Synthesis of High-Affinity, Hydrophobic Monosaccharide Derivatives and Study of Their Interaction with Concanavalin A, the Pea, the Lentil, and Fava Bean Lectins

Duraikkannu Loganathan, Scott E. Osborne,* Gary D. Glick,* and Irwin J. Goldstein

Department of Biological Chemistry and *Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109

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Concanavalin A (Con A) and agglutinins from the pea (PSA), lentil (LCH), and fava bean (VFA) constitute a group of D-mannose/D-glucose binding legume lectins. In addition to their sugar binding specificity, these lectins also contain sites that bind hydrophobic ligands. The present study explores a class of nonpolar binding sites reportedly present adjacent to the carbohydrate binding site in PSA, LCH, and VFA. A series of 2-O- and 3-O-substituted nitrobenzoyl and nitrobenzyl derivatives of methyl α-D-glucopyranoside and methyl α-D-mannopyranoside were synthesized. Evaluation of their binding to Con A, PSA, LCH, and VFA was carried out by the technique of hapten inhibition of precipitation reaction. The hapten inhibition assay results reveal that the presence of a methyl or methylene group at the O-2 or O-3 position of the sugar is essential for hydrophobic interaction with PSA, LCH, and VFA. The substitution of methyl by nitrobenzyl leads to enhanced binding (1.7-16.7 times for the 2-O-substituted compounds and 7.9-40.5 times for the 3-O-substituted compounds) with the m-nitrobenzyl group contributing to maximum binding. A hydrophobic interaction is also involved between Con A and 2-O-nitrobenzyl derivatives, resulting in enhanced binding, but the corresponding 3-O-isomers bind poorly due probably to steric reasons. These results may be rationalized on the basis of the recently published X-ray data of Con A and VFA. The nitrobenzyl derivatives, after transformation to their azido analogs, have potential applications in the photoaffinity labeling of these lectins.

Concanavalin A (Con A) from jack bean (Canavalia ensiformis) and agglutinins from the pea (Pisum sativum, PSA), the lentil (Lens culinaris, LCH), and the fava bean (Vicia faba, VFA) constitute a group of D-mannose/D-glucose binding legume lectins with high protein sequence homology and common carbohydrate binding properties. The molecular structure and carbohydrate binding specificity of these lectins have been extensively studied (1). Whereas Con A consists of four identical subunits, the other lectins are composed of two light (α) and two heavy (β) chains. With regard to their carbohydrate binding specificity, all four lectins require the pyranose forms of α-D-mannose/α-D-glucose. The hydroxyl groups most critical for Con A binding are those at positions C-3, C-4, and C-6 and it will tolerate considerable variations at the C-2 position. On the other hand, lectins from the pea, the lentil, and the fava bean require unmodified hydroxyl groups at C-4 and C-6.

The lectins PSA, LCH, and VFA, however, differ significantly from Con A in binding 3-O-substituted derivatives of D-mannose/D-glucose. Using the technique of hapten inhibition of hemagglutination, Allen and co-workers showed that the 3-O-methyl and 3-O-benzyl derivatives of glucose are 2-16 times more inhibitory than glucose for the pea, the lentil, and the fava bean lectins but are very poor or noninhibitors of Con A (2). Studies on the inhibition of the precipitation reaction of the pea and lentil lectins with a phosphomannan led to similar conclusions.

Abbreviations used: Con A, the lectin from jack bean (Canavalia ensiformis); PSA, the lectin from the pea (Pisum sativum); LCH, the lectin from the lentil (Lens culinaris); VFA, the lectin from fava bean (Vicia faba); me, methyl; manp, mannopyranoside; glcp, glucopyranoside; PBS, phosphate-buffered saline, pH 7.2; TLC, thin-layer chromatography; 1H NMR, proton magnetic resonance spectroscopy.


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2 To whom correspondence should be addressed. Fax: (313) 763-4936.
results (2, 3). However, the 3-O-linked disaccharides of
glucose, nigerose, and laminaribiose were found to be poor
inhibitors of the pea, the lentil, and the fava bean lectins
(2, 3). On the basis of these results, Allen and co-workers
suggested the presence of a hydrophobic area in the sugar
binding site of the lectin that interacts with the methyl
group of the 3-O-methyl glucoside and the methylene (or
benzyl) group of 3-O-benzyl glucose. The 3-O-substituted
D-mannose derivatives were not examined.

Sites which bind hydrophobic ligands are a common
feature of many plant lectins, notably those present in
leguminous plant seeds. Several classes of such sites have
been described from non- to highly specific. The objective
of the present study was to explore a class of nonpolar
binding sites adjacent to the carbohydrate binding sites
of PSA, LCH, and VFA. In designing the carbohydrate
ligands as probes of this hydrophobic site, we kept in mind
their potential application as intermediates in the prepa-
ration of photoaffinity labels for mapping the nonpolar
binding site in these lectins. Thus the nitrobenzyl deriv-
avives of methyl α-D-mannopyranoside and methyl α-D-glucopy-
ranoside were obtained from Pfannsteil Laboratories, Inc. (Waukegan,
IL). Methyl 4,6-O-benzylidene-α-D-glucopyranoside (1) and methyl 4,6-
O-benzylidene-α-D-mannopyranoside (2) were prepared by following the
literature procedures (4, 5). Methyl 2-O-methyl-α-D-mannopyranoside
(31) and methyl 3-O-methyl-α-D-mannopyranoside (32) were synthesized
according to the procedure of Nashed (6). Benzyl bromide, m-nitrobenzyl
bromide, p-nitrobenzyl bromide, p-nitrobenzoyl chloride, imidazole, and
2,4,6-tri(pyridyl)-1,3,5-triazine were from Aldrich (Milwaukee, WI).
Acetonitrile was distilled from calcium hydride under N2.
Berl-type IR-120 ion-exchange resin was purchased from Mallinckrodt
Chemical Co. Silica gel 60 (70~220 mesh) was from EM Science (Gibbstown,
NJ). Glass-hacked silica gel 60 plates precoated with a 0.25mm-thick layer of kieselgel 6OF-254, for thin-
layer chromatography, were available from other

MATERIALS AND METHODS

Materials. Methyl α-D mannopyranoside and methyl α-D glucopy-
ranoside were obtained from Pfannsteil Laboratories, Inc. (Waukegan,
IL). Methyl 4,6-O-benzylidene-α-D-glucopyranoside (1) and methyl 4,6-
O-benzylidene-α-D-mannopyranoside (2) were prepared by following the
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2,4,6-tri(pyridyl)-1,3,5-triazine were from Aldrich (Milwaukee, WI).
Acetonitrile was distilled from calcium hydride under N2. Amberlite IR-
120 ion-exchange resin was purchased from Mallinckrodt Chemical Co.
Silica gel 60 (70~220 mesh) was from EM Science (Gibbstown, NJ).
Silica gel 32/63 mesh (for flash) column chromatography was obtained
from ICN Biochemicals (Cleveland, OH). Glass-backed silica gel 60 plates
 precoated with a 0.25-mm-thick layer of kieselgel 60F-254, for thin-
layer chromatography, were obtained from E. Merck (Darmstadt,
Germany). Components were visualized by uv quenching (254 nm) or by
staining the plate in ceric ammonium molybdate (48.0 g ammonium
molybdate and 2.0 g cerium(II)sulfate in 1 liter of 10% aqueous sulfuric
acid). The organic layer was dried over MgSO4 and concentrated
under reduced pressure to give a white solid. This residue was chromato-
graphed on silica gel (eluting with a 2/1 mixture of petroleum ether/
ethyl acetate) to give methyl 2-O-(p-nitrobenzyl)-4,6-
O-benzylideneα-D-glucopyranoside (12) (71 mg, 12% yield) as white
solids. TLC (35% ethyl acetate in petroleum ether) Rf 0.33 (9),
0.20 (12), mp 106~107°C (12). 'H NMR data are listed in Table II.

Typical procedure for the synthesis of methyl 2-O-(p-nitrobenzyl)-4,6-
O-benzylideneα-D-glucopyranoside (5) and methyl 3-O-(p-nitrobenzyl)-
4,6-O-benzylideneα-D-glucopyranoside (6). The protected sugar (1) (400
mg, 1.4 mmol) and potassium carbonate (196 mg, 1.1 mmol, 1.0 eq)
were partially dissolved in acetonitrile (5.7 ml) followed by the ad-
dition of p-nitrobenzyl bromide (307 mg, 1.4 mmol). The reaction mixture
was stirred under N2 overnight at reflux, diluted with ethyl acetate, and
washed with 1 M citrate, saturated sodium bicarbonate, and saturated
NaCl solution. The organic layer was dried over MgSO4 and concentra-
ted under reduced pressure to give a white solid. This residue was chro-
matographed on silica gel (eluting with a 2/1 mixture of petroleum ether/
ethyl acetate) to give methyl 2-O-(p-nitrobenzyl)-4,6-O-benzylideneα-D-
glucopyranoside (9) (59 mg, 10% yield) and methyl 3-O-(p-nitrobenzyl-
4,6-O-benzylideneα-D-glucopyranoside (10) (75 mg, 11% yield) as white
solids. TLC (33% ethyl acetate in petroleum ether) Rf 0.33 (9),
0.20 (12), mp 106~107°C (12). 'H NMR data are listed in Table II.

Methyl 3-O-(p-nitrobenzyl) α-D glucopyranoside (21). Compound 9
(35 mg, 0.894 mmol) was dissolved in methanol (3 ml) and to this solution
cation-exchange resin (IR-120 H+, 0.3 g) was added. The reaction mixture
was stirred overnight at 30°C. The methanolic solution was filtered
through Whatman filter paper and the filtrate was concentrated under
reduced pressure to a syrup. Column chromatography of this syrup on
silica gel followed by lyophilization afforded the title compound (21)
as a pale yellow powder (>80%). TLC (chloroform/methanol, 9/1) Rf 0.31,
mp 115~116°C. 'H NMR data are given in Table III.

Methyl 3-O-(p-nitrobenzyl) α-D-glucopyranoside (24). Compound 12
(30 mg, 0.072 mmol) was treated in the same manner as above to obtain
24 as a pale yellow solid (90%). TLC (chloroform/methanol, 9/1) Rf 0.39,
mp 109~111°C. 'H NMR data are given in Table III.

Methods. Melting points were determined on a MELT-TEMP
melting apparatus (Laboratory Devices, Holliston, MA) and are uncor-
rected. All 'H NMR spectra were recorded on a Bruker AM 360 or AM
360 MHz spectrometer. Spectra were measured at ambient temperature
in CDCl3 or CD3OD using tetramethylsilane as the internal standard.
Mass spectra were recorded on a VG Analytical (70-S) mass spectrometer using ammonia as a reagent in the chemical ionization mode and p-nitrobenzyl alcohol as a matrix in the fast atom bombardment mode.

**Quantitative precipitation and precipitation inhibition assay.** A microprecipitation technique was employed for quantitative precipitation assay (9). Stock solutions of Con A (1 mg/ml), the lentil lectin (5 mg/ml), fava bean lectin (5 mg/ml), dextran B-1355 S (2 mg/ml), S. cerevisiae mannan (1 mg/ml), Pichia sp. phosphomannan (5 mg/ml), and H. holstii phosphomannan (5 mg/ml) were prepared in phosphate-buffered saline (PBS). In the case of the pea lectin, a stock solution (2 mg/ml) was prepared in PBS containing 0.1 mM MnCl₂. The precipitation reaction was conducted by adding Con A (15 µg/15 µl), pea lectin (60 µg/30 µl), lentil lectin (100 µg/20 µl), or fava bean lectin (50 µg/10 µl) to plastic vials (1.5 ml). Varying aliquots of polysaccharide solution were added and the mixtures were adjusted to a final volume of 100 µl using PBS solution containing 1 M NaCl and 0.1 mM MnCl₂. The reaction mixtures were kept at room temperature for 2 days and centrifuged (11,500 rpm, 10 min) and the supernatant solutions removed. The precipitates were washed three times with 150 µl of PBS containing 1 M NaCl. The protein content of the precipitate was determined by Lowry's method (10) using bovine albumin as the standard.

Stock solutions of the hapten standards (methyl α-D-mannopyranoside (1) or methyl 4,6-O-benzylidene-α-D-glucopyranoside (1) with N-benzoyl imidazole in chloroform gave a mixture of methyl 2-0-(p-nitrobenzoyl)-4,6-O-benzylidene-α-D-glucopyranoside (3) and methyl 3-0-(p-nitrobenzoyl)-4,6-O-benzylidene-α-D-glucopyranoside (4) in 30 and 20% yields, respectively. The mp and ¹H NMR data of 3 and 4 agree well with those reported by Ishido and co-workers (8). On stirring a suspension of 3 and 4 in 80% aqueous acetic acid at reflux for 3 h, complete debenzyldenation was effected. The crystalline products 5 and 6 showed a characteristic methine proton signal at δ 4.92 (dd, J₂,₁ = 3.7 Hz and J₂,₃ = 10 Hz, H-2) and at δ 5.36 (t, J = 9.5 Hz, H-3), respectively, confirming their structural assignment.

**Results**

Table I lists the physical properties of the products 19-30 and their ¹H NMR data are listed in Table II. Compound 8 was also analyzed by fast atom bombardment mass spectrometry in the positive ion mode and it showed the expected molecular ion peak at m/z 418 [M + H⁺].

**Debenzyldenation** was readily effected by stirring overnight a methanolic solution of each of the compounds 7-18 in the presence of IR-120 (H⁺ form) cation-exchange resin. The deprotection in each case was nearly quantitative and the resultant product was purified on silica gel by column chromatography. Table I lists the physical properties of the products 19-30 and their ¹H NMR data are listed in Table III. Additional proof for...
### Table II

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a) All spectra were measured at 300 MHz on CDCl3 solutions unless otherwise mentioned.

b) Measured at 360 MHz.

EXPLORING HYDROPHOBIC INTERACTIONS IN LEGUME LECTINS

The structure was also obtained from mass spectrometry. The chemical ionization mass spectrum of 24, methyl 3-O-(p-nitrobenzyl)α-D-glucopyranoside showed the expected molecular ion peak at m/z 347 ([M + NH\(_4\)]\(^+\)). Periodate oxidation of 20, methyl 2-O-(m-nitrobenzyl)-α-D-glucopyranoside, and 23, methyl 3-O-(m-nitrobenzyl)-α-D-glucopyranoside, was carried out according to the procedure of Avigad (11). Compound 20 consumed one equivalent of sodium periodate, whereas 23 did not react, thus providing unequivocal evidence that 20 and 23 are 2-O- and 3-O-isomers, respectively. Finally, methyl 2-O-methyl-α-D-mannopyranoside (31) and methyl 3-O-methyl-α-D-mannopyranoside (32) were prepared according to the procedure of Nashed (6).

Aqueous solutions of 5, 6, and 19–32 (7.5–40 mM) for hapten inhibition study were prepared by gentle warming and or vortexing. Quantitative precipitation of Con A by dextran B-1355-S, PSA by S. cerevisiae mannan, LCH by Pichia sp. or H. holstii phosphomannan, and VFA by S. cerevisiae mannan was carried out by adding increasing amounts of the polysaccharide to a fixed concentration of the lectin in suitable buffer solution (see Materials and Methods). Each of the compounds 5, 6, and 19–32 was assayed for its ability to inhibit the lectin precipitation reaction. Figures 2 and 3 show the typical inhibition curves obtained for LCH:phosphomannan system using 19–30 as haptens. The 50% inhibition concentration (IC\(_{50}\)) values obtained from these curves for 19–30 were normalized with respect to that of the standard monosaccharide haptens, methyl α-D-glcp and methyl α-D-manp to give relative inhibitory potencies (see Tables IV and V). For comparison, the relative inhibitory potencies of 2-O- and 3-O-methyl derivatives of methyl α-D-manp and 2-O- and 3-O-p-nitrobenzyl derivatives of methyl α-D-glcp are also listed in Tables IV and V.

The p-nitrobenzoyl derivatives 5 and 6 were poor inhibitors for both Con A and PSA. The 2-O- and 3-O-methyl derivatives of methyl α-D-manp, 31 and 32, were moderately more potent (2.4 and 6 times, respectively) than methyl α-D-manp for inhibiting the precipitation of PSA by S. cerevisiae mannan. All of the 2-O-benzyl and nitrobenzyl derivatives 19–21 and 25–27 were 1.3 to 16.7 times more inhibitory than the standards for all four lectins. The corresponding 3-O-isomers 22–24 and 28–30 were much more potent (2.8 to 40.5 times) than the standards for PSA, LCH, and VFA but were essentially noninhibitors of Con A.

**DISCUSSION**

Many legume lectins are known to possess at least three classes of nonpolar binding sites in addition to their car-
**TABLE III**

$^1$H NMR Data for 19–30

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<td>7.40–7.30</td>
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<tr>
<td>29</td>
<td>4.76</td>
<td>4.01</td>
<td>3.70</td>
<td>4.08</td>
<td>3.55</td>
<td>2.89</td>
<td>3.78</td>
<td>3.34</td>
<td>4.82</td>
<td>4.71</td>
<td>8.29</td>
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<tr>
<td>30</td>
<td>4.75</td>
<td>4.03</td>
<td>3.69</td>
<td>4.02</td>
<td>3.56</td>
<td>3.89</td>
<td>3.82</td>
<td>3.37</td>
<td>4.86</td>
<td>4.81</td>
<td>7.58</td>
<td>8.22</td>
<td>7.58</td>
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</table>

* All spectra were measured at 360 MHz on CDCl$_3$ solutions unless otherwise mentioned.
* Measured on CDCl$_3$ solution containing traces of CD$_3$OD.
* Measured at 300 MHz on CD$_3$OD solution.
* Spectral data of H-1 to H-6' measured on D$_2$O exchanged sample.

Bohydride and metal binding sites. Several legume lectins including lima bean lectin contain a specific, high-affinity binding site for adenine and its N-6 derivatives with association constants $10^5$-$10^6$ M$^{-1}$ (12). A low-affinity site that interacts with ligands such as tryptophan and indoleacetic acid ($K_a$ $10^2$-$10^4$ M$^{-1}$) is present in each subunit of Con A and many other lectins (13). The third class involves a hydrophobic site present adjacent to the carbohydrate binding site. In Con A, this site interacts with the phenyl groups of phenyl $\beta$-glucosides and mannosides.

**TABLE IV**

Hapten Inhibition by Methyl $\alpha$-D-Glucopyranoside and Derivatives

<table>
<thead>
<tr>
<th>Compound number</th>
<th>Hapten (Me $\alpha$-D-glucoside series)</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con A</td>
<td>Adsaxtan</td>
</tr>
<tr>
<td>19</td>
<td>Me $\alpha$-D-glucopyranoside</td>
<td>1.0</td>
</tr>
<tr>
<td>20</td>
<td>Me 2-O-benzyl-$\alpha$-D-glcp</td>
<td>6.4</td>
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<tr>
<td>21</td>
<td>Me 2-O-(m-nitrobenzyl)-$\alpha$-D-glcp</td>
<td>7.4</td>
</tr>
<tr>
<td>22</td>
<td>Me 2-O-(p-nitrobenzyl)-$\alpha$-D-glcp</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>23</td>
<td>Me 3-O-benzyl-$\alpha$-D-glcp</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>24</td>
<td>Me 3-O-(m-nitrobenzyl)-$\alpha$-D-glcp</td>
<td>0.3</td>
</tr>
<tr>
<td>25</td>
<td>Me 3-O-(p-nitrobenzyl)-$\alpha$-D-glcp</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>26</td>
<td>Me 3-O-(p-nitrobenzoyl)-$\alpha$-D-glcp</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>
TABLE V

Hapten Inhibition by Methyl α-D-Mannopyranoside and Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hapten</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Me α-D-mannopyranoside</td>
<td>1.0</td>
</tr>
<tr>
<td>26</td>
<td>Me 2-O-benzyl-α-D-manp</td>
<td>4.0</td>
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<tr>
<td>27</td>
<td>Me 2-O-(m-nitrobenzyl)-α-D-manp</td>
<td>5.9</td>
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<td>28</td>
<td>Me 2-O-methyl-α-D-manp</td>
<td>2.0</td>
</tr>
<tr>
<td>29</td>
<td>Me 3-O-benzyl-α-D-manp</td>
<td>0.1</td>
</tr>
<tr>
<td>30</td>
<td>Me 3-O-(p-nitrobenzyl)-α-D-manp</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>32</td>
<td>Me 3-O-methyl-α-D-manp</td>
<td>—</td>
</tr>
</tbody>
</table>

On the other hand, the methyl groups of 3-O-methyl-D-glucose and the methylene group of 3-O-benzyl-D-glucose have been reported to interact at a similar site in PSA, LCH, and VFA but not with Con A (2). Until now, D-mannose derivatives carrying hydrophobic substituents at C-3 position have not been examined.

In the present investigation we sought to obtain a better understanding of the site present in the legume lectins which interacts with hydrophobic ligands. A series of derivatives of methyl α-D-glcp and methyl α-D-manp containing 2-O- and 3-O-benzyl, m-nitrobenzyl, and p-nitrobenzyl substituents were synthesized according to the scheme shown in Fig. 1. The 2-O- and 3-O-methyl derivatives of methyl α-D-manp and 2-O- and 3-O-p-nitrobenzyl derivatives (see Materials and Methods) of methyl α-D-glcp were also prepared as probes for elucidating the structural requirements for the hydrophobic ligands. Nitro substituents in the phenyl ring were chosen so that these compounds could serve as precursors for the preparation of azido derivatives for affinity labeling of these hydrophobic sites. The yield of the isolated intermediates 7–18 was moderate. 1H NMR spectra of each of the intermediates 7–18 showed characteristic signals arising from aromatic ring protons, the methine proton of the benzylidene group (5.52–5.62 ppm), the anomeric proton (4.61–4.82), and the glycosidic methyl protons (3.36–3.45). Sugar ring protons of 2-O-substituted compounds 7 and 9 exhibited well-resolved resonances and hence were amenable to complete spectral assignment, whereas the remainder of the derivatives 10–18 showed overlapping signals in the sugar ring proton region (see Table II). 1H NMR spectrum of 9 provided convincing spectral evidence for its identity as methyl 2-O-(p-nitrobenzyl)-4,6-O-benzylidene-α-D-glucopyranoside. Three resonances appearing at δ 4.78 (d, 1H, J₁,₂ = 3.6 Hz), 4.20 (dt, 1H, Jₓ,₄ = 9.1 Hz, J₃,₂ = 9.3 Hz, J₃,₁ = 1.8 Hz), and 3.52 ppm (dd, 1H, J₁,₂ = 3.6 Hz, J₄,₃ = 9.3 Hz) were tentatively assigned to H-1, H-3, and H-2, respectively. On D₂O exchange, the signal at 4.20 ppm collapsed to a triplet and subsequent irradiation of the resonance at 4.78 ppm resulted in the simplification of the signal at 3.52 ppm into a doublet (Jₓ,₄ = 9.3 Hz), thus confirming the structural assignment. In contrast to compounds 7 and 9, the debenzylidenedated 3-O-substituted derivatives 28–30 showed well-resolved sugar ring proton resonances. The identity of compounds 19–21, 25–27 and 22–24, 28–30 as the 2-O- and 3-O-isomers, respectively, was also established unambiguously by periodate oxidation (11).
Using a hapten inhibition of precipitation assay, we generated a complete set of inhibition curves and derived IC₅₀ values for each derivative 5 and 6, 19–30, and 31 and 32 against each of the lectins, Con A, PSA, LCH, and VFA. An example of such a study is shown in Figs. 2 and 3, which portray the effect of various 2-O- and 3-O-substituted derivatives 19–30 in the LCH:phosphomannan precipitation system. The most dramatic effect on this system is shown by substitution at the O-3 position of both methyl α-D-glcp and methyl α-D-mannp. Methyl 3-O-(m-nitrobenzyl)-α-D-mannp was found to be 40 times more potent than the parent compound, methyl α-D-mannp.

The hapten inhibition assay results summarized in Table IV and Table V indicate the following: (i) the presence of a methyl or methylene group at the O-2 or O-3 position of methyl α-D-glcp and methyl α-D-mannp is essential for hydrophobic interaction with PSA, LCH, and VFA; (ii) the substitution of methyl by benzyl leads to enhanced binding with the m-nitrobenzyl group contributing to maximum binding; and (iii) a ligand–protein hydrophobic interaction is also involved between Con A and 2-O-methyl, 2-O-benzyl, and 2-O-nitrobenzyl derivatives, resulting in greater binding, but the corresponding 3-O-isomers bind poorly due to steric hindrance.

These results may be rationalized on the basis of the recently published high-resolution X-ray data of Con A (15) and VFA (16). As shown by Reeke and Becker (16), the replacement of two large side chains, Leu-99 and Arg-228, found in Con A with smaller groups, Ala-212 and Gly-100, in VFA results in a region of significant structural difference between these two lectins. Thus the considerably open carbohydrate binding site in VFA allows the binding of α-D-glcp/α-D-mannp derivatives with bulky substituents at the O-2 and O-3 positions unlike the situation with Con A. The two hydrophobic side chains Tyr-101 and Trp-129 in VFA could interact with Van der Waal contacts with the benzyl groups of 19–30, leading to enhanced binding. Experiments are under way to prepare photoaffinity-labeled as well as spin-labeled derivatives based on the results of the present study in order to map the carbohydrate binding site in these legume lectins.

ACKNOWLEDGMENTS

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REFERENCES