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5 **PURIFICATION AND PARTIAL CHARACTERIZATION OF SEED LECTINS FROM *VICIAS***
6 **BELONGING TO SUBGENUS *VICILLA* SECTION *CRACCA*.**

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ABSTRACT.

Lectins from the seeds of ten *Vicias* belonging to section *Cracca* (*V. benghalensis*, *V. dasycarpa*, *V. monantha*, *V. villosa*, *V. cracca*, *V. vicioides*, *V. pseudocracca*, *V. disperma*, *V. tenuifolia* and *V. monardii*) have been purified by ultrafiltration and gel filtration chromatography, and characterized. All lectins belonged to the single-chain legume lectin group, having four subunits with molecular weights around 35-40 kDa, and a native molecular weight around 160-170 kDa. A dendrogram based on amino acid composition matched the grouping based on quaternary structure. Agglutination assays indicated that affinity for N-acetyl-galactosamine was more frequent than affinity for glucose. N-acetyl-galactosamine is abundant in animal proteins such as mucins, and is part of the Tn antigen that has been related with malignancy, metastasis and bad prognosis in cancer. Hence, lectins from these *Vicia* seeds may be of interest for targeting cancerous cells and other animal cells that expose N-acetyl-galactosamine in their glycocalix.

KEY WORDS. *Vicia*, Lectins, N-Acetyl-galactosamine.

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53 **1. INTRODUCTION.**

54 Lectins are defined as carbohydrate binding proteins of non-immune origin and
55 without any enzymatic activity. Many plant lectins have been purified and
56 characterized (Van Damme, Peumans, Barre & Rougé, 1998). The seeds of some
57 legumes are rich in lectins, which have been purified from commercial legumes such as
58 soybean, peanut, broad bean, pea, and lentil (Rüdiger & Gabius, 2001). Different
59 functions have been proposed for seed lectins, including defense against predators
60 (Vasconcelos & Oliveira, 2004; Vandenborre, Smagghe & Van Damme, 2011), acting as
61 storage proteins (Van Damme, Peumans, Barre & Rougé, 1998), and stabilization of
62 protein bodies (Rüdiger & Gabius, 2001). Lectins in legumes are classified into two
63 major groups according to their structure: the single-chain and two-chain lectin groups
64 (Loris, Hamelryck, Bouckaert & Wyns, 1998). Single-chain lectins are composed of four
65 identical or very similar peptides of around 30 kDa, while two chain lectins are made of
66 two light and two heavy subunits of around 6 and 17-20 kDa, respectively (Van
67 Damme, Peumans, Barre, & Rougé, 1998). Lectins in soybean and peanut belong to the
68 first group while those in lentil and pea belong to the second (Rüdiger & Gabius, 2001).

69 The *Vicia* genera include both single and double chain lectins. For instance,
70 lectins in *V. faba* and *V. ervilia* belong to the two-chain group (Hemperly, Hopp, Becker
71 & Cunningham, 1979; Fornstedt & Porath, 1975) while lectins in *V. villosa* (Grubhoffer,
72 Tichá & Kocourek, 1981), *V. unijuga* (Yanagi, Ohyama, Yamakawa, Hashimoto &

Ohkuma, 1990), and *V. graminea* (Prigent & Bourrillon, 1976) belong to the single-chain group. Sometimes both types of lectins are present in the same species, e.g. lectins in *V. cracca* (Karhi & Gahmberg, 1980; Baumann, Strosberg & Rüdiger 1982). Lectins also differ in their affinity for sugars. Thus, single-chain lectins show, in general, higher affinity for N-acetyl-galactosamine (GalNAc), while two-chain lectins prefer mannose and glucose. For instance, the *V. faba* two-chain lectin shows affinity for mannose and glucose, while the single-chain lectins in *V. villosa*, *V. graminea*, and *V. cracca* display a higher affinity for GalNAc (Van Damme *et al.*, 1998). The lectin from *V. villosa* is particularly well known because it has been sequenced (Osinaga, Tello, Batthyany, Bianchet, Tavares, Duran, Cerveñansky, Camoin, Roseto & Alzari, 1997) and its crystal structure has been determined (Babino, Tello, Rojas, Bay, Osinaga & Alzari, 2003). It shows affinity for GalNAc that is part of the Tn cancer antigen (Fuster & Esko, 2005), and the crystal structure of the lectin-Tn antigen complex has been analyzed (Babino, Tello, Rojas, Bay, Osinaga & Alzari, 2003).

The Tn antigen is formed by GalNAc glycosylation of hydroxyl groups belonging to serine or threonine residues in proteins. This antigen can be found in secreted proteins such as mucins (Hollingsworth & Swanson, 2004) and in cell surface proteins (Fuster & Esko, 2005). In healthy cells, GalNAc is capped by addition of other sugars, but in malignant cells the Tn antigen is not capped due to an incomplete elongation of the O-glycan. Different studies have reported a positive correlation between cancer aggressiveness and the presence of the Tn antigen (Springer, 1997). The lectin from *V. villosa* has been used to quantify the Tn antigen in sera from cancer patients (Osinaga, Babino, Grosclande, Cairoli, Batthyany, Bianchi, Signorelli, Varangot, Muse & Roseto, 1996; Knoska, Guerry, Caldefie-Chezet, De Latour & Guillot, 2006; Lee, Muthusamy,

Abdul-Rahman, Bhavanandan & Hashim, 2013). Recently, a label-free biosensor has been developed using the lectin from *V. villosa* for detection of the Tn antigen (Silva & Rangel, 2017). This lectin has also been used to discriminate between different types of neurons (Ojima, Kuroda, Ohyama & Kishi, 1995; Ichikawa, Osada, Ikai, 1992).

V. villosa is grown for feed and as green manure, and belongs to subgen. *Vicilla* sect. *Cracca* (Schaefer, Hechenleitner, Santos-Guerra, Menezes de Sequeira, Pennington, Kenicer & Carine, 2012). Other taxonomically related *Vicia*, also belonging to sect. *Cracca*, are of interest from an agricultural and nutritional point of view. Thus, *V. dasycarpa*, *V. monantha*, *V. benghalensis*, *V. cracca*, and *V. tenuifolia* are or have been cultivated for food, feed or as green manure. Taxonomically related to these species are the wild species *V. disperma*, *V. pseudocracca*, *V. monardii*, and *V. vicioides* (Romero-Zarco, 1999). These *Vicia* species may represent new sources of lectins with biochemical and technological applications similar to those described for the lectin isolated from *V. villosa*. The objective of this work was to purify and characterize the lectins from these nine *Vicia* species related to *V. villosa* in order to determine whether these lectins may also be of interest because of potential biochemical and technological applications.

2. MATERIAL AND METHODS.

2.1. Materials.

Bromophenol blue, coomassie brilliant blue G, diethyl ethoxy-methylene-malonate, D-L- α -aminobutyric acid, glutaraldehyde, sodium azide, trypsin, *Lens culinaris* lectin and *Canavalia ensiformis* lectin were from Sigma Aldrich. All other

reagents were of analytical grade. *V. villosa* seeds (INIA-1383) were provided by CRF-INIA (Madrid, Spain). All other *Vicia* seeds were collected from the wild in southern Spain.

2.2. Purification of lectins.

A suspension of seed flour in water 1/10 (w/v) was stirred for 30 minutes at room temperature while pH was kept at 4. The solid residue after centrifugation at 10,000 x g was extracted once more. The combined extracts containing the solubilized lectins were concentrated by ultrafiltration using an Amicon cell filtration unit with a 3 kDa cut-off membrane, and applied to a Superose 12 10/300 (GE Life Sciences) gel filtration column mounted on an AKTA Purifier system (GE Life Sciences). Injection volume was 1 mL, and 50 mM Na₂PO₄, 0.5 M NaCl pH 7 buffer was used as eluent. Elution of the lectins was followed by SDS-PAGE of the collected fractions (0.5 mL), and those that did not contain pure lectin were chromatographed again.

2.3. SDS-PAGE.

Protein extracts were adjusted to 2 mg protein / mL, mixed (1:1 v/v) with solubilisation buffer (80 mM Tris, 0.57% EDTA, 0.26% DTT, 3.3% SDS, 0.008% bromophenol blue, 20% sucrose, pH 6.8), and heated at 100 °C for 10 min. Tricine-SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Schagger and von Jagow (1987) at a constant voltage of 60 V for the stacking gel, and 120 V for the separation gel. Gels were fixed in 20% methanol, 8% acetic acid for 15 min before staining using 0.25% coomassie brilliant blue G in 45% methanol 10% acetic acid. Molecular masses were determined using the low molecular weight standards from GE Healthcare Life Sciences.

2.4. Native PAGE.

Native PAGE was carried out using the Mini-PROTEAN TGX (4-20 %) precast gels from BIO-RAD (CA, U.S.A.). Protein extracts were adjusted to 2 mg protein / mL, mixed (1:1 v/v) with solubilisation buffer (running buffer containing 0.5% bromophenol blue, 20% sucrose). Native PAGE was performed at a constant voltage of 200 V with 25 mM Tris, 190 mM glycine pH 8 as running buffer. Gels fixation and staining was as for SDS-PAGE. Molecular masses were determined using the high molecular weight standards from Pharmacia LKB Biotechnology.

2.5. Amino acid analysis.

Samples were hydrolyzed by incubation in 6 N HCl at 110 °C for 20 h. Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC) according to the method described by Alaiz, Navarro, Girón, & Vioque (1992) using D-L- α -aminobutyric acid as internal standard. Samples (20 μ L) were injected in a reversed-phase column (Novapack C₁₈, 300 x 3.9 mm i.d., 4 μ m, Waters) that was kept at 18 °C. Tryptophan was determined by HPLC after basic hydrolysis according to Yust, Pedroche, Girón-Calle, Vioque, Millán & Alaiz (2004).

2.6. Erythrocytes agglutination assay.

Glutaraldehyde-fixed, trypsinized rat erythrocytes for the agglutination assays were prepared as follows. The pellet resulting from centrifugation of rat blood at 500 g for 10 min was washed three times by resuspension in PBS and centrifugation (500 g 10 min). The resulting pellet was resuspended 1/10 (v/v) in PBS containing 0.5% (v/v) glutaraldehyde and incubated in a shaker for 1 hour at room temperature. Erythrocytes were recovered by centrifugation and washed with PBS three times as

previously described, and were resuspended in PBS (20% v/v) containing sodium azide (0.1% w/v). The fixed erythrocytes (2 mL) were trypsinized by incubation with trypsin (2 mg) for 30 min at 37 °C, and washed with PBS three times as described above.

Agglutination assays were carried out by incubating these trypsinized, glutaraldehyde fixed rat erythrocytes (40 µL, 4 % w/v in PBS buffer) in the presence of increasing concentrations of lectin in 96 U shape well microplates for 1 hour at room temperature. Agglutinating activity was categorized as negative or positive for each well. *C. ensiformis* lectin (1 µg / well) was used as a positive control, and incubations of erythrocytes with no lectin added were used as negative control. Minimum lectin concentration that yield agglutination was used to assay agglutination inhibition by sugars. Decreasing amounts of galNAc and glucose were employed to determine the minimum concentration of sugar that inhibits the agglutination by lectins.

2.7. Statistical analysis.

Cluster analysis of *Vicia* lectins was performed according to the furthest neighbor method based on a euclidean distance matrix using the Stagraphics 5.1 software.

3. RESULTS AND DISCUSSION.

3.1. Purification of *Vicia* lectins.

Lectins in leguminous seeds are albumins according to the classic Osborne protein classification because they are soluble in plain water, in contrast to storage proteins (globulins) that are soluble in saline solutions but not in water. Hence, lectins in *Vicia* seeds were extracted in water at pH 4, corresponding to the isoelectric point of globulins. Figure 1A illustrates these differences in solubility by showing as an example

two different extracts from one of the *Vicia* species that were used for extraction of lectins, *V. benghalensis*. While extraction using SDS-PAGE running buffer (figure 1, left line) yields the most intense protein bands in the molecular weight range between 43 and 94 kDa corresponding to globulins, extraction in water at pH 4 (figure 1, right line) yields peptides around 30 kDa corresponding to lectins.

Because components that could interfere with the agglutination assay are also extracted with water, all lectin extracts were further purified by gel filtration chromatography. These potentially interfering components include polyphenols and sugars. Thus, soluble sugars can inhibit lectin agglutination by binding to the lectin active site, while polyphenols can actually have agglutinating activity by themselves which might lead to overestimate agglutination (Cortés-Giraldo, Girón-Calle, Alaiz, Vioque & Megías, 2012). Water extracts were applied to a gel filtration Superose 12 column, and fractions were pooled and concentrated to yield a chromatographically pure lectin preparation as shown in figure 1B for *V. benghalensis*.

The molecular weight of the purified lectin subunits is around 35-40 kDa, indicating that they belong to the single-chain legume lectin group (Figure 2A). This confirms previous reports concerning *V. villosa* (Grubhoffer, Tichá & Kocourek, 1981) and *V. cracca* (Rüdiger, 1977). *V. villosa* and *V. monardii* showed two different bands corresponding to 37 and 40 kDa, and 35 and 36 kDa, respectively. Native PAGE was carried out in order to further confirm the single-chain nature of the lectins (Figure 2B). A similar molecular weight of around 160-170 kDa was found in all lectins, similar to that reported for other single-chain lectins in *Vicia* (Grubhoffer, Tichá & Kocourek, 1981; Rüdiger, 1977).

The amino acid composition of the purified lectins shows that the most abundant residues in these proteins are Asx, Glx, Thr, Lys, and Leu (Table 1). The last column represents the mean amino acid composition for these ten lectins, and is plotted in figure 3 as compared to the mean amino acid composition of a group of two-chain lectins that were purified from the seeds of *Pisum sativum*, *Lens culinaris*, *Vicia faba*, *Lathyrus sativus*, and *Lathyrus cicera* (unpublished results). All these legumes are included, together with *Vicia*, in Tribe *Fabeae*. Significant differences ($P < 0.001$) were observed in the amounts of Asp, Ser, His, Gly, Thr, Arg, Ala, Val, Met, Ile, Trp and Lys between the two types of legume lectins. These differences between the single-chain and two-chain lectins were highlighted in a dendrogram that was constructed based on the amino acid composition using the furthest neighbor method (Figure 4). According to this dendrogram, the purified *Vicia* single-chain lectins are grouped in a single clade, clade A, while the two chain lectins of other legumes belonging to Tribe *Fabeae* are arranged in clade B. Clade C includes taxonomically distant legumes with single-chain type lectins. Clade D is a higher clade including clades C and A. Thus, the arrangement provided by the dendrogram based on amino acid composition discriminates between single-chain and two-chain lectins.

3.2. Erythrocytes agglutinating activity.

Table 2 shows the agglutinating activity of the purified *Vicia* lectins, and includes also the agglutinating activity of the lectins from *Lens culinaris* and *Canavalia ensiformis* for comparison. All *Vicia* lectins, except for those corresponding to *V. pseudocracca* and *V. monardii*, presented agglutinating activity at a concentration of 10 µg lectin / mL or bellow, which was also the case of the lectins from *Lens culinaris* and *Canavalia ensiformis* (Table 2). The activity was lower in the lectins belonging to *V.*

pseudocracca and *V. monardii*, with a minimum agglutinating lectin concentration of 59 and 140 µg /mL, respectively.

In general, binding to sugars by two-chain and one-chain lectins in Tribe *Fabeae* is specific for glucose and for GalNAc, respectively. Thus, the two-chain lectins in *V. faba*, *L. culinaris*, and *P. sativum* are specific for glucose, while the one-chain lectin in *V. villosa* is specific for GalNAc (Van Damme, Peumans, Barre & Rougé, 1998). The inhibition of agglutinating activity in the presence of glucose and GalNAc as shown in table 2 roughly follows this pattern. Thus, as previously reported for *V. villosa*, the agglutinating activity of the single-chain lectins from *V. benghalensis*, *V. dasycarpa*, *V. monantha*, *V. villosa*, *V. pseudocracca* and *V. monardii* was specific for GalNAc, while lectins from *V. cracca*, *V. disperma* and *V. tenuifolia* showed a similar specificity for GalNAc and glucose, and only the lectin from *V. vicioides* was more sensitive to inhibition by glucose.

Tribe *Fabeae* of Leguminosae includes genera *Vicia*, *Lathyrus*, *Pisum*, *Lens* and *Vavilovia*. Until now, most of the lectins purified from the seeds of legumes belonging to this Tribe were of the two-chain type, including the lectins from *P. sativum*, *L. culinaris*, *V. faba*, *V. cracca*, and several *Lathyrus* species (Van Damme, Peumans, Barre, & Rougé, 1998). On the contrary, single-chain lectins were only found in some species of genus *Vicia* such as *V. villosa* and *V. cracca* (Grubhoffer, Tichá & Kocourek, 1981; Rüdiger, 1977). Our results showing the presence of single-chain lectins in all of the ten *Vicia* species that have been studied indicate that single-chain lectins are more common in Tribe *Fabeae* than previously believed. This does not exclude the potential presence of two-chain lectins in these seeds in lower amounts than single-chain lectins, as has been described in *V. cracca* (Baumann, Strosberg & Rüdiger, 1982;

Rüdiger, 1977). The quaternary structure and molecular weight of the lectins purified from *V. cracca* and *V. villosa* are consistent with those previously described (Grubhoffer, Tichá, & Kocourek, 1981; Rüdiger, 1977). The preference for GalNAc in the lectins purified from *V. villosa*, *V. cracca* and *V. graminea* is also consistent with previous reports (Tollefsen & Kornfeld, 1983; Tollefsen & Kornfeld, 1984; Wu, 2005).

Although GalNAc is found in animal cells in many glycoproteins such as mucins (Gill, Clausen & Bard, 2011), it was not until few years ago that GalNAc was found in plant cells, namely in *Arabidopsis* cells cultures (Alonso, Piasecki, Wang, LaClair & Shachar-Hill, 2010). It is not known whether GalNAc is incorporated into glycans or other biomolecules in these cells (Bar-Peled & O'Neill, 2011). The presence of the Tn antigen in tumors correlates with cancer cell proliferation, metastasis, and a bad prognosis (Springer, 1977; Ju, Wang, Aryal, Lehoux, Ding, Kudelka, Cutler, Zeng, Wang, Sun, Heimburg-Molinaro, Smith & Cummings, 2013). Some methods to detect and quantify the Tn antigen in malignant cells are based on the affinity of lectins for GalNAc (Kakeji, Tsujitani, Mori, Maehara, & Sugimachi, 1991). Thus, the lectin from *V. villosa* has been used to study the expression of the Tn antigen in colon (Iitzkowitz, Yuan, Montgomery, Kjeldsen, Takahashi, Bigbee & Kim, 1989), breast (Konska, Guerry, Caldefie-Chezet, De Latour & Guillot, 2006) and ovarian tumors (Chen, Gentry-Maharaj, Burnell, Steentoft, Marcos-Silva, Mandel, Jacobs, Dawnay, Menon & Blixt, 2013). Interestingly, the lectin from *Morus nigra* covalently linked to a porphyrin has been used to target the Tn antigen in order to kill leukemia cells by photochemotherapy (Poiroux, Pitie, Culerrier, Lafont, Ségui, Van Damme, Peumans, Bernadou, Levade, Rougé, Barre & Benoist, 2011). Some of the *Vicia* lectins that are presented in this article may also represent a useful tool for targeting GalNAc,

288 especially those that present an affinity for this sugar even higher than the lectin from
289 *V. villosa*. That is the case for instance of the lectins from *V. benghalensis* and *V.*
290 *monantha*, with an affinity for GalNAc which is more than five times higher than the
291 affinity of the lectin from *V. villosa*. *V. benghalensis* and *V. monantha* are two forage
292 crops that have good agronomic characteristics including a high tolerance to biotic and
293 abiotic stress (Francis, Enneking & And el Moneim, 2000), fast growth, and high yield
294 (Bryant 2011). In conclusion, purification and characterization of the lectins from ten
295 species of *Vicia* species belonging to sect. *Cracca* subgen. *Vicilla* supports the view that
296 this section is rich in single-chain lectins. Some of these lectins bind to GalNAc with
297 high affinity. Some of these *Vicias* may be of interest as sources of lectins for research
298 in glycobiology with potential biomedical applications for targeting the Tn antigen.

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FIGURE LEGENDS.

Figure 1. A) SDS-PAGE of *V. benghalensis* seed proteins extracted using SDS-PAGE running buffer (left line) or in water adjusted to pH 4 (right line). Molecular weight markers are indicated on the right. **B)** Superose 12 gel filtration profile of the original *V. benghalensis* pH 4 water extract (dashed line) and after purification by using the same gel filtration procedure (full line).

Figure 2. A) SDS-PAGE of purified *Vicia* lectins: 1) *V. benghalensis*, 2) *V. dasycarpa*, 3) *V. villosa*, 4) *V. monantha*, 5) *V. cracca*, 6) *V. tenuifolia*, 7) *V. disperma*, 8) *V. vicioides*, 9) *V. pseudocracca*, 10) *V. monardii*. **B)** Native PAGE of purified *Vicia* lectins. 1) *V. benghalensis*, 2) *V. dasycarpa*, 3) *V. villosa*, 4) *V. monantha*, 5) *V. cracca*, 6) *V. monardii*, 7) *V. disperma*, 8) *V. vicioides*, 9) *V. pseudocracca*. Molecular weight standards are indicated on the side (kDa).

Figure 3. Average amino acid composition of the one-chain lectins from *Vicia* species in Table 1 (open bars), and two-chain lectins from *Pisum sativum*, *Lens culinaris*, *Lathyrus cicera*, *Lathyrus sativus* and *Vicia faba* (dark bars, unpublished results).

Figure 4. Dendrogram produced by cluster analysis of the amino acid composition of different one-chain and two-chain legume lectins. Amino acid composition of *Canavalia ensiformis* (ID 2098436), *Phaseolus vulgaris* (ID 3891966), *Phaseolus lunatus* (ID 8920387), *Dioclea grandiflora* (ID 5107577), *Robinia pseudoacacia*, (ID 538529), *Vigna unguiculata* (ID 388103), *Wisteria floribunda* (ID 1064245663) were calculated from their protein sequence. The amino acid composition of *Vicia faba*, *Pisum sativum*, *Lathyrus sativus*, *Lathyrus cicera* and *Lens culinaris* correspond to unpublished results.

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Table 1. Amino acids composition (g/100 g lectin) of purified *Vicia* seed lectins. Results are the average $\pm$ standard deviation of two independent determinations.

	<i>Vicia benghalensis</i>	<i>Vicia dasycarpa</i>	<i>Vicia monantha</i>	<i>Vicia villosa</i>	<i>Vicia cracca</i>	<i>Vicia vicioides</i>	<i>Vicia pseudocracca</i>	<i>Vicia disperma</i>	<i>Vicia tenuifolia</i>	<i>Vicia monardii</i>	x $\pm$ sd
Asx ^a	12.9 $\pm$ 0.01	14.2 $\pm$ 0.01	13.2 $\pm$ 0.10	15.4 $\pm$ 0.60	15.1 $\pm$ 0.04	14.1 $\pm$ 0.20	13.1 $\pm$ 0.02	13.2 $\pm$ 0.05	13.3 $\pm$ 0.18	15.4 $\pm$ 0.04	14.0 $\pm$ 0.94
Glx ^b	9.2 $\pm$ 0.03	11.6 $\pm$ 0.04	10.7 $\pm$ 0.34	8.0 $\pm$ 0.35	9.6 $\pm$ 0.06	10.0 $\pm$ 0.04	9.5 $\pm$ 0.01	9.6 $\pm$ 0.02	16.1 $\pm$ 0.04	12.6 $\pm$ 0.00	10.7 $\pm$ 2.17
Ser	7.7 $\pm$ 0.04	7.2 $\pm$ 0.02	7.2 $\pm$ 0.02	6.9 $\pm$ 0.05	7.6 $\pm$ 0.04	7.2 $\pm$ 0.08	7.9 $\pm$ 0.00	7.8 $\pm$ 0.03	6.4 $\pm$ 0.01	6.3 $\pm$ 0.01	7.2 $\pm$ 0.53
His	2.4 $\pm$ 0.01	2.4 $\pm$ 0.08	2.6 $\pm$ 0.01	3.0 $\pm$ 0.01	2.6 $\pm$ 0.03	2.6 $\pm$ 0.04	2.2 $\pm$ 0.01	2.3 $\pm$ 0.02	1.9 $\pm$ 0.03	2.4 $\pm$ 0.02	2.4 $\pm$ 0.28
Gly	3.4 $\pm$ 0.01	3.7 $\pm$ 0.02	4.1 $\pm$ 0.02	6.4 $\pm$ 0.09	3.8 $\pm$ 0.03	3.5 $\pm$ 0.01	3.5 $\pm$ 0.01	3.7 $\pm$ 0.01	6.2 $\pm$ 0.03	6.4 $\pm$ 0.02	4.5 $\pm$ 1.22
Thr	8.4 $\pm$ 0.01	8.2 $\pm$ 0.02	8.7 $\pm$ 0.05	6.6 $\pm$ 0.06	8.5 $\pm$ 0.04	8.3 $\pm$ 0.06	8.9 $\pm$ 0.00	7.8 $\pm$ 0.03	9.0 $\pm$ 0.01	8.2 $\pm$ 0.01	8.2 $\pm$ 0.66
Arg	2.8 $\pm$ 0.02	3.6 $\pm$ 0.10	3.9 $\pm$ 0.03	4.1 $\pm$ 0.04	3.4 $\pm$ 0.03	3.5 $\pm$ 0.03	4.0 $\pm$ 0.03	3.6 $\pm$ 0.00	3.9 $\pm$ 0.06	4.2 $\pm$ 0.05	3.7 $\pm$ 0.40
Ala	5.2 $\pm$ 0.01	5.9 $\pm$ 0.05	5.7 $\pm$ 0.00	5.2 $\pm$ 0.00	5.0 $\pm$ 0.07	4.9 $\pm$ 0.06	4.9 $\pm$ 0.07	5.9 $\pm$ 0.01	8.6 $\pm$ 0.00	7.5 $\pm$ 0.00	5.9 $\pm$ 1.16
Pro	3.3 $\pm$ 0.08	3.4 $\pm$ 0.11	3.0 $\pm$ 0.12	3.3 $\pm$ 0.08	3.2 $\pm$ 0.17	2.8 $\pm$ 0.02	2.7 $\pm$ 0.01	2.7 $\pm$ 0.00	2.7 $\pm$ 0.00	2.7 $\pm$ 0.06	3.0 $\pm$ 0.29
Tyr	3.3 $\pm$ 0.04	2.1 $\pm$ 0.01	2.3 $\pm$ 0.00	5.0 $\pm$ 0.01	2.2 $\pm$ 0.02	2.3 $\pm$ 0.03	3.1 $\pm$ 0.02	3.3 $\pm$ 0.00	1.9 $\pm$ 0.01	2.5 $\pm$ 0.01	2.8 $\pm$ 0.88
Val	8.0 $\pm$ 0.10	5.9 $\pm$ 0.05	7.0 $\pm$ 0.19	5.6 $\pm$ 0.02	7.1 $\pm$ 0.08	7.7 $\pm$ 0.01	6.4 $\pm$ 0.02	6.8 $\pm$ 0.01	4.8 $\pm$ 0.02	4.8 $\pm$ 0.00	6.4 $\pm$ 1.07
Met	0.0 $\pm$ 0.01	0.2 $\pm$ 0.00	0.1 $\pm$ 0.01	0.1 $\pm$ 0.02	0.4 $\pm$ 0.00	0.6 $\pm$ 0.01	0.1 $\pm$ 0.00	0.0 $\pm$ 0.00	0.1 $\pm$ 0.00	0.3 $\pm$ 0.01	0.2 $\pm$ 0.18
Cys	0.1 $\pm$ 0.01	0.2 $\pm$ 0.01	0.5 $\pm$ 0.00	1.0 $\pm$ 0.15	0.5 $\pm$ 0.00	0.3 $\pm$ 0.03	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.3 $\pm$ 0.03	0.3 $\pm$ 0.30
Ile	5.5 $\pm$ 0.02	6.6 $\pm$ 0.02	5.4 $\pm$ 0.10	5.2 $\pm$ 0.20	5.6 $\pm$ 0.00	6.5 $\pm$ 0.02	6.4 $\pm$ 0.00	5.9 $\pm$ 0.01	3.2 $\pm$ 0.04	3.8 $\pm$ 0.03	5.4 $\pm$ 1.07
Trp	1.9 $\pm$ 0.06	2.1 $\pm$ 0.05	2.0 $\pm$ 0.06	2.2 $\pm$ 0.06	2.2 $\pm$ 0.08	2.6 $\pm$ 0.02	2.7 $\pm$ 0.03	2.6 $\pm$ 0.04	2.4 $\pm$ 0.00	2.6 $\pm$ 0.00	2.3 $\pm$ 0.27
Leu	9.4 $\pm$ 0.02	7.5 $\pm$ 0.07	8.2 $\pm$ 0.07	9.8 $\pm$ 0.13	8.6 $\pm$ 0.12	7.6 $\pm$ 0.05	8.0 $\pm$ 0.00	7.5 $\pm$ 0.01	5.3 $\pm$ 0.02	6.7 $\pm$ 0.02	7.9 $\pm$ 1.24
Phe	9.2 $\pm$ 0.02	7.1 $\pm$ 0.03	8.0 $\pm$ 0.15	6.0 $\pm$ 0.02	7.6 $\pm$ 0.12	8.1 $\pm$ 0.02	8.7 $\pm$ 0.03	8.4 $\pm$ 0.04	3.3 $\pm$ 0.07	4.0 $\pm$ 0.03	7.0 $\pm$ 1.90
Lys	7.3 $\pm$ 0.02	8.2 $\pm$ 0.07	7.5 $\pm$ 0.03	6.4 $\pm$ 0.02	7.0 $\pm$ 0.06	7.4 $\pm$ 0.06	7.8 $\pm$ 0.01	8.9 $\pm$ 0.05	11.2 $\pm$ 0.09	9.4 $\pm$ 0.01	8.1 $\pm$ 1.34

^aAsp + Asn. ^bGlu + Gln.

Table 2. Agglutinating activity of the lectins purified from *Vicia*, *Lens culinaris* and *Canavalia ensiformis*, and inhibition by GalNAc and glucose. Agglutinating activity of lectins is expressed as the minimum concentration of lectins ($\mu\text{g/mL}$) that cause agglutination of a 4 % suspension of red blood cells. The inhibitory activity of the sugars is expressed as the minimum concentration of sugars (mM) that cause inhibition of agglutination. Results are the average of two determinations.

	[Lectin] ($\mu\text{g/mL}$)	GalNAc (mM)	Glucose (mM)
<i>V. benghalensis</i>	6.0	0.9	16.5
<i>V. dasycarpa</i>	9.0	3.6	65.0
<i>V. monantha</i>	5.6	0.9	65.0
<i>V. villosa</i>	3.4	5.0	33.0
<i>V. cracca</i>	4.0	14.4	16.5
<i>V. vicioides</i>	5.6	28.8	8.2
<i>V. pseudocracca</i>	58.8	0.9	33.0
<i>V. disperma</i>	3.6	3.6	6.7
<i>V. tenuifolia</i>	10.4	7.2	16.5
<i>V. monardii</i>	140.0	57.6	320.0
<i>L. culinaris</i>	4.0	90.4	6.7
<i>C. ensiformis</i>	8.0	90.4	6.7

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4