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PROTEIN–CARBOHYDRATE INTERACTION

ON THE MODE OF BINDING OF AROMATIC MOIETIES TO CONCANAVALIN A, THE PHYTOHEMAGGLUTININ OF THE JACK BEAN*

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Abstract—A number of *meta*-alkylphenyl β-D-glucopyranosides were synthesized and their ability to inhibit the concanavalin A-polysaccharide system was examined. The binding constants of these compounds as well as other substituted phenyl β-D-glucopyranosides were related to the hydrophobic (π) and electronic (σ) nature of the substituents utilizing the equations devised by Hansch§ and Hammett‖ respectively.

Regression analysis of these relationships revealed that: (1) no linear correlation between the binding constants and the electronic properties of the aromatic substituents was evident; (2) the molecular volume of mono-*ortho*-substituents does not significantly effect the binding of aromatic β-D-glucopyranosides to concanavalin A; and (3) the hydrophobic nature (π) of *ortho*- and *meta*- but not *para*-substituents is closely associated with the binding of aryl β-D-glucosides to concanavalin A.

It is proposed that apolar binding involving hydrophobic interactions associated with *ortho* and *meta* but not with the *para* positions of the aromatic nucleus are the predominant forces involved in the binding of the phenyl moiety of phenyl β-D-glucosides to concanavalin A.

CONCANAVALIN A, the jack bean hemagglutinin, has been shown to interact with a select group of polysaccharides in a specific manner analogous to an antibody–antigen reaction.[1–9] Recent reports from this laboratory[5,7,9,10] have indicated that the specificity of the protein involves the reversible polar binding (probably through hydrogen bonds) of the *oxygen atoms* of the C-1, C-2 and C-3 hydroxyl groups, the hydroxyl group at C-4 and the *hydrogen atom* of the C-6 hydroxyl of α-D-mannopyranosyl residues.

We have suggested that the aromatic aglycones of α- and β-D-gluco- and mannopyranosides also bind to concanavalin A. In a study of the reaction of this protein with bovine serum albumin *p*-azophenyl glycoside-conjugates,[7] it was noted that the azophenyl β-D-glucopyranoside conjugate interacted with concanavalin A almost as avidly as the analogous α-anomer although earlier results[5,9] indicated that methyl α-D-glucopyranoside binds to this protein to a much greater extent than the β-anomer. These results suggested that the aromatic moiety probably interacts with the protein

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§ C. HANSCH and E. W. DEUTSCH, *Biochim. biophys. Acta* **112**, 381 (1966).

‖ L. P. HAMMETT, in *Physical Organic Chemistry*, p. 184. McGraw-Hill, New York (1940).

in a non-specific fashion. A further demonstration of the influence of the aromaticity of the aglycone on the binding of saccharides to concanavalin A was the observation that phenyl β-D-glucopyranoside was 10 times more potent than cyclohexyl β-D-glucopyranoside as an inhibitor of the concanavalin A-levan system.[10] It was also noted that there was no increased binding to the protein when a series of alkyl β-D-glucopyranosides (methyl, ethyl, *n*-propyl, *n*-butyl) was examined, thus indicating the absence of apolar interactions involving the simple alkyl aglycones of these saccharides with a corresponding region of the protein molecule.

The present communication describes the results of investigations designed to elucidate the mode of interaction of the phenyl moiety of phenyl α- and β-D-glucopyranosides with concanavalin A. We will examine the relationship of the binding affinities of substituted aromatic glycosides to concanavalin A with respect to the electronic, steric and hydrophobic properties of the substituents.

Correlations involving regression analysis will be performed employing the Hammett σ values[11] as a measure of electronic effects, van der Waals' radii[12] as a steric parameter and the Fujita and Hansch π constant[13,14] as a measure of the hydrophobic character of substituents.

The present study may also assist in furthering our understanding of the binding mechanism whereby the plant protein concanavalin A exerts its mitogenic[15,16] and promising antitumor activity.[17,18]*

MATERIALS AND METHOD

Saccharides. Original aryl β-D-glucopyranosides (Table 1) employed in these studies and their 2,3,4,6-tetra-*O*-acetates (Table 2) were prepared by the fusion procedure. The preparation involves the fusion of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose with the appropriate phenol (molar ratio of 1:3·5) in the presence of *p*-toluenesulfonic acid.[20,21] This is followed by deacetylation[22] to yield the desired substituted phenyl β-D-glucopyranoside.

Phenyl and *p*-hydroxyphenyl β-D-glucopyranoside were obtained from Pfanstiehl Laboratories, Waukegan, Ill. Generous quantities of *p*-aminophenyl β-D-glucopyranoside, *p*-aminophenyl and phenyl α-D-glucopyranoside as well as *p*-aminophenyl and *p*-nitrophenyl α-D-mannopyranoside were the gift of Dr. R. N. Iyer of this laboratory. The remainder of the inhibitors employed in this study was the most generous gift of Dr. M. A. Jermyn. Levan NRRL B-512 was the gift of Dr. A. Jeanes.

Concanavalin A. A purified stock solution of concanavalin A was prepared as described previously.[23]

Quantitative inhibition. Analysis of the inhibitory potency of the test compounds was performed by the quantitative turbidimetric method as described previously.[8,10]

Regression analysis. Statistical analysis was conducted on variations of the relationship of log $1/C_{50}$ to π, σ and van der Waals' radii, where C_{50} is the experimentally determined quantity of compound (in micromoles) required to produce 50 per cent inhibition of the reaction between concanavalin A and levan B-512. Values for many of the hydrophobicity constants, π, were reported earlier[13,24] and values for σ and σ_0 were obtained from the literature.[11, 25] Van der Waals' radii were obtained by measuring the radius of the appropriate atom or group of atoms in Pauling–Corey–Koltum models. The fit between linear and quadratic functions of the above constants

* W. R. BRUCE, University of Toronto, private communication.

TABLE 1. PHYSICAL CONSTANTS OF ARYL β-D-GLUCOPYRANOSIDES

Aryl β-D-glucopyranoside (Elemental analysis: C,H)	Melting point (Crystallization solvent)		Specific rotation (Ethanol)	
	Observed	Literature	Observed	Literature
m-Ethylphenyl	153–154° (Water)	156–157°*	−59·2° (*c* 1·3)	−58·8°*
m-Isopropylphenyl	142·5–143·5° (Water)	†	−55·4° (*c* 1·1)	†
(Theory: 60·39; 7·43) (Found: 60·19; 7·34)				
m-*t*-Butylphenyl	133·5–134·5°	†	−54·9° (*c* 1·1)	†
(Theory: 61·52; 7·74) (Found: 61·62; 7·64)				
3,5-Dimethylphenyl	210–210·5° (Water)	†	−64·0° (*c* 1·0)	†
(Theory: 59·14; 7·09) (Found: 58·90; 7·04)				
3,5-Di-*t*-Butylphenyl	96–100° (Water)	†	−50·1° (*c* 0·7)	†
(Theory: 65·19; 8·75) (Found: 65·29; 8·90)				

* Helferich and Rullman.[19]
† New compound.

TABLE 2. PHYSICAL CONSTANTS OF ARYL 2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSIDES

Aryl 2,3,4,6-tetra-*O*-acetyl β-D-glucopyranosides	Melting point (Crystallization solvent)		Specific rotation (Chloroform)	
(Elemental analysis: C,H)	Observed	Literature	Observed	Literature
m-Ethylphenyl	121·5–122° (Ethanol)	120–121°*	−22·3° (*c* 2·6)	−19·2°*
m-Isopropylphenyl	132–133° (Ethanol)	†	−18·3° (*c* 2·5)	†
(Theory: 59·22; 6·48) (Found: 59·35; 6·43)				
m-*t*-Butylphenyl	107·5–108·5° (Ethanol)	†	−20·3° (*c* 2·7)	†
(Theory: 59·99; 6·71) (Found: 59·69; 6·64)				
3,5-Dimethylphenyl	142–143° (Ethanol)	†	−18·5° (*c* 2·5)	†
(Theory: 58·76; 6·18) (Found: 58·54; 6·12)				
3,5-Di-*t*-Butylphenyl	144–144·5° (Ethanol)	†	−7·92°	†
(Theory: 62·44; 7·44) (Found: 62·67; 7·51)				

* Helferich and Rullman.[19]
† New compound.

and the inhibiting power expressed as log $1/C_{50}$ was kindly tested by Dr. C. Hansch using an IBM 360/40 computer and employing a program developed by Hansch and Deutsch.[14] The equation which expresses the line of best fit (least squares method) will be used as a measure of the various correlations.

RESULTS

The inhibiting potencies (C_{50}) and other parameters of a variety of *ortho*, *meta* and *para* substituted phenyl β-D-glucopyranosides are listed in Table 3. Although the substituents range from the highly electron donating amino group to the highly electron withdrawing nitro function, it is apparent that the substituent effect of the *para*-substituted derivatives is relatively small. With regard to only the polar substituents, of the seven *p*-substituted phenyl β-D-glucopyranosides tested, the greater proportion were only 1·1 times more potent than the parent compound. On the other hand, polar *meta*-substitution appears to produce a much greater enhancement in the binding potencies of the resulting inhibitors. Thus, *m*-methoxyphenyl β-D-glucopyranoside is approximately 2·1 times more potent than phenyl β-D-glucopyranoside.

Although substitution at the *ortho* position does produce variations in the inhibiting power of the phenyl β-D-glucopyranosides, these differences are apparently not

TABLE 3. HYDROPHOBICITY (π), ELECTRONIC (σ), STERIC CONSTANTS AND BINDING AFFINITIES OF CONCANAVALIN A FOR VARIOUS AROMATIC SUBSTITUENTS

Substituent	C_{50}	log $1/C_{50}$	π	σ or σ°	van der Waals' radii
H	7·0	5·16	0	0	1·0
2-CH_3	5·5	5·26	0·55	−0·17	2·0
2-CH_3O	3·7	5·43	−0·33*	−0·39	2·9
2-CH_2OH	5·7	5·24	−0·51		2·9
2-I	2·8	5·55	0·98	0·21	2·15
2-NO_2	4·4	5·36	−0·07	0·80	2·6
2-NH_2	6·8	5·17	−0·52		1·9
3-CH_3	3·9	5·41	0·51	−0·070	
3-CH_2CH_3	3·0	5·53	1·02	−0·040	
3-$CH(CH_3)_2$	2·4	5·62	1·36		
3-$C(CH_3)_3$	1·8	5·75	1·72	−0·120	
3-CH_3O	3·4	5·47	0·19	0·120	
3-NO_2	4·5	5·35	0·20	0·710	
3-CF_3	3·3	5·48	1·20	0·420	
3,5-di-CH_3	2·4	5·63	0·97	−0·170	
3,5-di-$C(CH_3)_3$	0·70	6·11	3·44†	−0·240†	
2,3-$(CH)_4$	2·8	5·55	1·47	0·170	
4-CH_3	6·5	5·19	0·55	−0·170	
4-$C(CH_3)_3$	3·0	5·53	1·89	0·200	
4-CH_3O	5·1	5·29	−0·03	−0·270	
4-HO	6·2	5·21	−0·64	−0·360	
4-I	4·9	5·31	1·46	0·280	
4-Cl	6·0	5·23	0·97	0·230	
4-NO_2	6·5	5·19	0·27	0·780	
4-NH_2	6·8	5·18	−1·96	−0·660	
2,6-di-CH_3	29				
2,3,4,5,6-F	21				

* Fujita *et al.*[13]

† Twice the value of mono-*t*-butyl.

related to the electronic properties or molecular radii of the substituents. However, it was noted that 2,6-disubstitution of the phenyl moiety as in 2,6-dimethylphenyl and pentafluorophenyl β-D-glucopyranoside produced a drastic reduction in the inhibiting power of the saccharide. Apparently a specific orientation of the phenyl ring with respect to the pyranose moiety is required for the saccharide to interact maximally with a corresponding region on the protein molecule.

In contrast to the relative insensitivity to substitution of polar groups on the aromatic ring of phenyl β-D-glucopyranosides, there is a pronounced dependence of the inhibiting power of aryl α-D-glucopyranosides on the presence of substituents as illustrated in Table 4. Thus, in both the phenyl α-D-mannopyranoside and α-D-glucopyranoside series there is a 3- to 4-fold difference in inhibiting power between the *p*-nitro and *p*-amino substituted phenyl α-D-glucosides. Note that *p*-nitrophenyl α-D-glucopyranoside is only about one-half as potent an inhibitor as phenyl α-D-glucopyranoside.

TABLE 4. INHIBITION BY SOME ARYL α-D-MANNOPYRANOSIDES AND α-D-GLUCOPYRANOSIDES OF THE CONCANAVALIN A-LEVAN B-512 SYSTEM

Compound	Micromoles for 50 per cent inhibition
p-Aminophenyl α-D-mannopyranoside	0·054
p-Nitrophenyl α-D-mannopyranoside	0·17
Phenyl α-D-glucopyranoside	0·56
p-Aminophenyl α-D-glucopyranoside	0·26
p-Nitrophenyl α-D-glucopyranoside	0·98

Employing the entire range of substituted β-D-glucopyranosides for regression analysis of the data in Table 3, the line of best fit is given by equation (1).

$$\log 1/C_{50} = 0{\cdot}111\ \pi^2 + 0{\cdot}041\ (\text{van der Waals' radii}) + 5{\cdot}27 \qquad (1)$$

$R = 0{\cdot}847$, $S = 0{\cdot}132$, $N = 21$, where R represents the correlation coefficient, S the standard deviation, and N the number of points used to derive the equation.

However, the same relationship, without the van der Waals' radius function, is statistically no different from equation (1) and is expressed as:

$$\log 1/C_{50} = 0{\cdot}108\pi + 0{\cdot}036\pi^2 + 5{\cdot}29 \qquad (2)$$
$$R = 0{\cdot}830,\ S = 0{\cdot}135,\ N = 21.$$

This finding suggests that the term which describes the steric bulk of an *ortho* group does not contribute significantly to the correlation of these factors.

In contrast to equation (2) if σ, the electronic substituent constant, is considered in place of π, the hydrophobicity constant, a very poor correlation is obtained:

$$\log 1/C_{50} = 0{\cdot}051\sigma - 0{\cdot}380\sigma^2 + 5{\cdot}46 \qquad (3)$$
$$R = 0{\cdot}307,\ S = 0{\cdot}230,\ N = 21.$$

It appears, therefore, that the inhibiting potency does not vary substantially with respect to the electronic properties of the aromatic substituent, but rather correlates well with the hydrophobic nature of the substituent. However, the variations examined,

when tested with all the substituents, gave relatively poor correlation coefficients. Therefore, the variations of log $1/C_{50}$ with these constants were tested separately for the *para*, *meta* and *ortho* substituents.

Analysis of the relationships for the *para* substituted phenyl β-D-glucopyranosides produced 8 variations resulting in the best least squares line represented by:

$$\log 1/C_{50} = 0{\cdot}066\pi + 0{\cdot}039\pi^2 + 5{\cdot}19 \quad (4)$$
$$R = 0{\cdot}847,\ S = 0{\cdot}071,\ N = 9.$$

However, this quadratic equation containing a positive π^2 value (thus indicating that compounds possessing either large negative or positive values of π would be good inhibitors) may be misleading, and the equation may be represented better by the simplest variation:

$$\log 1/C_{50} = 0{\cdot}066\pi + 5{\cdot}24 \quad (5)$$
$$R = 0{\cdot}662,\ S = 0{\cdot}922,\ N = 9.$$

On the other hand, a similar type of correlation which is extremely poor may be expressed as:

$$\log 1/C_{50} = 0{\cdot}56\sigma + 5{\cdot}25 \quad (6)$$
$$R = 0{\cdot}214,\ S = 0{\cdot}120,\ N = 9.$$

It can be seen from Table 3 that *p*-*t*-butylphenyl β-D-glucopyranoside possesses the largest binding constant of all the *para* substituted derivatives examined. In fact, analysis of the data for the *para* derivatives, neglecting the *para* *t*-butyl function, produces as the best relationship for these compounds the following equation:

$$\log 1/C_{50} = 0{\cdot}026\pi + 5{\cdot}22 \quad (7)$$
$$R = 0{\cdot}517,\ S = 0{\cdot}050,\ N = 8.$$

Examination of the above equations for the *para* substituents indicates that these relationships yield very poor correlations. Furthermore, the equations contain very small and possibly insignificant coefficients for π (slope of the line). This is highly indicative of the absence of any relationship between log $1/C_{50}$ for the *para* derivatives and π.

In contrast to the absence of a linear relationship in the *para* series, the best linear equation expressing the relationship of π for the *meta* derivatives with log $1/C_{50}$ indicates an excellent correlation:

$$\log 1/C_{50} = 0{\cdot}241\pi + 5{\cdot}30 \quad (8)$$
$$R = 0{\cdot}949,\ S = 0{\cdot}091,\ N = 9.$$

This equation suggests a moderate dependence of the inhibiting potencies for *meta* substituted phenyl β-D-glucopyranosides on the hydrophobicity constant, and is diagrammed in Fig. 1. Kiehs, Wedding *et al.*,[26,27] obtained slopes in the range of 0·68 to 0·80 for the binding of aromatic substances to a variety of proteins, but only about 0·30 for the slope of the line relating π to the binding affinity of phenyl β-D-glucopyranosides to almond emulsion.[28]

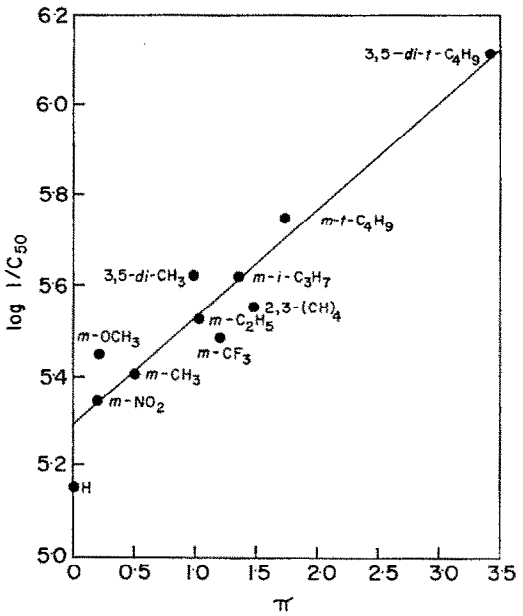

FIG. 1. Correlation of log $1/C_{50}$ of *meta*-substituted phenyl β-D-glucopyranosides with the hydrophobic substituent constant (π). C_{50} is described in the text.

The electronic properties of the *meta* substituents, on the other hand, correlate very poorly with log $1/C_{50}$, as is apparent from the following equation:

$$\log 1/C_{50} = -0{\cdot}445\sigma + 5{\cdot}57 \qquad (9)$$
$$R = 0{\cdot}509,\ S = 0{\cdot}249,\ N = 9.$$

This, again, is indicative of the importance of the hydrophobic rather than the electronic properties of the substituent for maximal inhibition of the concanavalin A system by phenyl β-D-glucopyranosides.

Since relatively few *ortho* substituted phenyl β-D-glucopyranosides were examined, the statistical analysis of these substances was conducted simultaneously with the *meta* derivatives. As before, the best correlation appeared to be the simplest type of variation of log $1/C_{50}$ with π alone.

$$\log 1/C_{50} = 0{\cdot}218\pi + 5{\cdot}33 \qquad (10)$$
$$R = 0{\cdot}899,\ S = 0{\cdot}109,\ N = 13.$$

This relationship is shown in Fig. 2, and displays a slope and intercept similar to the relationship involving only the *meta* derivatives (Fig. 1).

Although this correlation is poorer than that for equation (8) ($R = 0{\cdot}899$ and 0·949, respectively), it is still a relatively good one, and may indicate a mode of binding for the *ortho* derivatives similar to that deduced for the *meta* substituents. However, too few *ortho* substituted derivatives were examined to demonstrate this conclusively.

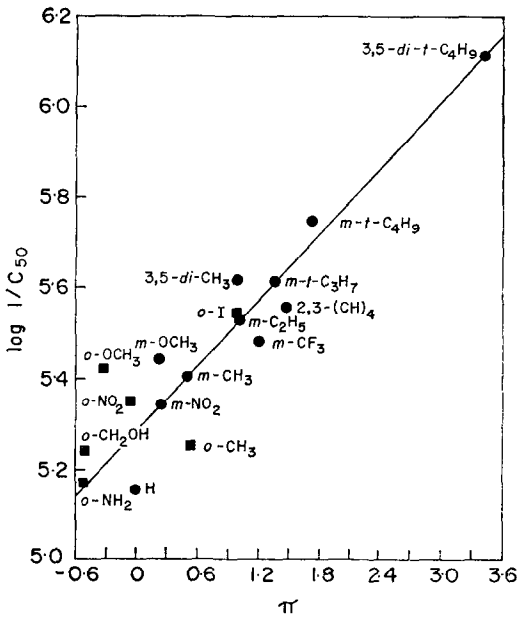

FIG. 2. Correlation of log $1/C_{50}$ for *ortho* and *meta* substituted phenyl β-D-glucopyranosides with the hydrophobic substitutent constant (π), *meta*-derivative, ●; *ortho*-derivative, ■.

By comparing the following equation involving van der Waals' radii and π,

$$\log 1/C_{50} = 0{\cdot}228\pi + 0{\cdot}021 \text{ (van der Waals' radii)} + 5{\cdot}30 \qquad (11)$$
$$R = 0{\cdot}904,\ S = 0{\cdot}112,\ N = 13,$$

with equation (10) it is apparent that the addition of this extra term does not represent any significant improvement in the relationship. This is consistent with the previously discussed results for the regression analysis for all 21 compounds, and indicates that the binding affinity of the saccharide to concanavalin A is relatively independent of the molecular volume of the *ortho* substituent. Similarly, the equation involving log $1/C_{50}$ and σ:

$$\log 1/C_{50} = 0{\cdot}218\sigma + 5{\cdot}52 \qquad (12)$$
$$R = 0{\cdot}332,\ S = 0{\cdot}235,\ N = 13.$$

when compared to equations (9) and (10), indicates that the binding affinity (expressed as log $1/C_{50}$) of phenyl β-D-glucopyranosides to concanavalin A is also unrelated to the electronic properties of the *ortho* substituent.

DISCUSSION

Aromaticity of the aglycone of β-D-glucopyranosides is not the sole condition for the binding of phenyl β-D-glucopyranosides to concanavalin A. This is apparent from a consideration of the lower inhibiting power of benzyl β-D-glucopyranoside compared to phenyl β-D-glucopyranoside (37 and 7 μmoles, respectively, for 50 per cent inhibition). The difference in the inhibitory power of these two compounds may indicate

the necessity for an optimal distance between the aromatic nucleus and the glycosidic oxygen atom; in fact, the aromatic residue must be joined directly to the anomeric oxygen atom for maximum binding. A specific orientation of the aromatic moiety with respect to the glycose residue is also indicated by the very poor inhibitory power of 2,6-disubstituted phenyl β-D-glucopyranosides (e.g. 2,6-dimethylphenyl and pentafluorophenyl β-D-glucopyranosides). Examination of CPK space filling molecular models shows that di-*ortho* substitution limits the number of stable conformations that can be assumed by the D-glucopyranosyl residue, possibly restricting it to conformations unfavorable for maximum complementation of the binding site on the concanavalin A protein.

π–π Charge-transfer complexation is often invoked to explain the binding of aromatic substrates to proteins. Preliminary examination of a π–π charge-transfer complex model for the binding of phenyl β-D-glucopyranoside to concanavalin A in which one or both partners in the complex are aromatic residues suggests intuitively that the equilibrium constant of such a complex would be sensitive to the electronic properties of substituents on the aromatic ring. Indeed, earlier workers[29–33] have demonstrated excellent correlations between the wavelengths of the charge-transfer absorption band and the electronic properties of the aromatic substituent (usually expressed as the Hammett substituent constant, σ) in both inter- and intra-, molecular charge-transfer complexation.

However, regression analysis clearly indicates a poor correlation between the inhibiting potencies of aryl β-D-glucopyranosides and the Hammett electronic substituent constant (equation 3). On the other hand, *para*-substituents of phenyl α-D-glycopyranosides greatly affect the inhibiting power of the saccharide. Iyer[34] has demonstrated that the inhibition values of these compounds appear to vary linearly with the Hammett constants.

Wallace *et al.*[35] also have noted an absence of a relationship between the Hammett constants and the binding of aromatic inhibitors to chymotrypsin. These workers concluded either that π–π charge-transfer bonding is not a dominant factor in determining the magnitude of the inhibition constants, or else that the locus for the aromatic moiety in chymotrypsin is hermaphroditic and contains both a π acid and a π base. Recently Hansch and Coats[36] described a moderate dependence of binding on the σ value (when analyzed in conjunction with the hydrophobic properties) of substituents of subsite P_2 variant inhibitors of chymotrypsin.

Even if the binding site of concanavalin A was hermaphroditic, it would be expected for this version of the charge-transfer model that differences in the binding affinity of the saccharide would be observed, depending upon whether an amino acid that is a Lewis acid or base is involved in the binding, though these differences would not be directly related to the Hammett equation. Since inhibitory potencies of the *para*-substituted phenyl β-D-glycopyranosides are apparently independent of the electronic nature of the substituent, it may be concluded that a hermaphroditic site, and therefore, charge-transfer binding, is not responsible for the binding of the aromatic moiety to concanavalin A.

A second possible explanation for the effect of aromatic aglycones in stabilizing the binding is that a planar aromatic ring, having an assumed greater effective area in its interaction with the binding site, will be a more potent inhibitor of concanavalin A than the glycoside bearing the corresponding non-planar hydroaromatic aglycone.

In fact, Hymes *et al.*[37] have shown that the inhibition constants for aromatic hydrocarbons interacting with α-chymotrypsin may be described as a linear function of the surface area of the inhibitor. However, examination of the inhibitory power of cyclopentyl β-D-glucopyranoside[10] indicates it to be a more potent inhibitor than cyclohexyl β-D-glucopyranoside, a compound possessing an aglycone of larger surface area. It appears, therefore, that the surface area of a hydrophobic aglycone is not the dominant factor responsible for the binding of the aromatic moiety to concanavalin A.

In a recent series of reports Fujita, Hansch, Iwasa *et al.*[13,14,38,39] attempted to quantitate the relative hydrophobicity that a substituent contributes to the total hydrophobic character of an aromatic substance. They have demonstrated that the relative strength of binding involving hydrophobic interactions may be estimated for aromatic compounds by an approach similar to that employed by Hammett in evaluating the electronic effect of aromatic substituents.[40] Hansch's method involves comparison of the partition coefficient, in a 1-octanol–water system, of a substituted compound with that of the parent compound.[13] Thus, π (the hydrophobicity constant) may be considered to be a measure of the apolar binding power of an aromatic substituent just as σ is an indication of the electronic properties of an aromatic substituent, Wedding, Hansch *et al.*[27,28] have also applied the technique of regression analysis to study the relationship between π and the binding constants in a variety of systems in which they include measures of the electronic properties and steric nature of a substituent.

Employing the Hansch equation it may be seen (equation 7) that a poor correlation ($R = 0{\cdot}517$) exists for π and the inhibiting potencies of *para*-substituted phenyl β-D-glucopyranosides in the concanavalin A-levan system, this being indicative of the absence of apolar bonding between the *para* position of the aromatic moiety and a complementary area of the concanavalin A molecule. On the other hand, equation (8) and Fig. 1 illustrate an excellent correlation ($R = 0{\cdot}949$) of π with the binding constant for *meta*-substituted compounds. This is highly suggestive of the presence of apolar interactions involving the *meta* portion of the aromatic β-D-glucopyranoside and concanavalin A. Similarly, Fig. 2 and equation (10) illustrate, although with somewhat less certainty, the dependence of the binding constants of *ortho*-substituted phenyl β-D-glucopyranosides on the hydrophobicity of the substituent. It appears, therefore, that hydrophobic interactions involving apolar amino acyl side chains of concanavalin A, and substituents associated with the *ortho* and *meta* positions, but not the *para* position of phenyl β-D-glucopyranoside, assist in the binding of the aromatic residue to the protein.

Hansch *et al.*[28] suggested that hydrophobic interactions involving substituents of the *para* but not the *meta* position are responsible for the binding of the aromatic residue of phenyl β-D-glucopyranoside to almond β-glucosidase. Similarly, Kiehs *et al.*[26] demonstrated that bovine hemoglobin binds aromatic compounds in proportion to the lipophilic character of the substance. Recently Wedding *et al.*[27] illustrated the correlation of π with the binding of various inhibitors to malate dehydrogenase, and thus deduced that the binding of pyrimidine nucleotides to this enzyme is assisted by hydrophobic interactions. Sarma and Woronick[41] also found that hydrophobic interactions involving the *meta* but not the *ortho* and *para* positions of various aromatic inhibitors were partially responsible for the stabilization of the inhibitor–alcohol dehydrogenase complex. Employing techniques similar to those of Kiehs, Hansch *et al.*,

[26,28,42] and Schlamowitz *et al.*[43] indicated that hydrophobicity is the prominent feature that enhances the binding of inhibitors to pepsin.

It is therefore apparent that the binding of aromatic substances to proteins of diverse origins and functions involves apolar bonding assisted by hydrophobic interactions. Similarly, the finding that concanavalin A possesses an apolar area complementary to the *ortho* and *meta* positions of the aromatic moiety of bound phenyl β-D-glucopyranosides, indicates, as Goldstein and Iyer[7] suggested previously, that hydrophobic interactions may assist in the stabilization of the saccharide–lectin complex.

Of the various models which may be postulated to rationalize and describe the enhanced binding produced by aromatic aglycones of β-D-glucopyranosides to concanavalin A one of the most attractive involves the specific apolar bonding of the aromatic moiety of the bond glycoside with alkyl side chains of amino acyl residues such as valine, leucine, and isoleucine, or the polar functions of threonine and serine. Dispersion forces acting between the benzene ring and various alkyl groups have been discussed by Brown,[44] who indicated that the interaction energy of a methyl residue with benzene may be as large as 2·2 kcal/mole. When these aromatic molecules are in solution, however, it would be expected that the solvent would somewhat reduce the interaction energy. This value may be compared to a $\Delta(\Delta F)$ value of 1·0–1·2 kcal/mole for the binding of the phenyl residue to concanavalin A. Recently Hansch and Anderson[38] have demonstrated intramolecular hydrophobic bonding involving a polar function and the aromatic moiety of compounds of the family of ω-X-alkylbenzenes. McDonald and Phillips[45] first suggested the possibility of a methyl-aromatic interaction to explain certain stabilizing forces responsible for the maintenance of the tertiary structure of lysozyme. Later Sternlicht and Wilson[46] concluded from nuclear magnetic resonance studies of this enzyme that such an interaction does indeed represent an important stabilizing force in the lysozyme molecule.

The data cited above as well as previous publications indicate the existence of two types of loci on concanavalin A capable of binding phenyl β-D-glucopyranosides; a highly specific polar site for the binding of the carbohydrate moiety[5,9,10,47] and a relatively apolar area adjacent to the carbohydrate binding site which specifically accommodates aromatic structures joined directly to the anomeric oxygen atom.[7,10]

Singer[48] has suggested that the cooperative interaction of adjacent polar and apolar regions of a protein molecule may affectively assist the binding of a small molecule which interacts with the polar site of the proteins. He pointed out that, "The effect of hydrophobic interactions may be to bring into close contact, in a relatively nonpolar micro-environment from which water has effectively been excluded, a pair of oppositely charged ionic groups or a hydrogen bond donor and acceptor. . . ." These complementary groups could then interact strongly in the relatively nonpolar medium in which they have become embedded.

Such a cooperative effect is quite plausible and may be effective in the binding of aromatic β-D-glucopyranosides to concanavalin A. The finding of contiguous polar and apolar regions on a variety of proteins may suggest that such a cooperative effect is of general occurrence in the interaction of a protein with other molecules. Others also have noted the enhancement of saccharide binding to proteins when alkyl aglycones are replaced by aromatic ones.[49–51] Strim *et al.*[49] found that *p*-aminophenyl glycosides inhibited the interaction of an enterobacterium polysaccharide with its homologous antibody more readily than the corresponding methyl glycoside. Springer

et al.[50] made an observation even more relevant to ours in showing that *p*-aminophenyl α-L-fucopyranoside is a more potent inhibitor of the hemagglutinin (lectin) from *Lotus tetragonologus* than methyl α-L-fucopyranoside.

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