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# BINDING OF PARA-SUBSTITUTED PHENYL GLYCOSIDES TO CONCANAVALIN A

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ABSTRACT

The binding of para-substituted phenyl glycopyranosides of α-D-glucose, β-D-glucose, and α-D-mannose by concanavalin A has been related to the electronic and hydrophobic nature of the substituents by multiparameter regression analysis. Hydrophobicity is an important factor for the binding of the β-D-glucosides, especially in the *p*-alkyl series; a smaller but mutually comparable dependence on hydrophobicity is noted for each of the *p*-halogeno, *p*-alkoxy, and *p*-acyl substituent series. In the last two series, an additional substituent interaction with the protein might occur. The more tightly bound α-D-mannosides and α-D-glucosides show a constant binding ratio for all *p*-phenyl substituents. Here, hydrophobic contributions are negligible when compared with electronic effects. Hammett relations ($\rho = -0.5$) are valid for α-D-glucosides and α-D-mannosides, and can be improved by considering inductive and mesomeric contributions of the substituents. These observations are compatible with crystallographic data at a resolution of 2 Å. Their relevance for the α-D-anomeric specificity, governed by a protein electrophile, is discussed.

## INTRODUCTION

Concanavalin A[1], the hemagglutinin from the Jack bean (*Canavalia ensiformis*), has two carbohydrate binding sites per molecular weight of 55,000[2,3]. Because of their anomeric specificity, this class of proteins has been proposed for use in a microtest for the glycosidic linkage[4]. The binding specificity of concanavalin A for polysaccharides[5], monosaccharides, and glycosides[6] has been studied extensively. Poretz and Goldstein[7] have reported the influence of aryl substitution in phenyl β-D-glucopyranosides upon interaction with the agglutinin. For these β-D-glucosides, which are less firmly bound than their α-D anomers, the contribution of the aryl substituent seemed aspecific because the binding efficiency correlates well with the hydrophobic parameter $\pi$ for ortho and meta substituents, but not for para substituents.

We now present an extension of this para-substituted phenyl β-D-glucopyranoside series to reveal that the hydrophobic contribution is maintained and is comparable with ortho and meta substitution in the *p*-alkyl series, whereas in

*p*-halogeno, *p*-alkoxy, and *p*-acyl derivatives the effect is weakened but mutually comparable. In addition, the effect of para-substitution in phenyl α-D-manno-pyranosides and phenyl α-D-glucopyranosides, the anomers favoured for binding, is reported to be almost exclusively dependent on the electronic properties of the substituent. These results are in agreement with the recently proposed[2], different orientation of α- and β-D-glucopyranosidic rings in the binding site of concanavalin A.

## EXPERIMENTAL

Methyl and *p*-nitrophenyl glycopyranosides were commercial samples and were recrystallized from methanol. All para-substituted phenyl glycosides, except *p*-bromo-phenyl α-D-glucopyranoside[8], were prepared by deacetylation[9] of their corresponding acetates and crystallisation from methanol. Para-substituted phenyl α-D-manno-pyranosides were prepared as reported[10].

Para-substituted phenyl tetra-*O*-acetyl-α-D-glucopyranosides were prepared by fusion[10,11] of α-D-glucose penta-acetate (0.1 mole) and phenol (0.15 mole) with zinc chloride (10 g) for 60 min at 135°/200 mmHg. Excess phenol was first removed by distillation at 0.1 mmHg, and then by successive treatments of the chloroform extract with charcoal and with Polyclar (a polyamide purchased from Gaf). The resulting[10] syrup was triturated with 1.5 vol. of hot methanol. Slow crystallisation at

TABLE I

PARA-SUBSTITUTED PHENYL β-D-GLUCOPYRANOSIDES AND THEIR 2,3,4,6-TETRA-ACETATES

| p-*Phenyl substituent* | *M.p.* (*degrees*) | $[\alpha]_{589}^{22}$ (*degrees*) | $[\alpha]_{436}^{22}$ (*degrees*) | *Found* (%) | | *Formula* | *Calc.* (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | | *C* | *H* | | *C* | *H* |
| *2,3,4,6-Tetra*-O-*acetyl*-β-D-*glucopyranosides* | | | | | | | | |
| Ethoxy | 110–111 | −16.0 | −35.3 | 56.1 | 5.9 | $C_{22}H_{28}O_{11}$ | 56.4 | 6.0 |
| Butoxy | 113–114 | −14.0 | −34.0 | 57.8 | 6.5 | $C_{24}H_{32}O_{11}$ | 58.1 | 6.5 |
| Acetyl | 172–173 | −28.4 | −65.4 | 56.6 | 5.6 | $C_{22}H_{26}O_{11}$ | 56.7 | 5.6 |
| Propionyl | 156–157 | −25.8 | −61.7 | 57.5 | 5.8 | $C_{23}H_{28}O_{11}$ | 57.5 | 5.9 |
| Ethyl | 144–146 | −17.3 | −37.4 | 58.3 | 6.2 | $C_{22}H_{28}O_{10}$ | 58.4 | 6.2 |
| Propyl | 139–140 | −15.4 | −34.2 | 59.1 | 6.5 | $C_{23}H_{30}O_{10}$ | 59.2 | 6.5 |
| *sec*-Butyl | 165–166 | −13.8 | −31.9 | 60.0 | 6.7 | $C_{24}H_{32}O_{10}$ | 60.0 | 6.7 |
| Fluoro | 125–126 | −21.6 | −48.4 | 54.2 | 5.3 | $C_{20}H_{23}FO_{10}$ | 54.3 | 5.2 |
| β-D-*Glucopyranosides* | | | | | | | | |
| Ethoxy | 169–171[a] | −56.4 | −120 | 55.9 | 6.7 | $C_{14}H_{20}O_7$ | 56.0 | 6.7 |
| Butoxy | 151–154[b] | −50.9 | −108 | 58.6 | 7.5 | $C_{16}H_{24}O_7$ | 58.5 | 7.4 |
| Acetyl | 192–193[a] | −82.1 | −185 | 56.2 | 6.0 | $C_{14}H_{18}O_7$ | 56.4 | 6.1 |
| Propionyl | 163[a] | −75.6 | −170 | 57.1 | 6.4 | $C_{15}H_{20}O_7$ | 57.7 | 6.5 |
| Ethyl | 163–164[a] | −60.6 | −127 | 58.9 | 7.0 | $C_{14}H_{20}O_6$ | 59.1 | 7.1 |
| Propyl | 151–152[c] | −54.4 | −115 | 60.2 | 7.4 | $C_{15}H_{22}O_6$ | 60.4 | 7.4 |
| *sec*-Butyl | 131–133[d] | −52.5 | −112 | 61.1 | 7.8 | $C_{16}H_{24}O_6$ | 61.5 | 7.7 |
| Fluoro | 160–162[a,e] | −59.0 | −124 | 52.4 | 5.7 | $C_{12}H_{15}FO_6$ | 52.5 | 5.5 |

[a]Methanol, [b]methanol–isopropyl ether, [c]water, [d]ethanol, [e]*p*-dioxane.

room temperature, 4°, and −15° was monitored by t.l.c. to yield successive crops of pure α-D anomer (25–35%). Physical constants were as given in Table I, or in accordance with the literature[11–13].

Para-substituted phenyl tetra-*O*-acetyl-β-D-glucopyranosides were prepared[14] under anhydrous conditions by adding tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.1 mole dissolved in 250 ml of acetone) to a dry (7 g of finely powdered Sikkon) solution of sodium phenolate (0.1 mole)–phenol (15 mmoles) in 100 ml of methanol–acetone (1:3) and stirring for 2 h at room temperature. Filtration and evaporation in *vacuo* yielded a syrup that was immediately reacetylated[15] at 70° for 10 min. Crystallisation occurred when the cooled mixture was poured into 2 litres of ice–water. Recrystallisation from 100 ml of methanol yielded the β-acetates (50–60%). They were further purified by recrystallisations from isopropyl ether and from methanol. Physical constants are as given in Table II, or are in accordance with the literature[12,16].

TABLE II

PARA-SUBSTITUTED PHENYL α-D-GLYCOPYRANOSIDES AND THEIR 2,3,4,6-TETRA-ACETATES

| p-*Phenyl substituent* | *M.p.* (*degrees*) | $[\alpha]_{589}^{22}$ (*degrees*) | $[\alpha]_{436}^{22}$ (*degrees*) | *Found* (%) | | *Formula* | *Calc.* (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | | *C* | *H* | | *C* | *H* |
| *2,3,4,6-Tetra*-O-*acetyl*-α-D-*glucopyranosides* | | | | | | | | |
| Ethoxy | 66–69 | +153 | +311 | 55.7 | 6.0 | $C_{22}H_{28}O_{11}$ | 56.4 | 6.0 |
| *tert*-Butyl | 150–151 | +154 | +312 | 60.0 | 6.8 | $C_{24}H_{32}O_{11}$ | 60.0 | 6.7 |
| Chloro | 98–99 | +165 | +334 | 52.3 | 5.1 | $C_{20}H_{23}ClO_{11}$ | 52.3 | 5.1 |
| α-D-*Glucosides* | | | | | | | | |
| Ethoxy | 175–177 | +177 | +359 | 56.0 | 6.6 | $C_{14}H_{20}O_7$ | 56.0 | 6.7 |
| *tert*-Butyl | 95–97[a] | +153 | +310 | 61.5 | 7.6 | $C_{16}H_{24}O_6$ | 61.5 | 7.7 |
| Chloro | 194–196 | +189 | +383 | 49.6 | 5.4 | $C_{12}H_{15}ClO_6$ | 49.6 | 5.2 |
| Bromo | 193–194 | +169 | +343 | 42.8 | 4.5 | $C_{12}H_{15}BrO_6$ | 43.0 | 4.5 |
| *2,3,4,6-Tetra*-O-*acetyl*-α-D-*mannopyranoside* | | | | | | | | |
| Acetyl | 119–120 | +94 | | 56.6 | 5.6 | $C_{22}H_{26}O_{11}$ | 56.7 | 5.6 |
| α-D-*Mannoside* | | | | | | | | |
| Acetyl | 178–180 | +151 | +334 | 56.4 | 6.1 | $C_{14}H_{18}O_7$ | 56.4 | 6.1 |

[a]Acetone.

The melting points were determined with a Mettler FP2 instrument and are uncorrected. The optical rotations of 1% solutions in chloroform (acetylated glycosides) or methanol (glycosides) were measured with a Perkin–Elmer 141 photoelectric polarimeter. The purity of the products was tested by t.l.c. on Silica Gel G (Merck) with ethyl acetate–benzene (3:7) for the acetates, and acetic acid–water–ethyl acetate (1:1:3) for the glycosides. Detection was effected with 5% sulphuric acid in ethanol (10 min at 120°).

Glycogen (Merck) was precipitated with ethanol[17], and had $[\alpha]_D^{22}$ +175° (*c* 1, water). A stock solution (15 mg/ml) was stored frozen in small portions until needed.

Concanavalin A was isolated[18] from Jack-bean meal (Serva). Its purity was confirmed by disc electrophoresis[19] at pH 4, and by determination[20] of the number of carbohydrate binding sites[2,3,21,22]. The latter number was practically constant upon a second adsorption on Sephadex G-75 (Fig. 1). Protein concentrations were determined in M sodium chloride at 280 nm in a Zeiss PMQII–M4QIII spectrophotometer, using[23] $\varepsilon = 1.14\ cm^2/mg$ of protein and a molecular weight[24] of 55,000. Turbidity contributions[25] were 0.5% or less.

Binding parameters for *p*-nitrophenyl α-D-mannopyranoside were determined by equilibrium dialysis in 0.6-ml cells[26], sandwiched in a holder[27], using 2.5–3.0 mg of protein/ml in 0.1M sodium acetate–acetic acid, 0.2M sodium chloride, 0.1mM manganese(II) chloride, and 0.1mM calcium chloride (pH 5.35). Concentrations of *p*-nitrophenyl α-D-mannopyranoside were determined in an Eppendorf photometer at 313 nm in the range 0.025–0.14mM, using $\varepsilon = (9.87 \pm 0.02) \times 10^3\ M^{-1}.cm^{-1}$ at 22°. To obviate spectral shift[20] corrections upon ligand binding, the extent of binding was calculated from the amount of ligand added and the concentration of free ligand at equilibrium. Six dialyses were performed for each ligand concentration. The number ($n$) of independent binding sites and the association constant $K$ were calculated from the equation[28] $r/L_f = nK - rK$, in which r is the moles of ligand bound per mole of protein, and $L_f$ is the concentration of free ligand at equilibrium.

TABLE III

$M_{50}$ VALUES FOR PARA-SUBSTITUTED PHENYL β-D-GLUCOPYRANOSIDES

| *Compound No.* | *Para-substituent* | $10^3 M_{50}$ | $\pi$ | $\sigma_I$ | $\sigma_R$ |
|---|---|---|---|---|---|
| 1* | Hydroxy | (4.8)[a] | −0.64 | — | — |
| 2* | Methoxy[37,38] | 3.96 | −0.04 | 0.25 | −0.518 |
| 3 | Ethoxy | 3.46 | 0.46 | 0.27 | −0.51 |
| 4 | Butoxy | 2.58 | 1.46 | 0.27 | −0.59 |
| 5* | Methyl[16,39] | 5.10 | 0.52 | −0.05 | −0.120 |
| 6 | Ethyl | 3.47 | 0.97 | −0.05 | −0.101 |
| 7 | Propyl | 3.12 | 1.47 | −0.03 | — |
| 8 | *sec*-Butyl | 2.66 | 1.82 | −0.03 | — |
| 9* | *tert*-Butyl[16,40] | 2.33 | 1.68 | −0.007 | −0.127 |
| 10* | Hydrogen[41] | 5.89 | 0 | 0 | 0 |
| 11 | Fluoro | 5.48 | 0.15 | 0.52 | −0.458 |
| 12* | Chloro[16,42] | 4.40 | 0.70 | 0.47 | −0.243 |
| 13 | Bromo[8] | 4.14 | 1.02 | 0.45 | −0.218 |
| 14* | Iodo[13] | 3.62 | 1.26 | 0.30 | −0.21 |
| 15 | Acetyl | 3.19 | −0.37 | 0.29 | 0.212 |
| 16 | Propionyl | 2.76 | 0.13 | — | — |
| 17 | Cyano[16] | 4.46 | −0.32 | 0.58 | 0.080 |
| 18* | Nitro[41] | 4.53 | 0.24 | 0.76 | 0.018 |
| 19* | Amino | (5.56)[a] | −1.96 | — | — |

[a] ( ) calculated as $M_{50}$ from reported * values[10].

The turbidity of mixtures of glycogen and concanavalin A was measured in selected, cylindrical Klett tubes at 400 nm in a Beckman colorimeter C. The test mixture (3 ml, 25.0°, final pH 7.0) was M sodium chloride–33mM sodium phosphate, and contained 1.5 mg of glycogen and $0.368 \pm 0.002$ mg of protein. Uninhibited reference tests ($E = 0.608$, $s = 0.007$, $n = 45$ over several weeks) were measured after 25 min. Tests containing glycoside as inhibitor were measured after 15 min. About seven concentrations of a given inhibitor in the range 40–60% inhibition were measured for graphical determination of the molarity giving 50% turbidity ($M_{50}$) of a simultaneously determined reference. Up to four such series were measured for the α-D-glycosides. Inhibitor blank corrections were necessary for *p*-nitrophenyl glycosides.

The Hammett substituent constant[29] ($\sigma_H$), its inductive[30] ($\sigma_I$) and resonance ($\sigma_R = \sigma_{H,para} - \sigma_I$) contributions, and the Hansch hydrophobic parameter $\pi$ for phenoxyacetic acid[31] (see Table III) were used in multiparameter regression analyses of $-\log M_{50}$. In these, $n$ is the number of points used in the regression, $s$ the standard error of the estimate, and $r$ the regression coefficient; values in brackets are the partial correlation products.

## RESULTS AND DISCUSSION

The binding parameters of *p*-nitrophenyl α-D-mannopyranoside for concanavalin A were obtained by equilibrium dialysis at pH 5.35 (Fig. 1): (*a*) the number of binding sites $n = 2.016 \pm 0.105$, and the intrinsic association constant $K = (3.54 \pm 0.15) \times 10^4 M^{-1}$ at 4° for the protein used in the turbidimetric experiments (recycling of this protein through Sephadex G-75 increased the number of binding sites only slightly); (*b*) $n = 2.135 \pm 0.109$, $K = (3.37 \pm 0.14) \times 10^4 M^{-1}$ at 4°; (*c*) $n = 2.143 \pm 0.123$, $K = (1.494 \pm 0.075) \times 10^4 M^{-1}$ at 25°.

The last value of $K$ is comparable with that obtained under the conditions of the turbidimetric test: at pH 7.0 and 25°, $K = (1.44 \pm 0.09) \times 10^4 M^{-1}$. These values can be used as a reference for the turbidimetric $M_{50}$ values; these are the binding affinities of glycosides, expressed as the glycoside molarity giving 50% inhibition in the turbidimetric test. As obtained from such an ill-defined, mutual depletion[32] system, $M_{50}$ values have no simple relation with $K$. Nevertheless, linear free-energy relations apply to such $M_{50}$ series. $M_{50}$ values are reproducible and, expressed as relative values, they are comparable under slightly different experimental conditions. This can be judged for the methyl pyranosides of α-D-mannose, α-D-glucose, and β-D-glucose, yielding $10^3 \times M_{50}$ values equal to 0.304, 1.15, and 33.9, respectively; these are consistent with the corresponding, relative values 1:3.7:110 obtained by Goldstein's group[33] with Dextran NRRL B-1335 S. Our $M_{50}$ values were completed with those for the *p*-amino- and *p*-hydroxylphenyl β-D-glucopyranosides[7]. This is feasible on account of the excellent agreement of the values for seven mutually corresponding derivatives (* in Table III).

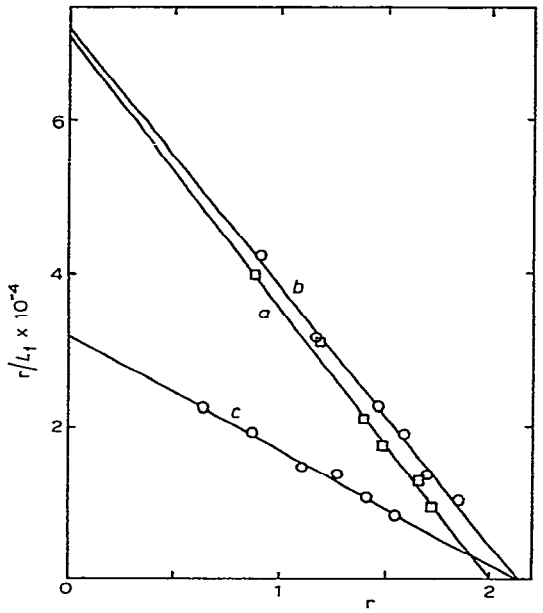


Fig. 1. Evaluation of the binding parameters of *p*-nitrophenyl α-D-mannopyranoside for concanavalin A at 4° for a single protein purification (*a*), and at 4° (*b*) and 25° (*c*) for a protein sample purified twice.

*Para-substituted phenyl β-D-glucopyranosides*

$M_{50}$ values for para-substituted phenyl β-D-glucopyranosides are given in Table III. Here, para-substitution has only a minor effect on the inhibitory power of the less tightly bound β-D-glucosides; extreme $M_{50}$ values differ only by a factor of 2.2.

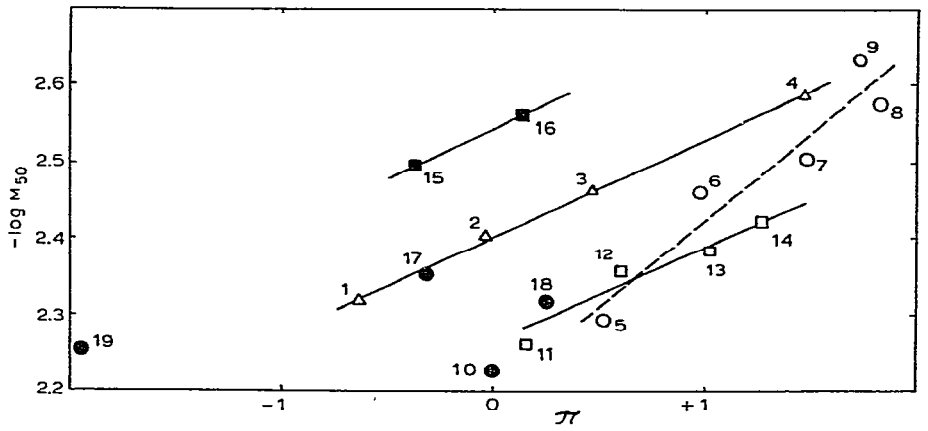


Fig. 2. Correlations for para-substituted phenyl β-D-glucopyranosides. The substituents are numbered as in Table III: *p*-alkyl (○), *p*-halogeno (□), *p*-alkoxy (△), and *p*-acyl (■) series. Binding increases along the ordinate.

As observed by Poretz and Goldstein[7], correlation of $-\log M_{50}$ with electronic parameters, expressed as the Hammett substituent constant $\sigma_H$, is extremely poor. Only a moderate change in $M_{50}$ value is observed when the strongest electron-acceptor of the series, the *p*-nitro group, is substituted for the *p*-amino group which is the most powerful electron-donor, indicating that polar effects have a minor influence. Among the best inhibitors are the butyl derivatives **4**, **8**, and **9** (Table III); these are the substituents with the highest $\pi$ values in the series. Poretz and Goldstein[7] observed that the binding ability of a substituted phenyl β-D-glucoside is closely associated with the hydrophobic nature of the substituent in the meta, and most probably in the ortho positions, but not in the para-position. From our results, however, it is obvious that the hydrophobic nature of the substituent is an important factor for the para position also. When the para derivatives are classified in homologuous sub-series, *i.e.*, alkoxy, alkyl, halogen, and acyl, the $M_{50}$ value systematically decreases with $\pi$ (Fig. 2).

Of the sub-series of para-substituted phenyl β-D-glucosides, it is the alkyl derivatives that show the most pronounced, though moderate, positive dependence on $\pi$:

$$-\log M_{50} = 2.195 + (0.229 \pm 0.045)\pi, \quad \text{with } n = 5,\ r = 0.947, \text{ and } s = 0.048. \tag{1}$$

The value for the regression coefficient +0.229 is strikingly similar to that (0.241) for the ten meta derivatives[7], among which were six alkyl substituents. Of the few polar meta-substituents, only the methoxy derivative binds noticeably better than calculated; this is also true for the ortho series[7]. In our para series, a considerable number of derivatives behave differently from equation **1**. Only the *p*-halogeno series could fit this equation. It is informative, however, to consider the latter separately without (equation **2**) or together with the *p*-nitro derivative (equation **3**), and to compare the regression coefficient with that in the alkoxy (equation **4**, *p*-hydroxy and *p*-cyano derivatives included) and in the acyl correlations (equation **5**):

$$-\log M_{50} = 2.2393 + (0.1548 \pm 0.0154)\pi, \quad \text{with } n = 4,\ r = 0.990, \text{ and } s = 0.0128; \tag{2}$$

$$-\log M_{50} = 2.2597 + (0.136 \pm 0.021)\pi, \quad \text{with } n = 5,\ r = 0.965, \text{ and } s = 0.0204; \tag{3}$$

$$-\log M_{50} = 2.4011 + (0.1295 \pm 0.004)\pi \quad \text{with } n = 5,\ r = 0.999, \text{ and } s = 0.0066; \tag{4}$$

$$-\log M_{50} = 2.541 + 0.127\pi, \quad \text{with } n = 2. \tag{5}$$

All of the regression coefficients in equations **2–5** are mutually comparable and smaller than in equation **1**. Thus, deviation from the purely aliphatic nature of the substituent causes the dependence of $-\log M_{50}$ on $\pi$ to be almost halved in the *p*-halogeno, *p*-alkoxy, and *p*-acyl sub-series. Superimposed on this diminished dependence on $\pi$ is an enhanced binding for the alkoxy and, most prominantly, for

the acyl derivatives. The latter have negative or small $\pi$ values, but are among the best inhibitors of the series, demonstrating the total inadequacy of $\pi$ in correlating for all derivatives. This cannot be improved satisfactorily by multi-parameter relations, including $\sigma_H$ or dipole moments, for all of the polar substituents considered. An additional interaction of the para substituent with the protein seems possible. A hydrogen bond could be formed, especially with a keto function and to a lesser degree with the alkoxy derivatives, a decreased hydrophobicity dependence of $-\log M_{50}$, remaining. The *p*-amino derivative, which is extremely susceptible to protonation, also binds far better than predicted from equation **1** alone. An analoguous situation apparently occurs upon binding of phenols to bovine serum albumin[34], where *p*-nitro-, *p*-methoxy-, and *m*-hydroxy-phenols bind better than calculated from the hydrophobicity dependence, although *m*-nitro-, *m*-methoxy-, and *m*-cyano-phenols do not deviate from the relation. Whether such effects are due to a supplementary interaction of the substituent with a protein group or to the inadequacy of $\sigma_H$ and $\pi$ parameters in describing the phenomenon remains unanswered.

*Para-substituted phenyl α-D-glycopyranosides*

The effect of para substitution in phenyl α-D-glucopyranosides, the anomer favoured by concanavalin A, is totally different from the β-D-glucosides. This had been observed previously for the polar *p*-amino and *p*-nitro substituents in phenyl α-D-glucosides and α-D-mannosides[7]. The results with the present derivatives confirm and extend this observation (Table IV). For the α-D-glycosides tested, it is characteristic that, for each substituent, the glucoside–mannoside $M_{50}$ ratio is practically constant and equal to 0.05. A noticeable deviation was systematically observed for the *p*-nitro derivatives, as shown by independent $M_{50}$ determinations for the α-D-glucoside (0.643, 0.652, and 0.657mM) and for the α-D-mannoside (0.1205 and 0.1216mM). Both α-D-glycoside types seem to bind in a similar way, as far as para substitution in the phenyl ring is concerned.

TABLE IV

$M_{50}$ VALUES FOR PARA-SUBSTITUTED PHENYL α-D-GLUCOPYRANOSIDES AND PHENYL α-D-MANNOPYRANOSIDES

| *Compound No.* | *Para-substituent* | α-D-*Mannosides*[10] $10^5 \times M_{50}$ | α-D-*Glucosides* $10^4 \times M_{50}$ | *Ratio* ($\times 10^2$) |
|---|---|---|---|---|
| **2** | Methoxy | 3.498 | — | — |
| **3** | Ethoxy | 2.841 | 1.301 | 4.58 |
| **5** | Methyl | 5.618 | 2.530[43,44] | 4.51 |
| **6** | Ethyl | 4.552 | — | — |
| **9** | *tert*-Butyl | 3.275 | 1.607 | 4.91 |
| **10** | Hydrogen | 10.79 | 5.142[41] | 4.76 |
| **12** | Chloro | 6.440 | 3.063 | 4.76 |
| **13** | Bromo | 5.953 | 2.955[8] | 4.96 |
| **15** | Acetyl | 8.751 | — | — |
| **18** | Nitro | 12.05 | 6.52[41] | 5.51 |

α-D-*Mannopyranosides.* In contrast to the β-D-glucosides, electronic factors have an almost exclusive contribution in determining the effectiveness of a para-substituted phenyl α-D-mannoside; *p*-alkoxy derivatives bind ~4 times better than the *p*-nitro derivative. Regression analysis for all but the unsubstituted phenyl α-D-mannopyranoside, with $\sigma_H$ as single parameter, yields (Fig. 3):

$$-\log M_{50} = 4.315 - 0.513\sigma_H, \quad (6)$$
with $n = 9$, $r = -0.935$, and $s = 0.112$.

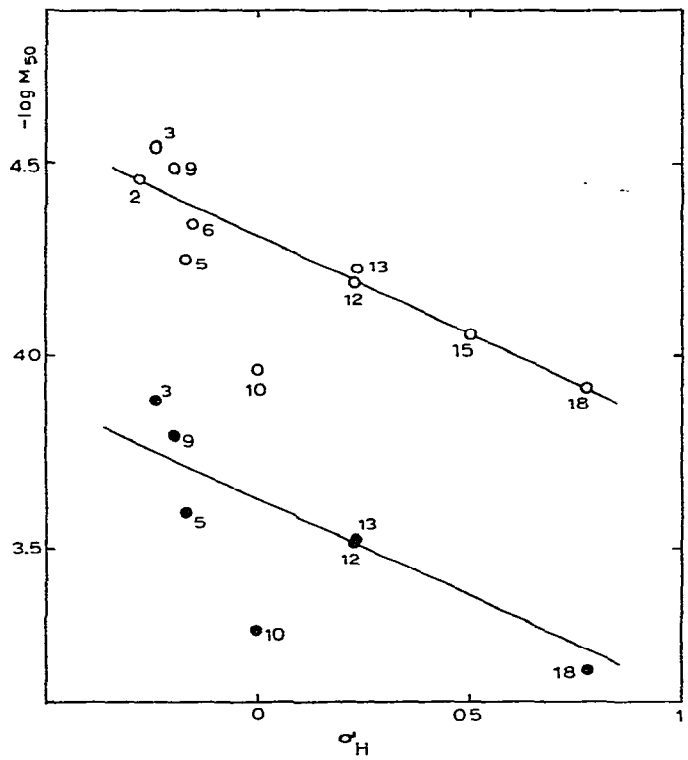


Fig. 3. Correlations for para-substituted phenyl α-D-mannopyranosides (upper graph, equation 6) and phenyl α-D-glucopyranosides (parallel lower graph). The substituents are numbered as in Table IV. Binding increases along the ordinate.

The Hammett reaction constant $\rho$ is negative, indicating that para-substituted phenyl α-D-mannopyranosides interact favourably with an electron-deficient "site" on the protein and that binding is enhanced by electron-releasing *p*-phenyl substituents. Introduction of $\pi$ next to $\sigma_H$ yields:

$$-\log M_{50} = 4.300 - 0.499\sigma_H + 0.023\pi \quad (7)$$
$$(0.849) \quad (0.029) \quad r^2 = 0.877,$$
with $n = 9$, $r = -0.937$, and $s = 0.078$.

This is an insignificant improvement of $r$, as $\pi$ correlates for less than 3% only. Separation of $\sigma_H$ into $\sigma_I$ and $\sigma_R$ is somewhat more significant, $\sigma_R$ having the larger, partial correlation product:

$$-\log M_{50} = 4.275 - 0.434\sigma_I - 0.625\sigma_R \qquad (8)$$
$$(0.375) \quad (0.524) \quad r^2 = 0.899,$$
with $n = 9$, $r = -0.948$, and $s = 0.071$.

If the three parameters are introduced, $r$ amounts to 0.953, $\pi$ still contributing for less than 5%:

$$-\log M_{50} = 4.248 - 0.404\sigma_I - 0.616\sigma_R + 0.037\pi \qquad (9)$$
$$(0.349) \quad (0.514) \quad (0.045) \quad r^2 = 0.908,$$
with $n = 9$, $r = -0.953$, and $s = 0.075$.

A hydrophobicity contribution in the binding of para-substituted phenyl α-D-mannopyranosides is therefore insignificant, compared with electronic effects.

α-D-*Glucopyranosides.* The dependence of $-\log M_{50}$ on $\sigma_H$ is comparable with the corresponding α-D-mannosides (Fig. 3). A formal mathematical treatment of the material from Table IV can be restricted to:

$$-\log M_{50} = 3.076 - 0.488\sigma_I - 0.878\sigma_R \qquad (10)$$
$$(0.477) \quad (0.465) \quad r^2 = 0.942,$$
with $n = 5$, $r = -0.970$, and $s = 0.07$.

The correlation is better than for the corresponding equation **8** for the α-D-mannosides, caused by the slightly different behaviour of the *p*-nitro substituent (compound **18**) and by the unavailablility of the α-D-glucosides **6** and **15**.

The reaction constant $\rho$ for the binding of aryl α-D-glycosides to concanavalin A can be compared in magnitude and sign to the $\rho$ value for the acid hydrolysis of meta- and para-substituted phenyl β-D-glucosides[35], in which protonation of the glycosidic oxygen atom occurs. The favourable effect of electron-releasing substituents on the formation of this conjugate acid is partially cancelled, however, by their opposing effect on the ensuing unimolecular heterolysis of the glucosidic bond.

*Anomeric specificity.* It is plausible that a causal relationship exists between the specificity of concanavalin A for the α over the β anomer and the dependence of binding efficiency on the electronic nature of the para substituent observed in phenyl α-D-glycosides and absent in phenyl β-D-glucosides. The increase in electron density around the glycosidic oxygen atom, caused by electron-releasing substituents, would favour the interaction of an α-D-glycoside with a protein electrophile, *e.g.* a hydrogen bridge. Apart from any special binding interaction of HO-2, protonation of the α-D-glycosidic oxygen atom would be less hindered by an axial hydroxyl group at C-2, as in α-D-mannopyranosides in the *CI*(D) conformation, than by an equatorial hydroxyl group as in α-D-glucopyranosides, the weaker bound α-D-hexose type. This is consistent with the specificity of concanavalin A for these α-D-glycosides. Such a hydrogen bridge could contribute in the anomeric specificity, for electronic effects are completely absent upon binding of para-substituted phenyl β-D-glucosides. For

the latter, hydrophobicity of the para substituent is an essential factor, as with ortho and meta substituents. To recognise the contribution of the hydrophobicity parameter upon substitution in the para position, it is necessary to consider the substituents in homologuous sub-series; a supplementary interaction seems to occur with the *p*-alkoxy, *p*-acyl, and, probably, *p*-amino derivatives.

The observed effects of *p*-phenyl substitution are in agreement with the different binding orientation of the anomeric D-glucopyranoside rings, as deduced from crystallographic data[2] at a resolution of 2 Å. In the β-D-glucosides, as in *o*-iodophenyl β-D-glucopyranoside, the phenyl group is located at the bottom of the cleft, allowing several possible hydrophobic contacts. None of the bulky *p*-phenyl substituents reported here suffer from steric hindrance. In α-D-glucosides, the anomeric carbon atom points towards the surface, and an influence of $\pi$ is no longer valid for *p*-phenyl substituted aglycon groups. Instead, electronic substituent effects, with $\rho = -0.5$, are predominant; the α-D-glucosidic oxygen atom could make a new contact, sharing a proton with any of the electrophiles forming the saccharide binding site: Ser 190, Ser 56, Asx 83, Tyr 56, or Ser 114.

Alternatively, $\pi$–$\pi$ charge complex formation with aryl α-D-glycosides as $\pi$ bases might account for the observed dependence on $\sigma_H$. Here, the apparent relation with anomeric specificity would merely result from a chemical artifact of the glycoside, namely the aryl ring itself. It has been reported[36] that, for charge-transfer adsorption bands, the spectral shift correlates well with the polar nature of the substituent expressed as $\sigma_H$, the intensity of the spectral shift being comparable for all substituents. It seems, however, that such a relation is not valid for concanavalin A. Spectral binding-studies[20] with *p*-amino, *p*-nitro, and unsubstituted phenyl α-D-mannopyranoside show that both the *p*-amino and *p*-nitro derivatives show a blue shift over identical wavelength, that is independent of $\sigma_H$. The unsubstituted phenyl α-D-mannopyranoside, however, shows a red shift of small intensity, comparable with the difference spectrum of protein chromophores on binding of methyl α-D-glycosides. These observations do not favour the interpretation of charge complex formation on binding of para-substituted phenyl α-D-glycosides on to concanavalin A. In fact, the similarity of the difference spectra of *p*-nitrophenyl α-D-mannopyranoside on binding to concanavalin A, and in *p*-dioxane, led Hassing[20] to interpret the change of the chromophore spectrum as resulting from a change in dielectric constant on binding with the protein.

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