trypsinogen "X," then the data of Table I indicate that, at least at low ionic strength, the effect of the trypsin to zymogen ratio extends to much higher ratios in the case of "X" as opposed to the A-zymogen.

The observation that the difference in the effect of the trypsin to zymogen ratio on the activation of A and "X" largely disappears in the presence of KCl (Table I) could be related to the fact that, at the applied pH, the two zymogens have an opposite net charge. In the absence of salt, the charge difference would affect the interaction of the zymogens with the positively charged trypsin to a greater extent.

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The Hydrophobic Character of Phenyl Glycosides and Its Relation to the Binding of Saccharides to Concanavalin A

In his studies on the interaction of organic compounds with proteins, Hansch (1, 2) has introduced the concept of π, a parameter indicative of the hydrophobicity of an atom or group of atoms. π has been defined as $\log P_X/P_H$, where P_X is the

TABLE I

HYDROPHOBIC SUBSTITUENT CONSTANTS FOR A VARIETY OF SUBSTITUENTS

Substituent	Observed substituent constants		
	Phenyl β-D-gluco-pyranoside	Phenoxyacetic acid[a]	Phenol[a]
H[b]	0	0	0
3-Methyl	0.51	0.51	0.56
4-Methyl	0.55	0.52	0.48
3-Ethyl	1.02	0.97	0.94
3-Isopropyl	1.36	1.30	—
3-t-Butyl	1.72	1.68	—
4-Methoxy	−0.02	−0.04	−0.12
4-Hydroxy	−0.64	−0.61	−0.87
4-Chloro	0.97	0.70	0.93
4-Iodo	1.46	1.26	1.45

[a] Values taken from Ref. 7.

[b] π_H is equal to zero by the definition, $\pi = \log P_X/P_H$.

partition coefficient of a substituted solute in a water-lipophilic binary system and P_H is the partition coefficient of the parent compound. The successful correlation of the hydrophobicity constants, π, with the binding constants of various aromatic compounds to proteins has been demonstrated by Hansch (2–4).

Recent studies (5) in our laboratory have suggested the presence of a region on the concanavalin A molecule, the jack bean hemagglutinin, adjacent to the specific saccharide binding site, which is capable of interacting specifically with the aromatic moiety of phenyl β-D-glucopyranosides. In an effort to elucidate the mode of interaction between the protein and the aglycone of the bound saccharide, we have experimentally determined π for a variety of aryl-substituted phenyl β-D-glycopyranosides. The partition of the glycosides between water and 1-octanol was performed in duplicate, in a manner similar to that employed by Hansch et al. (1), except on a considerably reduced scale. The concentration of the sugar in the water layer, after partition, was determined by the phenol-sulfuric acid method (6) with a reproducibility of ±3%. The average value for duplicate partition experiments was employed to calculate the partition coefficient of the solute and is accurate to ±3%. The corresponding concentration of the solute in the 1-octanol phase was calculated as the difference between the concentration of the sugar in the aqueous layer and the total quantity of saccharide employed in the partition experiment (about 8 mM).

TABLE II

ADDITIONAL π VALUES FOR SUBSTITUENTS OF
VARIOUS SUBSTITUTED PHENYL
β-D-GLUCOPYRANOSIDES

Substituent	π
2-CH$_3$	0.55
2-CH$_2$OH	−0.51
2-I	0.98
2-NO$_2$	−0.07
2-NH$_2$	−0.52
3-CH$_3$O	0.19
3-NO$_2$	0.20
3-CF$_3$	1.20
3,5-di-CH$_3$	0.97
2-CH(CH$_3$)$_2$-5-CH$_3$	1.78
2,3-(CH)$_4$	1.47
4-C(CH$_3$)$_3$	1.89
4-NO$_2$	0.27
4-NH$_2$	−1.96

TABLE III

PARTITION COEFFICIENTS OF VARIOUS ARYL AND
ALKYL GLYCOSIDES

Compound	P
p-Nitrophenyl β-D-glucopyranoside	0.366
p-Nitrophenyl α-D-glucopyranoside	0.410
p-Nitrophenyl β-D-galactopyranoside	0.256
p-Nitrophenyl α-D-mannopyranoside	0.656
Phenyl β-D-glucopyranoside	0.196
Benzyl β-D-glucopyranoside	0.198
Cyclohexyl β-D-glucopyranoside	0.236

Table I compares some π values obtained for the phenyl β-D-glucopyranoside system, with the values obtained by Fujita et al. (7) for the phenoxyacetic acid and phenol systems. It may be seen that our values compare very favorably with those reported in the literature. It appears, as Fujita et al. (7) have suggested, that π is indeed a constant which is indicative of the properties of the substituent and which is relatively independent of the remainder of the molecule. Additional π values for substituents in the phenyl β-D-glucopyranoside system are listed in Table II.

Furthermore, as assumed by Hansch and Fujita (8), the π value for a molecule containing multiple substituents is apparently equivalent to the sum of the π values for the individual substituents. Thus, as shown on Tables I and II, the π value for 3,5-dimethylphenyl β-D-glucopyranoside is approximately twice the value for the 3-methylphenyl derivative. Similarly, the π value for 2-iso-propyl-5-methylphenyl β-D-glucopyranoside is equal to the sum of the π values for the isopropyl and methyl groups. (Since the π value for the 2-isopropyl function was not determined, the π value for its positional isomer, the 3-isopropyl group, was used for these calculations. Examination of Tables I and II (cf. ref. 7) indicates that this is probably valid since the π values for positional isomers of hydrocarbon groupings are almost identical.)

It is interesting to note, as shown in Table III, that alteration of the glycosyl moiety of the solute may, though not necessarily, alter the partitioning properties of the molecule in a water-1-octanol system. Thus, p-nitrophenyl β-D-glucopyranoside displays a significantly higher partition coefficient than its C-4 epimer, p-nitrophenyl β-D-galactopyranoside, but is essentially no different from its α-anomer, i.e., p-nitrophenyl α-D-glucopyranoside. Similarly, inversion of the configuration about two carbon atoms of the glycose moiety dramatically alters the hydrophobic character of the saccharide. Thus, p-nitrophenyl α-D-mannopyranoside displays a considerably higher partition coefficient than either the analogous β-D-galactopyranoside or α-D-glucopyranoside. These properties may be highly significant in the transport of sugars through lipophilic cellular membranes.

Table III also demonstrates that phenyl β-D-glucopyranoside possesses a partition coefficient essentially no different from that of the corresponding glycoside containing a hydro-aromatic aglycone (cyclohexyl β-D-glucopyranoside) or for benzyl β-D-glucopyranoside which possesses an aromatic moiety not attached directly to the glycosidic oxygen atom.

We are presently investigating the binding of a large number of sugar glycosides containing a wide variety of aglycones to concanavalin A in an effort to determine a possible correlation between their binding constants and the π values of substituents on the phenyl ring. Preliminary studies indicate an excellent linear relationship between the appropriate π values and the negative common logarithm of the molar concentrations of meta (but not para) substituted phenyl β-D-glucopyranosides required to produce 50% inhibition of the concanavalin A-polysaccharide interaction (5). An insufficient number of ortho substituted phenyl β-D-glucopyranosides have been examined to discern any definite relationship.

These data are consistent with the hypothesis that concanavalin A possesses an apolar region, adjacent to the polar saccharide binding site, which is capable of interacting specifically with the meta, but not para, portion of aromatic nuclei

joined directly to the β-glycosidic oxygen atom, presumably by means of hydrophobic forces.

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