The seed lectin from \textit{Dioclea grandiflora} and jack bean lectin concanavalin A (ConA) are both members of the Diocleinae subtribe of Leguminosae lectins. Both lectins have recently been shown to possess enhanced affinities and extended binding sites for the trisaccharide, 3,6-di-O-(a-D-mannopyranosyl)-D-mannose, which is present in the core region of all asparagine-linked carbohydrates (Gupta, D., Oscarson, S., Raju, S., Stanley, P., Toone, E. J. and Brewer, C. F. (1996) \textit{Eur. J. Biochem.} 242, 320–326). In the present study, the binding specificities of seven other lectins from the Diocleinae subtribe have been investigated by hemagglutination inhibition and isothermal titration microcalorimetry (ITC). The lectins are from \textit{Canavalia brasilensis}, \textit{Canavalia bonariensis}, \textit{Cratylocacia floribunda}, \textit{Dioclea rostrata}, \textit{Dioclea virgata}, \textit{Dioclea violacea}, and \textit{Dioclea guianensis}. Hemagglutination inhibition and ITC experiments show that all seven lectins are Man/Glc-specific and have high affinities for the core trimannoside, like ConA and \textit{D. grandiflora} lectin. All seven lectins also exhibit the same pattern of binding to a series of monodeoxy analogs and a tetra-deoxy analog of the trimannoside, similar to that of ConA and \textit{D. grandiflora} lectin. However, \textit{C. bonariensis}, \textit{C. floribunda}, \textit{D. rostrata}, and \textit{D. violacea}, like \textit{D. grandiflora}, show substantially reduced affinities for a biantennary complex carbohydrate with terminal GlcNAc residues, while \textit{C. brasilienisis}, \textit{D. guianensis}, and \textit{D. virgata}, like ConA, exhibit affinities for the oligosaccharide comparable with that of the trimannoside. Thermodynamic data obtained by ITC indicate different energetic mechanisms of binding of the above two groups of lectins to the complex carbohydrate. The ability of the lectins to induce histamine release from rat peritoneal mast cells is shown to correlate with the relative affinities of the proteins for the bi-antennary carbohydrate.

The thermodynamic data (7) identified the 3-, 4- and 6-OH of the \textit{α}(1–3)-Man, the 2- and 4-OH of the \textit{α}(1–3)-Man, and the 2- and 4-hydroxyls of the central Man of 1 in binding, confirming the hemagglutination results (4). Importantly, both the hemagglutination inhibition (4) and ITC data (7) agree with the recently determined x-ray crystal structure of ConA complexed with the trimannoside (8), which shows binding of the above hydroxyls of the trisaccharide (Fig. 2).

Phytohemagglutinins from the Leguminosae family comprise one of the largest group of homologous proteins with carbohydrate binding properties (see Ref. 1). Despite similarities in their physicochemical properties and their relatively conserved primary sequences, Leguminosae lectins display considerable diversity in their carbohydrate binding properties (2). This diversity is present not only in terms of recognizing different monosaccharides but also in lectins with the same nominal monosaccharide specificity. For example, Man-specific Leguminosae lectins have been isolated from the Diocleinae subtribe, which include the jack bean lectin concanavalin A (ConA) and seed lectin from \textit{Dioclea grandiflora}, and from the Vicieae tribe, which includes the sweet pea, garden pea, lentil, and fava bean lectins. However, ConA and \textit{D. grandiflora} lectin have recently been shown to possess substantially enhanced affinities for the “core” trimannoside, 3,6-di-O-(a-D-mannopyranosyl)-D-mannose, which is present in all asparagine-linked (N-linked) carbohydrates (3, 4). In addition, recent hemagglutination inhibition studies have reported that ConA and \textit{D. grandiflora} lectin have nearly the same pattern of binding to deoxy analogs 2–11 of the trimannoside (Fig. 1) (4). These studies indicate that two nominal Man/Glc-specific lectins from the Diocleinae subtribe (Scheme I), possess extended binding sites and high affinities for the trimannoside, unlike members of the Vicieae tribe (1). In addition, the hemagglutination inhibition study showed that, while ConA possesses high affinity for a biantennary complex carbohydrate (14, Fig. 1), \textit{D. grandiflora} lectin shows much lower affinity for the oligosaccharide (4). These results indicate a further divergent specificity of these two Diocleinae subtribe lectins for complex type carbohydrates.

Isothermal titration microcalorimetry (ITC) has been used to determine the thermodynamics of carbohydrate binding to ConA (3, 5, 6), including binding of the methyl \textit{α}-anomer of the core trimannoside (1) and its deoxy analogs (2–11) (7). The thermodynamic data (7) identified the 3-, 4- and 6-OH of the \textit{α}(1–6)-Man, the 3- and 4-OH of the \textit{α}(1–3)-Man, and the 2- and 4-hydroxyls of the central Man of 1 in binding, confirming the hemagglutination results (4). Importantly, both the hemagglutination inhibition (4) and ITC data (7) agree with the recently determined x-ray crystal structure of ConA complexed with the trimannoside (8), which shows binding of the above hydroxyls of the trisaccharide (Fig. 2).
Seven other lectins of the subtribe Diocleinae from different genera and different species (Scheme I) have recently been described. The lectins are from Canavalia brasiliensis, Canavalia bonariensis, Cratylix floribunda, Dicocle rostrata, Dicocle virgata, Dicocle violacea, and Dicocle guianensis. The SDS-polyacrylamide gel electrophoresis patterns of the subunit structures of the lectins resemble ConA and D. grandiflora lectin, with molecular masses ranging from 26 to 30 kDa (9–15). The x-ray crystal structure of C. brasiliensis has recently been reported (16) and is similar to ConA (17, 18). Although the complete primary sequences of all of the Diocleinae lectins are not known, the high degree of sequence homologies of ConA, D. grandiflora, and C. brasiliensis suggests that other members of the Diocleinae subtribe possess relatively conserved sequences.

Despite their phylogenetic proximity and apparently conserved sequences, the above Diocleinae lectins possess different biological activities such as histamine release from rat peritoneal mast cells (19), lymphocyte proliferation and interferon-γ production (20), peritoneal macrophage stimulation and inflammatory reaction (21), and induction of paw edema and peritoneal cell immigration in rats (22). Thus, it is important to determine the fine carbohydrate binding specificities of this group of lectins.

The present study reports hemagglutination inhibition and ITC studies of the binding of the above seven new Diocleinae lectins to a variety of mono- and oligosaccharides, trimannoside 1, deoxy analogs 2–12, Man5 oligomannose carbohydrate 13, and biantennary complex carbohydrate 14 (Fig. 1). Together with results for ConA and D. grandiflora lectin, the present findings indicate that nine members of the Diocleinae subtribe of Leguminosae lectins possess conserved binding specificities toward 1 but differential specificities for 14. Furthermore, the relative affinities of the lectins for 14 correlate with their abilities to stimulate histamine release from the rat peritoneal mast cells.

**MATERIALS AND METHODS**

MeeGlC, MeeMan, methyl-β-D-glucopyranoside, 2-deoxy-D-glucose, Gal, Fuc, Manα1–2Man, Manα1–3Man, Manα1–6Man, maltose, trehalose, lactose, lactulose, melibiose, salicic acid, and 1 were purchased from Sigma. Methyl-2-deoxy-α-D-mannopyranoside was a gift from Dr. S. Sabesan (DuPont). Biantennary complex carbohydrate 14, GlcNAcβ1–2Man, and Manβ5 oligosaccharide (13) were obtained from Dextra Laboratories Ltd. The synthesis of oligosaccharides 2–11 (Fig. 1) has been described elsewhere (23). Synthesis of 12 will be presented elsewhere. The concentrations of carbohydrates were measured by the phenol-sulfuric acid method using an appropriate mixture of Man, Glc, and Gal as the standard (24, 25). The purity of the oligosaccharides was checked by 500-MHz 1H NMR spectroscopy. Seeds of all of the species were obtained from the States of Ceara and Rio Grande do Sul, Brazil.

**Purification of the Lectins—** Lectins were purified by affinity chromatography using Sephadex G-50, as described previously (see Ref. 26). Concentrations of the lectins were determined spectrophotometrically at 280 nm and expressed in terms of monomer. The elution of carbohydrate required for complete inhibition of four hemagglutination doses was determined by visual inspection.

**Hemagglutination Inhibition Assay—** The assay was performed at room temperature using a 2-fold serial dilution technique (27) and 3% (v/v) rabbit erythrocytes in HEPES buffer (0.1 mM HEPES, 0.15 mM NaCl, 1 mM CaCl₂, and 1 mM MgCl₂, pH 7.2). The minimum concentration of saccharide required for complete inhibition of four hemagglutination doses was determined by visual inspection.

**Isothermal Titration Microcalorimetry—** ITC experiments were performed using an OMEGA Microcalorimeter from Microcal, Inc. (Northampton, MA). In individual titrations, injections of 4 μl of carbohydrate were added from the computer-controlled 250-μl microsyringe at an interval of 4 min into the lectin solution (cell volume = 1.3424 ml).
dissolved in the same buffer as the saccharide, while stirring at 350 rpm. An example of an ITC experiment is shown in Fig. 3 for binding of 1 to D. violacea at 27 °C. Control experiments performed by making identical injections of saccharide into a cell containing buffer with no protein showed insignificant heats of dilution. The experimental data were fitted to a theoretical titration curve using software supplied by Microcal, with $\Delta H$ (enthalpy change in kcal mol$^{-1}$), $K_a$ (association constant in M$^{-1}$), and $n$ (number of binding sites/monomer), as adjustable parameters. The quantity $c = K_a M(0)$, where $M(0)$ is the initial macromolecule concentration, is of importance in titration microcalorimetry (28). All experiments were performed with $c$ values $1 < c < 200$. The instrument was calibrated using the calibration kit containing ribonuclease A (RNase A) and cytidine 2-monophosphate supplied by the manufacturer. Thermodynamic parameters were calculated from the equation,

$$\Delta G = \Delta H - T \Delta S = -RT \ln K_a \tag{1}$$

where $\Delta G$, $\Delta H$, and $\Delta S$ are the changes in free energy, enthalpy, and entropy of binding. $T$ is the absolute temperature, and $r = 1.98$ cal K$^{-1}$ M$^{-1}$.

RESULTS AND DISCUSSION

Monosaccharide Binding Specificities—The monosaccharide binding properties of ConA (29) and D. grandiflora lectin (4, 30) are well defined, with both lectins showing preferential binding to the $\alpha$-pyranosides of Man and Glc. The seven other Dioceinae lectins in Table I generally show similar preferential binding to the $\alpha$-pyranosides of Man and Glc, and not to Gal, Fuc, lactose, melibiose, or sialic acid. The C-2 hydroxyl group of Man is not essential for binding to the Dioceinae lectins, since methyl 2-deoxy-$\alpha$-D-mannopyranoside is as potent as MeoMan. Methyl 2-deoxy-$\alpha$-D-mannopyranoside was previously reported to not inhibit D. grandiflora lectin (4); however, a reinvestigation shows that it does inhibit the lectin (Table I). 2-D-Glc inhibits C. floribunda, D. rostrata, and D. guianensis more poorly than Glc. GlcNAc at a relatively high concentration (150 mM) shows some inhibition of C. brasilienis, C. bonariensis, and D. virgata but did not inhibit the other four lectins. 3-deoxy mannose, 4-deoxy mannose, and 6-deoxy mannose show no inhibitory activity, suggesting that Dioceinae lectins recognize the 3-, 4-, and 6-hydroxyl groups of Man, as observed for ConA (29) and D. grandiflora lectin (4).

The thermodynamic binding parameters of the seven new Dioceinae lectins to MeoMan determined by ITC measurements are listed in Table III. The thermodynamic parameters for ConA (5) and D. grandiflora lectin (3) binding to MeoMan have previously been reported and are listed in Table III for comparison. The values for D. grandiflora lectin binding to MeoMan have been reexamined and confirmed in the present study. C. brasilienis displays the highest $K_a$ value ($1.3 \times 10^4$ M$^{-1}$), with D. rostrata possessing the lowest $K_a$ value ($1.7 \times 10^3$ M$^{-1}$). C. brasilienis, D. guianensis, D. violacea, and D. virgata have $\Delta H$ values between $-5.8$ and $-4.9$ kcal mol$^{-1}$, while C. bonariensis, C. floribunda, D. rostrata, ConA, and D. grandiflora possess $-\Delta H$ values between $-6.9$ and $-8.9$ kcal mol$^{-1}$. However, the relative $K_a$ values of the lectins for binding MeoMan do not correlate with their respective $-\Delta H$ values, indicating compensating entropic factors.

Disaccharide Binding Specificities—Inhibitory potencies of most of the disaccharides for the seven Dioceinae lectins were comparable with that of ConA and D. grandiflora lectin (Table I). None of the lectins were inhibited by lactose and melibiose, as expected. It has previously been shown that the affinity of ConA for Man(1–2)Man, a disaccharide moiety found in N-linked oligomannose carbohydrates, is 5-fold greater than MeoMan, as compared with weaker binding of D. grandiflora lectin to the disaccharide relative to the monosaccharide (4). The seven new Dioceinae lectins display a range of relative affinities for Man(1–2)Man. D. virgata shows 16-fold greater affinity for the disaccharide relative to MeoMan, whereas D. guianensis and D. violacea show enhanced affinities for the disaccharide comparable with that of ConA. The remaining four lectins show lower relative affinities for the disaccharide.
Binding Specificity of Diocleinae Lectins

Inhibitory potencies of monosaccharides and disaccharides for ConA and D. grandiflora lectins are shown in Table I. Values in parenthesis are normalized with respect to MeMan for each lectin.

| Saccharide          | Chr$^a$ | Chor$^a$ | Clfo$^d$ | Droc$^e$ | Dgu$^f$ | Dvio$^g$ | Dvir$^h$ | ConA$^i$ | Dgr$^{c,j}$ |
|---------------------|---------|---------|----------|---------|--------|----------|----------|----------|-----------|
| Man                 | 12 (0.26)| 18 (0.27)| 37 (0.24)| 37 (0.33)| 37 (0.48)| 18 (0.5) | 19 (0.94)| 15 (0.2) | 15 (0.2)  |
| MecMan             | 3.2 (1) | 5 (1)   | 9 (1)    | 12.5 (1) | 18 (1)  | 9 (1)    | 18 (1)   | 3.1 (1)  | 3.1 (1)   |
| Mez2-DMan$^a$      | 2 (1.6) | 4 (1.25)| 14 (0.64)| 10 (1.2) | 10 (1.8) | 6.1 (1.4)| 1.5 (12) | 6 (0.5)  | 10 (0.3)  |
| Glc                 | 50 (0.06)| 75 (0.06)| 125 (0.07)| 125 (1)  | 125 (1.4)| 75 (1.2) | 36 (0.5) |          |           |
| ManGlce            | 12 (0.26)| 25 (0.2) | 40 (0.2) | 50 (0.25) | 50 (0.36)| 25 (0.36)| 25 (0.7) | 13 (0.25) | 25 (0.12) |
| 2-Deoxyglucose     | 75 (0.04)| 85 (0.05)| NI$^i$   | NI       | NI      | NI       | 100 (0.09)| 60 (0.3) |          |
| Man1–2Man          | 1.1 (2.9)| 2.3 (2.1)| 4.6 (1.9)| 4.6 (2.7)| 2.3 (7.8)| 1.7 (5.2)| 1.1 (16) | 0.6 (5.4) | 4.6 (0.67) |
| Man1–3Man          | 2.3 (1.4)| 3.1 (1.6)| 6.2 (1.4)| 6.2 (2)  | 6.2 (2.9)| 3.1 (2.9)| 3.1 (5.8)| 3.1 (1)  | 3.1 (1)   |
| Man1–6Man          | 2.5 (1.3)| 2.8 (1.8)| 3.9 (2.3)| 3.9 (3.2)| 3.9 (4.6)| 1.9 (4.7)| 1.9 (9.4)| 2.0 (1.58)| 2.0 (1.58) |
| Glc1–4Glc         | 12 (0.26)| 12 (0.41)| 37 (0.24)| 26 (0.48)| 26 (0.69)| 12 (0.75)| 9 (2.0)  | 25 (0.12) | NI at 50 mX|
| Glc1–1LeGlc       | 6 (0.5)  | 9 (0.5)  | 37 (0.24) | 18 (0.69)| 18 (1)   | 9 (1)    | 6 (3)    |          |           |
| Maltotriose        | 12 (0.26)| 12 (0.4)| 37 (0.24)| 26 (0.48)| 26 (0.69)| 13 (0.69)| 6 (3)    | 25 (0.12) | 50 (0.06) |
| GNj1–2Man         | 10 (0.32)| NI       | NI       | NI       | 33 (0.54)| NI       | 24 (0.75)| 6.5 (0.48)| NI        |

$^a$ Defined under "Materials and Methods."
$^b$ C. brasiliensis.
$^c$ C. bonariensis.
$^d$ C. floribunda.
$^e$ D. rostrata.
$^f$ D. guianensis.
$^g$ D. violacea.
$^h$ D. virgata.
$^i$ Data from Ref. 4.
$^j$ D. grandiflora.

Methyl 2-deoxy-α-D-mannopyranoside.

NI, not inhibitory at 100 mX.

Large differences in the binding specificity of ConA and D. grandiflora lectin toward GlcNAc(1–2)Man, a disaccharide moiety found in a variety of N-linked carbohydrates, have been reported (4). While ConA binds to the disaccharide, no binding was detected for D. grandiflora lectin. This is consistent with the difference in relative affinities of ConA and D. grandiflora lectin for biantennary complex carbohydrate (14) (Fig. 1) (Table II), with ConA showing high affinity for the pentasaccharide (4). The other Diocleinae lectins exhibit distinct patterns of binding to GlcNAc(1–2)Man. While C. brasiliensis, D. guianensis, and D. virgata bind the disaccharide, the remaining lectins show little or no affinity for it, similar to D. grandiflora lectin. This observation is significant in light of the results for binding of the lectins to biantennary carbohydrate (14), discussed below.

Binding to Trimannoside 1 and Its Deoxy Analogs—ConA is known to possess high affinity for the trisaccharide, 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannose, which is present in the core region of all asparagine-linked carbohydrates (31). ITC data established that ConA binds to the trimannoside and its methyl α-anomer (1) with a nearly 6 kcal mol$^{-1}$ greater −ΔH and a 60-fold greater $K_a$ than MeMan (5). These results suggested extended site binding of ConA to the trimannoside, which was confirmed by the x-ray crystal structure of the trimannoside-ConA complex (Fig. 3) (8). ITC studies of ConA binding to deoxy analogs 2–11 established the binding energetics of the various hydroxyl groups of trimannoside 1 to ConA (7). The results also demonstrated that the solution complex of the trimannoside involves binding of the same hydroxyl groups of 1 observed in the x-ray crystal complex. Thus, ConA binds to 1 via the 3-, 4-, and 6-hydroxys of the α(1–6)-Man residue, the 2- and 4-hydroxys of the central Man residue, and the 3- and 4-hydroxys of the α(1–3)-Man residue (Fig. 2).

Chervenak and Toone (3) reported similar enhanced −ΔH and $K_a$ values for D. grandiflora lectin binding to 1 relative to MeMan, which were confirmed in the present study (Table III). In addition, hemagglutination inhibition experiments with deoxy analogs 2–11 established that the pattern of binding of the hydroxyl groups of 1 to D. grandiflora lectin is similar to that for ConA (Table II for comparison) (4). These findings suggest similar extended sites for both lectins to the trimannoside.

Hemagglutination inhibition data in the present study (Table II) show that the seven new Diocleinae lectins exhibit similar enhanced affinities for 1 relative to MeMan, as observed for ConA and D. grandiflora lectin. ITC data shown in Table III indicate that all seven new Diocleinae lectins show enhanced $K_a$ and −ΔH values for 1 relative to MeMan. The enhanced $K_a$ values of the lectins for 1 relative to MeMan are shown in Fig. 4. The −ΔH values for all seven lectins binding to 1 are −5 to −7 kcal mol$^{-1}$ greater than that for MeMan, similar to the differences observed for ConA and D. grandiflora lectin (Table III). These data strongly suggest similar extended binding sites for all nine Diocleinae lectins.

In order to determine which hydroxyl groups of 1 are involved in binding to the Diocleinae lectins, hemagglutination inhibition experiments were performed using monodeoxy analogs 2–11 and tetradeoxy analog 12 (Table II). As an example, hemagglutination inhibition data for the C. floribunda lectin are shown in Fig. 5. The results indicate the involvements of the 3-, 4-, and 6-hydroxys of the α(1–6)-Man, the 3- and 4-hydroxys of the α(1–3)-Man, and the 2- and 4-hydroxys of the central Man of trimannoside 1 in binding. There is also an indication of possible participation of the 2-hydroxyl of the α(1–6)-arm, as observed for a few of the lectins in Table II. As expected, tetradeoxy analog 12 shows very little inhibition potency relative to 1 and is comparable with that of MeMan. The data in Table II show a similar pattern of inhibition by the analogs for the seven new Diocleinae lectins as observed for ConA and D. grandiflora lectin (4). These results indicate highly conserved binding sites for 1 in all nine Diocleinae lectins.

Binding of Man5 Oligomannose Carbohydrate—Hemagglutination inhibition data in Table II show that the Diocleinae lectins bind Man5 oligosaccharide 13 with almost the same inhibitory potency as 1. This indicates that the trimannoside moiety on the α(1–6)-arm is the primary epitope for interaction, as observed for ConA and D. grandiflora lectin (4).
### Binding of Biantennary Complex Oligosaccharide

| Saccharide        | Chr\(^a\) | Chon\(^b\) | Cbio\(^c\) | Dros\(^d\) | Dgu\(^e\) | Dvio\(^f\) | ConA\(^g\) | Dgr\(^h\) |
|-------------------|-----------|------------|------------|-----------|-----------|-----------|-----------|-----------|
| 1 Trimmannoside   | 27 (1)    | 9 (1)      | 20 (1)     | 27 (1)    | 40 (1)    | 13 (1)    | 27 (1)    | 28 (1)    |
| 2 α(1–3)–deoxy    | 24 (1.1)  | 8 (1.1)    | 16 (1.2)   | 20 (1.3)  | 35 (1.1)  | 8 (1.6)   | 19 (1.4)  | 25 (1.1)  |
| 3 α(1–3)–deoxy    | 156 (0.17)| 120 (0.07)| 250 (0.08)| 250 (0.1) | 312 (0.12)| 108 (0.12)| 108 (0.25)| 290 (0.09)|
| 4 α(1–3)–deoxy    | 45 (0.6)  | 29 (0.31)  | 45 (0.4)   | 53 (0.5)  | 75 (0.5)  | 38 (0.34)| 41 (0.65)| 26 (1.0)  |
| 5 α(1–3)–deoxy    | 20 (1.3)  | 7 (1.2)    | 19 (1.05)  | 25 (1.08)| 38 (1.05) | 18 (0.8)  | 29 (0.9)  | 32 (0.78)|
| 6 α(1–2)–deoxy    | 30 (0.9)  | 12 (0.75)  | 30 (0.6)   | 39 (0.9)  | 30 (1.3)  | 10 (1.3)  | 30 (0.9)  | 36 (0.7)  |
| 7 α(1–6)–deoxy    | 900 (0.03)| 1025 (0.008)| 1500 (0.01)| 3000 (0.09)| NI\(^i\)  | 900 (0.01)| 3000 (0.09)| 200 (0.14)|
| 8 α(1–6)–deoxy    | 580 (0.04)| 580 (0.01)| 900 (0.02) | 1250 (0.02)| NI\(^i\)  | 620 (0.02)| 1250 (0.02)| 230 (0.12)|
| 9 α(1–6)–deoxy    | 580 (0.04)| 620 (0.01)| 900 (0.02) | 1250 (0.02)| NI\(^i\)  | 620 (0.02)| 900 (0.03) | 240 (0.1) |
| 10 "Core" 2–deoxy| 45 (0.6)  | 45 (0.2)   | 62 (0.3)   | 125 (0.21)| 180 (0.2) | 45 (0.28)| 90 (0.3)  | 59 (0.4)  |
| 11 "Core" 4–deoxy| 125 (0.21)| 62 (0.14)  | 125 (0.16)| 125 (0.21)| 250 (0.16)| 62 (0.2) | 90 (0.3)  | 120 (0.2) |
| 12 Tetramannoside | 1500 (0.018)| 2000 (0.004)| 3200 (0.006)| 4300 (0.006)| 4600 (0.009)| 2400 (0.005)| 5800 (0.005)| 2200 (0.012)|
| 13 Man-5\(^j\)    | 17 (1.5)  | 6 (1.5)    | 11 (1.8)   | 17 (1.5)  | 34 (1.1)  | 9 (1.4)   | 22 (1.2)  | 27 (0.9)  |
| 14 GN\(_{Man}\)^k | 13 (2.07) | 790 (0.01)| 790 (0.02)| 790 (0.03)| 20 (2)    | 790 (0.01)| 25 (1.08)| 6 (4.2)   |

\( ^a \) Defined under "Materials and Methods."

\( ^b \) C. brasiliensis.

\( ^c \) C. bonariensis.

\( ^d \) C. floribunda.

\( ^e \) D. rostrata.

\( ^f \) D. guianensis.

\( ^g \) D. violacea.

\( ^h \) D. virgata.

\( ^i \) Data from Ref. 4.

\( ^j \) Data from T. K. Dam, S. Oscarson, and C. F. Brewer (unpublished results).

\( ^k \) Binding of Biantennary Complex Oligosaccharide 14—The affinities of \( D. \) grandiflora lectin for biantennary complex oligosaccharide 14 and the longer chain analog with terminal Gal residues have been reported to be weak compared with that of ConA (4). These results are related to the lack of \( D. \) grandiflora lectin binding to the disaccharide GlcNAcβ1–2Man, which is present in 14 (Table I) (4). All of the Diocleinae lectins tested showed distinct correlated binding affinities toward this disaccharide and 14. Hemagglutination inhibition data in Table II indicates that 14 has much higher inhibition potencies with \( C. \) brasiliensis, \( D. \) guianensis, and \( D. \) virgata as compared with the other new lectins. Longer chain analogs of 14 also show a similar pattern (data not shown). This parallels the binding activities of the lectins toward GlcNAcβ1–2Man (Table I). In addition, ITC data in Table III show an order of magnitude greater \( K_a \) values of \( C. \) brasiliensis, \( D. \) guianensis, and \( D. \) virgata for 14 relative to the other four lectins. Among the nine Diocleinae lectins, ConA shows the highest \( K_a \) value for 14, with \( D. \) grandiflora lectin showing a relatively low \( K_a \). The relative \( K_a \) values for all nine lectins binding to 14 (along with
with respect to Man are shown in Fig. 4. Table III also shows that *C. brasiliensis*, *D. guianensis*, and *D. virgata* possess greater 2D values for 14 of the seven new lectins, and that ConA possesses the greatest 2D value of the nine lectins. Importantly, an enthalpy-entropy compensation plot (2D versus −TΔS) of the data in Table III for 14 shows different slopes for the above two groups of the Diocleinae lectins (Fig. 6B). The lectins from *C. brasiliensis*, *D. guianensis*, and *D. virgata* fall on a line with a slope of 1.44 (correlation coefficient 0.85), while the lectins from *C. bonariensis*, *C. floribunda*, *D. rostrata*, and *D. violacea* fall on a line with a slope of 0.85 (correlation coefficient 0.98). Although the *D. grandiflora* data point appears to intersect both plots, it is associated with the latter group of lectins because of its relatively low affinity and −2D values for 14. By comparison, a similar plot of the lectins binding to 1 shows a single line with a slope of 1.21 (correlation coefficient 0.97) (Fig. 6A). These
results indicate different energetic mechanisms of binding of the four relatively high affinity lectins to ConA, as compared with the five lower affinity lectins. Thus, although all nine Dio- cleinae lectins show conserved high affinities binding for 1, four of the lectins show relatively high affinities to ConA, with the other five lectins showing relatively low affinities. Therefore, binding discrimination among this group of lectins occurs toward biantennary complex carbohydrates. The structural basis for this discrimination is currently under investigation.

**Histamine Release Activities of the Diocleinae Lectins Are Correlated with Relative Affinities for ConA**—ConA has long been known for its ability to induce histamine release from cells (see Refs. 32 and 33). Recently, Gomes and co-workers (19) investigated the histamine release properties from rat peritoneal mast cells of several other lectins from the same subtribe. At the level of 10 μg/ml lectin concentration, ConA, C. brasilensis, D. guianensis, and D. virgata induced a higher level of histamine release from rat peritoneal mast cells, whereas D. grandiflora, C. bonariensis, C. floribunda, D. rostrata, and D. violacea displayed lower abilities for induction. A significant correlation between the histamine releasing properties of these lectins and their affinity constants for ConA is apparent from the present study. Fig. 7 shows that the 1/K values of the Diocleinae lectins for ConA and the amount of histamine released by the lectins at 10 μg/ml are correlated. The strong histamine-inducing lectins ConA, C. brasilensis, D. guianensis, and D. virgata exhibit relatively high affinities (1/K) for ConA. On the other hand, the remaining relatively inactive lectins possess lower affinities for the complex carbohydrate. It appears, therefore, that induction of histamine release from rat peritoneal mast cells by ConA, C. brasilensis, D. guianensis, and D. virgata involves binding of the lectins to a biantennary complex carbohydrate and/or structurally homologous epitope present on the cell surface.

**Conclusions**—The present study demonstrates that nine lectins from the Diocleinae subtribe are Man/Glc-binding proteins with conserved binding specificities for the core trimannoside of N-linked carbohydrates. Using deoxy analogs of the trimannoside, all nine lectins were shown to possess conserved binding sites that recognize the 3-, 4-, and 6-hydroxyl groups on the α(1–6)-Man, the 3- and 4-hydroxyl groups on the α(1–3)-Man, and the 2- and 4-hydroxyl groups of the central Man of the trimannoside. While the binding specificities of the lectins are conserved for the trimannoside, their specificities are different for biantennary complex carbohydrate and longer chain analogs. Thermodynamic data from ITC experiments indicate different energetic mechanisms of binding of the Diocleinae lectins to ConA. The relative affinities of the lectins for ConA correlate with their induced histamine release activities from rat peritoneal mast cells, suggesting that the lectin receptors on the cells involve a carbohydrate(s) structure similar to ConA.

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