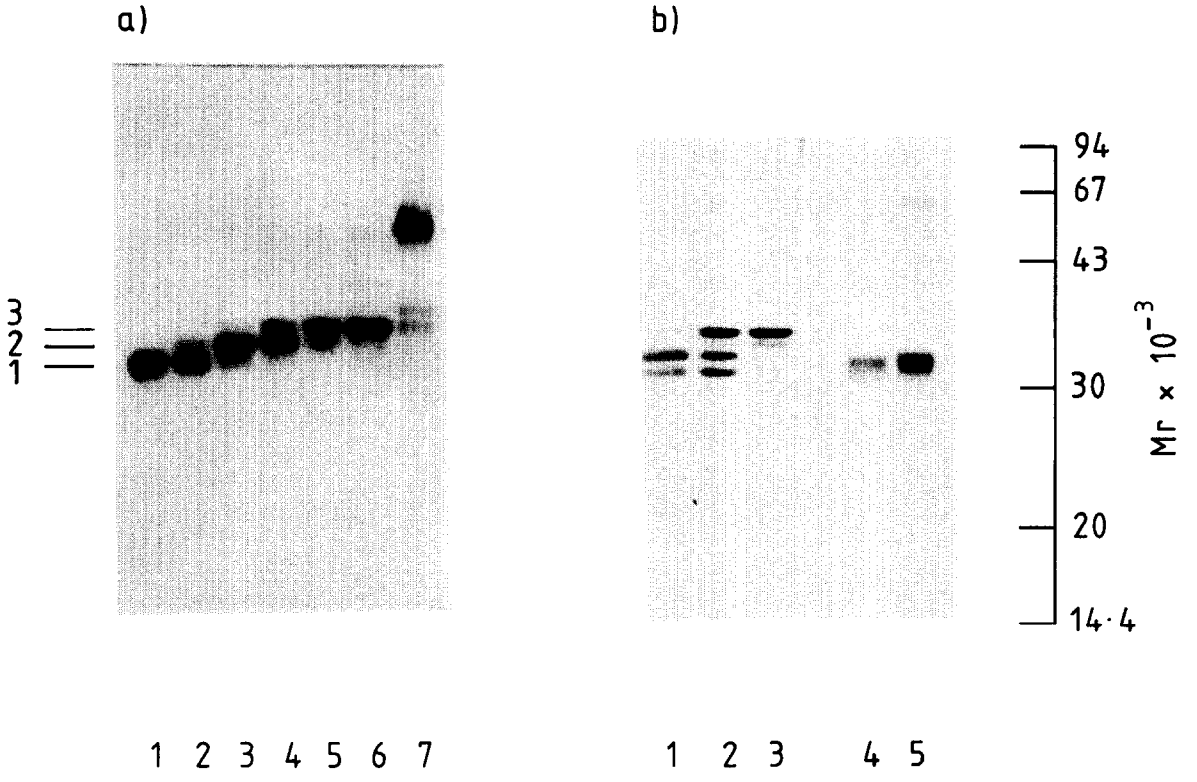

Fig. 6. (a) PAGE of *P. scandens* acidic lectin fractions at pH 8.8 from Fig. 5. (1) lectin A1 (fractions 53–61); (2) fractions 62–65; (3) lectin A2 (fractions 66–71); (4) fractions 72–75; (5) fractions 76–79; (6) lectin A3 (fractions 80–87); (7) fractions 95–110. (b) SDS-PAGE of *P. scandens* lectins. (1) lectin A1; (2) lectin A2; (3) lectin A3; (4) lectin B1; (5) lectin B2.

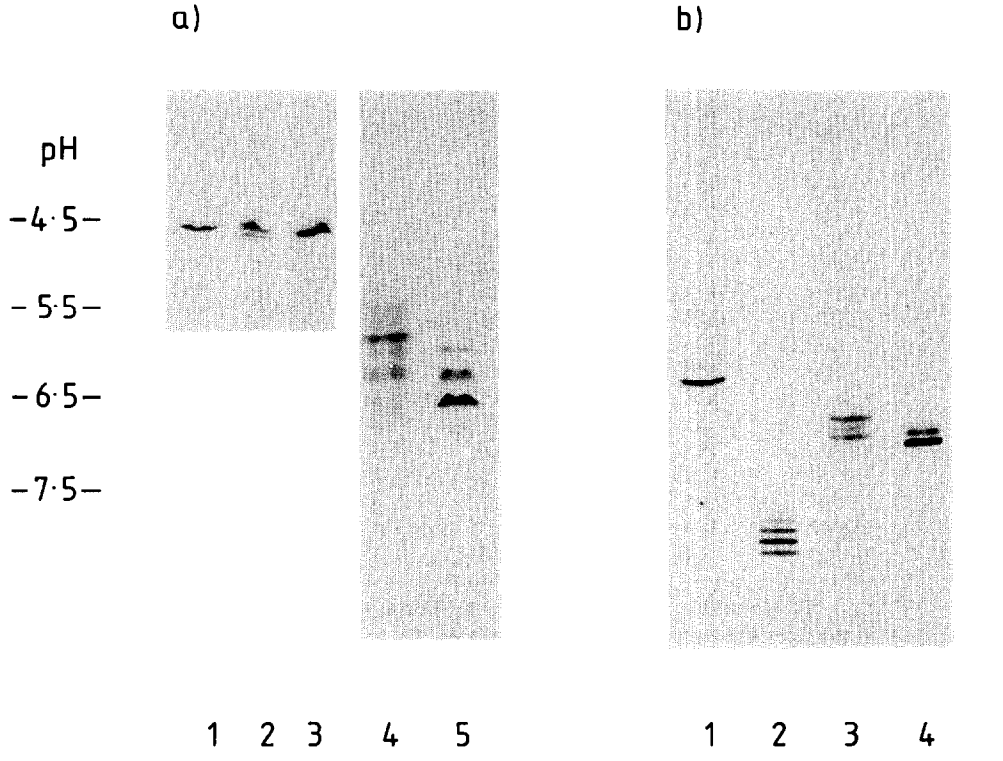

Fig. 7. (a) Isoelectric focusing of *P. scandens* lectins on Ampholine polyacrylamide gel plates (pH 3.5–9.5 gradient) of (1) lectin A1; (2) lectin A2; (3) lectin A3; (4) lectin B1; (5) lectin B2; (b) Isoelectric focusing on a polyacrylamide gel slab in 8 M urea of (1) *P. tetragonolobus* acid lectins, affinity purified; (2) *P. tetragonolobus* basic lectins, affinity purified; (3) *P. scandens* lectin B1; (4) *P. scandens* lectin B2.

Table 2. Amino acid compositions of *P. scandens* acidic lectins

	Residues/subunit*			Residues/100 residues	
	A1	A2	A3	A1	*P. tetragonolobus*† acidic lectins
Lys	13.7	14.4	14.1	5.0	4.8
His	4.7	4.9	5.0	1.7	2.2
Arg	4.8	4.4	4.9	1.8	1.9
Asp	39.2	38.6	37.5	14.3	12.5
Thr	22.9	25.6	23.3	8.4	7.3
Ser	20.5	21.1	18.8	7.5	7.5
Glu	26.5	27.4	27.3	9.7	10.6
Pro	18.9	17.9	18.9	6.9	6.0
Gly	20.4	21.1	19.9	7.5	9.2
Ala	17.5	16.5	17.3	6.4	6.4
½Cys	0	0	0	0	0
Val	19.6	19.8	19.6	7.2	8.8
Met	1.3	0.9	1.2	0.5	0.5
Ile	16.3	15.4	17.5	6.0	4.6
Leu	22.0	21.4	21.6	8.0	7.2
Tyr	7.6	7.2	8.0	2.8	2.5
Phe	17.8	17.3	18.5	6.5	6.4
Trp	3.5	3.2	3.1		

*Results are based on a subunit M_r of 30 000 with 6% carbohydrate. Values are from 24 hr hydrolysates and are uncorrected.

†Taken from ref. [16].

Table 3. Amino acid compositions of *P. scandens* lectins B1 and B2

	Residues/subunit*		Residues/100 residues		
	B1	B2	B1	B2	*P. tetragonolobus*† basic lectins
Lys	10.6	13.8	3.8	4.8	3.8
His	7.5	5.7	2.7	2.0	2.2
Arg	8.6	7.8	3.1	2.7	3.4
Asp	35.2	32.8	12.6	11.3	12.3
Thr	21.9	25.4	7.9	8.8	8.4
Ser	20.7	21.3	7.4	7.4	7.8
Glu	21.7	26.6	7.8	9.2	7.8
Pro	18.5	20.2	6.6	7.0	8.1
Gly	24.0	21.6	8.6	7.5	7.9
Ala	21.0	22.9	7.5	7.9	6.5
½Cys	0	0	0	0	0
Val	23.8	23.9	8.5	8.3	7.7
Met	0	1.8	0	0.6	0
Ile	15.5	14.2	5.6	4.9	6.4
Leu	17.4	18.1	6.3	6.3	6.4
Tyr	6.9	7.0	2.5	2.4	2.6
Phe	21.4	22.5	7.7	7.8	7.1
Trp	3.9	3.6	1.4	1.2	1.3

*Results are based on a subunit M_r of 30 000 with 9% carbohydrate. Values are from 24 h hydrolysates and are uncorrected.

†Taken from ref. [16].

anomer over the β-anomer. Glycosides of D-galactose with an aromatic aglycone were better inhibitors than those with alkyl aglycones. The α-galactosides, melibiose, raffinose and stachyose were excellent inhibitors whereas the β-galactosides such as lactose and 3-*O*-β-galactopyranosyl-D-arabinose, for example, were poor inhibitors. Similar results were obtained with both trypsinized rabbit and human type A erythrocytes. Lectin B2 agglutination of trypsinized rabbit and human type A erythrocytes was also inhibited by glycosides of D-galactose. However, in

Table 4. Carbohydrate composition of *P. scandens* lectins

	Composition (mol/mol)				
Monosaccharide*	A1	A2	A3	B1	B2
Fucose	0.5	0.4	0.3	2.3	3.0
Xylose	1.2	0.7	0.2	1.8	2.6
Mannose	14.2	13.2	7.2	18.2	16.2
Galactose	1.2	0.2	0.3	0.4	0.6
Glucose	2.8	1.9	6.9	0.7	0.5
N-Acetylglucosamine	4.0	4.0	4.0	8.0	8.0
% CHO	6.0	6.3	4.3	8.8	8.8

*The monosaccharides were determined by gas chromatography as the alditol acetate derivatives. The value for *N*-acetylglucosamine is based on the glucosamine content determined after hydrolysis of the protein in 3 M methanesulphonic acid for 24 hr. The results are based on a M_r of 60000.

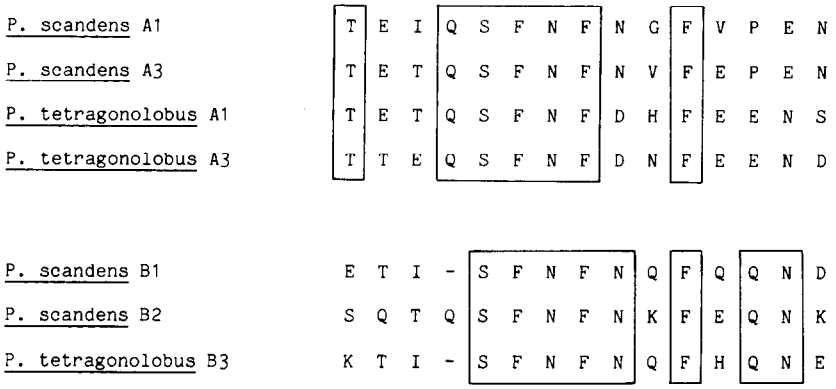

Fig. 8. Amino-terminal sequences of *P. scandens* lectins A1, A3, B1 and B2 compared with the sequences of *P. tetragonolobus* acidic lectins A1 and A3 [12], and basic lectin B3 [11].

contrast to lectin B1 there was no clear preference for either the α- or the β-anomer (Table 5) and both α- and β-galactosides were excellent inhibitors.

The erythroagglutination of *P. scandens* acidic lectins was also inhibited by glycosides of D-galactose and only a slight preference for the β-anomer over the α-anomer was noted (Table 5). The *P. scandens* acidic lectins have much lower affinities for the inhibitory sugars than *P. scandens* lectins B1 and B2, and in this regard they closely resemble the *P. tetragonolobus* acidic lectins [12]. While the affinity of the *P. scandens* acidic lectins for lactose is similar to that found for the *P. tetragonolobus* acidic lectins, it is interesting to note that the *P. scandens* acidic lectins did not bind to lactose-Sepharose whereas the *P. tetragonolobus* acidic lectins did [16]. This suggests that non-specific binding of the *P. tetragonolobus* acidic lectins to the lactose-Sepharose affinity matrix may contribute to their binding.

DISCUSSION

The seeds of *P. scandens*, like those of *P. tetragonolobus*, contain two distinct groups of lectins characterized by different erythrocyte specificities. The *P. scandens* acidic lectins (pI ~4.7) resemble the *P. tetragonolobus* acidic lectins (pI ~5.5) with regard to their erythrocyte and sugar specificities, as well as their amino acid compositions and amino-terminal sequences. The acidic lectins from both species of *Psophocarpus* are preferentially inhibited by β-galactosides, such as lactose, with comparable binding affinities, but unexpectedly the *P. scandens* acidic lectins fail to bind to lactose-Sepharose under conditions which quantitatively adsorb the *P. tetragonolobus* acidic lectins. A notable difference between the acidic lectins of *P. scandens* and *P. tetragonolobus* is in their protomer and subunit M_rs. The M_r of the *P. scandens* acidic lectins (M_r ~60000) is greater than that reported for the *P. tetragonolobus* acidic lectins (M_r ~54000) [12, 15], and the *P. scandens* lectins A1, A2 and A3 are composed of four discrete subunits of different apparent M_r's (32400, 33500, 34500 and 35500) compared with the single subunit of apparent M_r ~32000 of the *P. tetragonolobus* acidic lectins. While the amino-terminal sequence and amino acid composition data indicate that the subunits of the acidic lectin protomers are similar, more structural information is required to elucidate their apparent differences in size as seen on SDS-PAGE which may be due to variation in polypeptide chain length, sequence differences or carbohydrate content. SDS-PAGE analyses have shown that the protomers of soybean lectin [19] and *Erythrina cristagalli* lectin [20] are also composed of subunits of slightly different size with no evidence of sequence heterogeneity.

Table 5. Inhibition of haemagglutinating activity of *P. scandens* lectins by various sugars

	Minimum inhibitory concentration (mM)*				
	Lectin B1		Lectin B2		Lectin A1
N-Acetyl-D-galactosamine	0.2†	0.8‡	0.2†	0.8‡	12.5‡
D-Galactose	3.9	7.8	2.0	7.8	62.5
D-Galactosamine	7.8	15.6	7.8	7.8	250.0
D-Fucose	15.6	62.5	7.8	7.8	125.0
Methyl-α-D-galactoside	2.0	15.6	2.0	7.8	62.5
Methyl-β-D-galactoside	15.6	31.3	7.8	7.8	62.5
p-Nitrophenyl-α-D-galactoside	0.1	0.8	0.8	1.6	12.5
p-Nitrophenyl-β-D-galactoside	0.4	3.1	0.4	0.8	6.2
Thiodigalactoside	3.9	15.6	2.0	3.9	15.6
Melibiose	0	3.9	1.0	7.8	31.3
Raffinose	2.0	7.8	2.0	7.8	62.5
Stachyose	3.9	31.3	3.9	31.3	250.0
Lactose	250.0	250.0	15.6	3.9	15.6
Lactobionic acid	125.0	>250.0	3.9	0.5	15.6
Lactulose	>250.0	>250.0	15.6	7.8	15.6
3-*O*-β-Galactopyranosyl-D-arabinose	>250.0	>250.0	3.9	3.9	31.3

*The concentration of lectin was such that two two-fold dilutions would give the end point agglutination in the absence of inhibitors. Trypsinized rabbit† and human type A‡ erythrocytes were used. Basic lectin 2 yielded the same values with trypsinized human type O erythrocytes as obtained with the type A. The 3 acidic lectins gave the same values with trypsinized human type A and O erythrocytes and results only for acidic lectin 1 are shown. The following sugars were not inhibitory at 0.5 M; methyl-α-D-glucoside, *N*-acetylglucosamine, glucose methyl-α-D-mannoside and L-fucose.

The second group of lectins present in the seeds of *P. scandens*, lectins B1 and B2, share some properties in common with the *P. tetragonolobus* basic lectins but are quite distinct in some other properties. These *P. scandens* lectins which are adsorbed by immobilized melibiose have lower isoelectric points (pI range 5.9–6.7) than the *P. tetragonolobus* basic lectins (pI > 9.5) and are composed of two distinct components (lectins B1 and B2) with different erythrocyte and sugar specificities. While both lectins are effectively inhibited by α-galactosides, such as melibiose, lectin B2 is also effectively inhibited by β-galactosides, such as lactose, which does not inhibit lectin B1. This difference in sugar specificity enables these two lectins to be quantitatively separated by gel filtration on Ultrogel AcA 44, an agarose gel matrix, which interacts preferentially with β-galactoside specific lectins. A comparison of the erythrocyte and sugar specificities shows that *P. scandens* lectin B1 resembles the *P. tetragonolobus* basic lectins whereas *P. scandens* lectin B2 has no corresponding activity in the seeds of *P. tetragonolobus*.

Both *P. scandens* lectins B1 and B2, with native M_r's of ~62 000, are glycoproteins composed of two identical or nearly identical subunits. SDS-PAGE of these lectins yields a single subunit (in contrast to the *P. scandens* acidic lectins) which migrates with an apparent $M_r \sim 32\,000$, which is greater than the apparent $M_r \sim 29\,000$ observed for the subunits of *P. tetragonolobus* basic lectins. Carbohydrate analyses suggest that the higher carbohydrate content of lectins B1 and B2 may account for this difference in apparent M_r's. Isoelectric focusing of lectins B1 and B2 on polyacrylamide gel plates (pH 3.5–9.5 gradient) shows the presence of several isolectins and isoelectric focusing in 8 M urea establishes that charge heterogeneity at the subunit level accounts for the different isolectin forms. Similar results are observed with the *P. tetragonolobus* basic lectins.

Although the amino acid compositions of lectins B1 and B2 show some differences they are clearly related and comparison with *P. tetragonolobus* basic lectin B3 shows that these lectins from the two species of *Psophocarpus* are related in composition. Furthermore, the amino-terminal sequences of lectins B1 and B2 show extensive homology with the *P. scandens* acidic lectins, the *P. tetragonolobus* lectins and other legume lectins.

In summary, the seeds of the two species of *Psophocarpus* studied to date contain two distinct groups of lectins which can be distinguished on the basis of their specificity towards α- and β-galactosides, erythrocyte specificities and molecular properties. In addition *P. scandens* contains an extra lectin (lectin B2) not found in *P. tetragonolobus* seed which binds α- and β-galactosides with approximately equal affinity.

EXPERIMENTAL

The seeds of *Psophocarpus scandens* var. Matete were obtained from Dr J. J. Paulus (Dept. de Biologie, Campus Universitaire de Kinshasa, Kinshasa XI, Zaire). Immobilized melibiose (Selectin 4) was purchased from Pierce Chemical Co. and lactose-Sepharose 6B was prepared according to ref. [21]. Sugars were purchased from Sigma and British Drug Houses. The sources of other materials were as previously described [16].

Purification of the lectins. Finely ground seed meal (5 g), defatted with petrol, was extracted for 4 hr, at room temp. with 200 ml 0.05 M Tris–HCl–0.1 M NaCl, pH 8.0. The extract was clarified by centrifugation and the supernatant was concd (Amicon Diaflo, YM-10 membrane) to 40 ml. A 10 ml aliquot of the concentrated extract was applied to the melibiose Bio-Gel P150 (Selectin 4) column (6.0 × 1.5 cm) equilibrated with 0.01 M Pi–0.15 M NaCl, pH 7.1 (PBS); however, not all of the haemagglutinin activity in the extract was adsorbed by this affinity resin, even at lower loadings. The adsorbed activity was eluted

with 0.3 M galactose-PBS, dialysed exhaustively against deionized H_2O and the protein recovered by lyophilization. The melibiose Bio-Gel P150 adsorbed protein was rechromatographed on an Ultrogel AcA 44 column (150 × 1 cm) in PBS to separate two components with haemagglutinin activity (lectins B1 and B2).

The bulk of the extract (peak 1, Fig. 1) not adsorbed by the melibiose Bio-Gel P150 affinity column was reconcentrated and applied to an Ultrogel AcA 44 column (150 × 2 cm) in PBS. The fractions comprising the peak agglutinating trypsinized human (type O) erythrocytes (the acidic lectins) were pooled, concentrated, dialysed against 0.01 M citrate–0.01 M $CaCl_2$, pH 5.1 and rechromatographed on SP-Sephadex C-25. The chromatography experiments were performed at room temp.

Erythrocyte agglutination and inhibition assays. The erythrocyte agglutination activity was determined by measuring the ability of the lectin to agglutinate trypsinized human (type A, B and O) and trypsinized rabbit erythrocytes as described [11]. The sugar inhibition studies were performed as described [11].

Electrophoresis. Gel electrophoresis was carried out in 3 mm slabs [7.5% (w/v) polyacrylamide] at pH 8.8 [22]. SDS-PAGE was carried out in 3 mm slabs [12% (w/v) polyacrylamide] by the method of ref. [23]. Gels were run without reducing agent (2-mercaptoethanol) unless stated otherwise. Gel isoelectric focusing was carried out in the pH range 3.5–9.5 using Ampholine (LKB) polyacrylamide gel plates according to the manufacturer's instructions. Isoelectric focusing in 8 M urea was carried out in 0.8 mm thick slabs of 5% (w/v) polyacrylamide with 3% (v/v) Servalyt AGS 2-11 (Serva). The gels were prefocused for 1.25 hr at 200 V and run for 2 hr at 1000 V (at 4°). The pH gradients were measured with a surface electrode. Gels were soaked in 12% (w/v) trichloroacetic acid to wash out the carrier ampholytes prior to staining.

Amino acid and carbohydrate analyses. Amino acid compositions and carbohydrate compositions of the lectins were determined using methods previously described [16].

Amino-terminal sequence analyses. The amino-terminal sequences were determined in an Applied Biosystems sequencer using 1 nmol of protein and the phenylthiohydantoin (PTH) derivatives were identified by HPLC.

M_r *determination.* The M_r of the lectins was estimated on a calibrated Ultrogel AcA 44 column (150 × 1 cm) in PBS, with and without 0.3 M galactose. The M_r of *P. scandens* lectin B2 was determined in PBS by the meniscus depletion method of Yphantis [24] in a Beckman Model E ultracentrifuge equipped with Rayleigh interference optics. The partial specific volume of 0.73 ml/g was calculated from the amino acid composition [25]. The subunit M_r was estimated by SDS-PAGE using Pharmacia LMW standards.

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