Carbohydrate Research, 78 (1980) 317–326

BINDING OF ALKYL β-D-XYLOPYRANOSIDES, CONTAINING BRANCHED-CHAIN, CYCLIC, AND SUBSTITUTED AGLYCON GROUPS, TO β-D-XYLOSIDASE FROM *Bacillus pumilus* PRL B12

ELIZA VAN DOORSLAER, HILDA KERSTERS-HILDERSON, AND CLEMENT K. DE BRUYNE
Lab. Algemene en Biologische Scheikunde, Ledeganckstraat 35, B-9000 Gent (Belgium)
(Received April 20th, 1979; accepted for publication, May 16th, 1979)

ABSTRACT

For a number of alkyl β-D-xylopyranosides having branched-chain, cyclic, and substituted aglycon groups, and binding to β-D-xylosidase from *B. pumilus* PRL B12, the binding constant K_i and (for some of them) the thermodynamic equilibrium parameters ΔH^0, ΔS^0, and ΔG^0 have been determined. Although the aglycon is bound through hydrophobic forces, no simple relationships between the binding parameters and the relative hydrophobicity of the alkyl β-D-xylopyranosides could be demonstrated. All of the available evidence suggests that the aglycon sub-site has a highly specific structure which forces the atoms of the aglycon group to occupy well-defined positions. The supplementary energy-requirements resulting from the imposed restrictions seem to be the main reason for the irregular way in which the binding parameters depend on the aglycon structure.

INTRODUCTION

In previous papers[1,2], we reported on the binding of aryl and *n*-alkyl β-D-xylopyranosides to the β-D-xylosidase (β-D-xyloside xylohydrolase, EC 3.2.1.37) from *Bacillus pumilus* PRL B12. Binding of the aglycon group of these compounds occurs through hydrophobic forces. However, it became clear from these studies (*1*) that, even for the series of simple *n*-alkyl xylosides, hydrophobic forces alone could not explain the differences between the binding parameters, and (*2*) that the effect of a CH_2 group was dependent on its position in the chain. This effect is possible if the active centre of the enzyme has a highly specific structure, so that the atoms of the alkyl chain are forced to take up well-defined positions, and are excluded from other positions. If this explanation is correct, xylosides having branched-chain and cyclic aglycon groups must bind in a still more irregular way. Therefore, we determined the binding constants and thermodynamic equilibrium parameters for a number of β-D-xylopyranosides having branched-chain and cyclic-alkyl aglycon groups. Since these compounds behave as fully competitive inhibitors of the β-D-xylosidase-catalysed hydrolysis of, for example, *p*-nitrophenyl β-D-xylopyranoside, the inhibition constant K_i (association), being the equilibrium constant for the

equilibrium enzyme + inhibitor $\rightleftharpoons$ enzyme–inhibitor complex, is a true measure of the affinity of the inhibitor molecule for the enzyme.

RESULTS AND DISCUSSION

The K_i values (association) at 25° (pH 7.15), ΔG^0 (25°), and some hydrophobicity parameters are collected in Table I. Table II shows the thermodynamic equilibrium parameters, as calculated from K_i values at five different temperatures. For comparison, values for *n*-alkyl derivatives[2] are included.

Alkyl β-D-xylopyranosides having a branched-chain aglycon

Aryl β-D-xylopyranosides are good substrates[1] for β-D-xylosidase of *B. pumilus* and thus the phenyl group must fit into the active centre of the enzyme. Since the thickness of plane of an aromatic-hydrocarbon ring (π-cloud thickness)[3] is 0.34 nm,

TABLE I

K_i VALUES AT 25°, AND HYDROPHOBICITY PARAMETERS

Compound	*Aglycon group*	K_i (M^{-1})	$-\Delta G°$ *(25°)* *(kJ.mol^{-1})*	π^a	*log P (alcohols)*[b]	*(Å)*[2 c]
	β-D-Xylopyranosides					
1	Isopropyl	18	7.2	1.30	0.14	
2	2-Methyl-1-propyl	517	15.5	1.80	0.64	249.10
3	3-Methyl-1-butyl	667	16.1	2.30	1.14	274.60
4	Neopentyl	195	13.1		1.36	270.14
5	2,2-Dimethyl-1-butyl	327	14.3			290.76
6	2-Butyl	39	9.1	1.82		
7	3-Pentyl	47	9.5	2.20		
8	*tert*-Butyl	4	3.4	1.68	0.37	
9	*tert*-Pentyl	8	5.2		0.89	
10	Benzyl	1 160	17.5	2.01	1.10	240.71
11	2-Phenylethyl	667	16.1			273.90
12	3-Phenylpropyl	439	15.1			302.27
13	Cyclohexyl	205	13.2	2.51		279.10
14	Cyclopentyl	81	10.9	2.14		255.40
15	3-Methylcyclohexyl	426	15.0	3.0		304.85
16	2-Chloroethyl	166	12.6			
17	2-Hydroxyethyl	13	6.4			
18	*p*-Nitrobenzyl	1 084	17.3			
	1-Thio-β-D-xylopyranosides					
19	Benzyl	448	15.1			
20	*p*-Nitrobenzyl	161	12.6			

[a]Hansch hydrophobicity parameter (from Ref. 6). [b]Partitition coefficients for corresponding alcohols (from Ref. 9). [c]Cavity surface-area for corresponding alkanes (from Ref. 5).

TABLE II

K_i VALUES AND EQUILIBRIUM PARAMETERS (pH 7.15)

Inhibitor	K_i (M^{-1})					$-\Delta H^\circ$ (*kJ.mol*$^{-1}$)	$-\Delta G^\circ$ (25°) (*kJ.mol*$^{-1}$)	ΔS° (25°) (*J.K*$^{-1}$.*mol*$^{-1}$)
	10°	*15°*	*20°*	*25*	*28°*			
Benzyl β-D-xylopyranoside	2038	1617	1350	1160	1012	27.7 ± 0.9	17.5	− 35 ± 3
Benzyl 1-thio-β-D-xylopyranoside	623	528	498	448	420	14.7 ± 1.1	15.0	+ 1 ± 4
D-Xylose				23		50.0 ± 3.0	7.7	−142 ± 10
Methyl β-D-xylopyranoside				23		28.0 ± 2.0	7.7	− 67 ± 6
Ethyl β-D-xylopyranoside				42		25.6 ± 1.0	9.3	− 54 ± 4
Propyl β-D-xylopyranoside				235		30.1 ± 1.1	13.5	− 54 ± 4
Butyl β-D-xylopyranoside				784		35.2 ± 0.8	16.5	− 63 ± 3
Methyl 1-thio-β-D-xylopyranoside				116		34.1 ± 0.4	11.8	− 71 ± 1
Ethyl 1-thio-β-D-xylopyranoside				336		31.3 ± 0.6	14.4	− 58 ± 2

and the Van der Waals radius of a methyl group[3] is 0.2 nm, the aglycon group of methyl β-D-xylopyranoside probably also fits into the active centre. Addition of a methyl group at C-1 of the aglycon (methyl→ethyl) increases K_i, and thus this methyl group contributes to the binding. However, when a second and third group are added at C-1 (isopropyl, *tert*-butyl, *tert*-pentyl), K_i decreases. The effect of the third group (*tert*-butyl) is such that even the first CH_2-group no longer contributes to the binding (*cf.* the methyl derivative). On the other hand, benzyl β-D-xylopyranoside is a good inhibitor (Table I). Thus, when two hydrogen atoms are bound at C-1, even the large phenyl-ring can position itself so that it is "bound" without hindrance. With the symmetrical *tert*-butyl group, rotation around the C-1(alkyl)–O(exocyclic) axis has no effect, and the strongly hindered rotation around the O(exocyclic)–C-1(xylose) axis is probably insufficient to allow the correct positioning of the tertiary group. The above findings clearly indicate that the enzyme structure around the C-1(alkyl) position involves much more than a simple "cleft".

As *tert*-butyl and *tert*-pentyl xylosides bind less effectively than D-xylose, the glycon moiety of the inhibitor must have lost part of its binding capacity, probably because the bulky aglycon prevents its correct positioning. Another possible explanation of the unfavourable effect of tertiary (and, to a lesser extent, secondary) alkyl groups would be that they hinder a conformational change of the xyloside from $^4C_1$(D) (in solution) to, for example, $^1C_4$(D) (on the enzyme). However, there is no evidence that such conformational change is necessary for binding.

That CH_2-groups, added as spacer groups between the exocyclic oxygen and the branching point, increase K_i far more than can be accounted for by the increase in relative hydrophobicity ($\Delta\Delta G^0 \sim 3.4$ kJ.mol^{-1}/per CH_2)[4] is clearly illustrated by the following examples:

isopropyl→2-methyl-1-propyl: $\Delta\Delta G^0(K_i) = -8.3$ kJ.mol^{-1};
tert-butyl→neopentyl: $\Delta\Delta G^0(K_i) = -9.6$ kJ.mol^{-1};
isopropyl→3-methyl-1-butyl: $\Delta\Delta G^0(K_i) = -9.0$ kJ.mol^{-1}.

Moreover, the last example, and the value $\Delta\Delta G^0(K_i) = 0.7$ kJ.mol^{-1} for 2-methyl-1-propyl→3-methyl-1-butyl show that the contribution to the binding of the second spacer-group is small compared to that of the first. The main effect clearly results from the fact that C-1 now bears two hydrogen atoms.

When a methyl group is added at C-2 of propyl (→2-methyl-1-propyl), K_i increases from 235 to 517 M^{-1} and $\Delta\Delta G^0(K_i) = 2$ kJ.mol^{-1}. Comparison with log P (alcohols) shows (Table I) that this increase can be explained by the enhanced [$\Delta\Delta G^0$(log P) = −1.7 kJ.mol^{-1}] relative hydrophobicity. However, addition of a methyl group at C-3 of butyl (→3-methyl-1-butyl) decreases K_i from 784 to 667 M^{-1}.

When a second methyl group is added at C-2 of 2-methyl-1-propyl (→neopentyl), K_i decreases from 517 to 195 M^{-1} and $\Delta\Delta G^0(K_i) = +2.4$ kJ.mol^{-1}. As, in this case, the branching point is separated from the exocyclic oxygen-atom by a CH_2-group, an unfavourable effect on a conformational change of the glycon part of the inhibitor is less likely. Substitution by a methyl group on an already branched

chain (neopentyl→2,2-dimethyl-1-butyl) increases K_i from 195 to 327 M^{-1} and $\Delta\Delta G^0(K_i) = -1.3$ kJ.mol^{-1}. When a methyl group is added to isopropyl (→2-butyl), K_i increases from 18 to 39 M^{-1} and $\Delta\Delta G^0(K_i) = -1.9$ kJ.mol^{-1}. However, addition of a methyl group to 2-butyl (*i.e.*, to the other "branch" of isopropyl) has practically no effect.

Replacement of a hydrogen atom at C-2 by an hydroxyl group (ethyl→2-hydroxyethyl) slightly decreases K_i, whereas replacement by a chlorine atom (→2-chloroethyl) increases K_i, although the 2-chloroethyl derivative is less hydrophobic.

The foregoing examples clearly indicate that, besides the relative hydrophobicity of the alkyl chain, other factors influence the binding of the inhibitor. Probably, an important energy-contribution stems from the conformation forced on the alkyl chain by the structure of the active site. Since no information about this structure is available, further analysis of the influence of branching alkyl-chains would be too hypothetical. However, it seems very clear that the active site (especially around C-1 of the alkyl chain) has a highly specific structure.

Alkyl β-D-xylopyranosides having a cyclic aglycon

Since the cyclic alkyl-chains in derivatives **13–15** possess the branched-chain "isopropyl structure", the binding constants will be lower than would be expected from their relative hydrophobicity. However, the effect of branching will remain constant within the series.

Fig. 1 shows that, for derivatives **13–15**, $\Delta G^0(K_i)$ is linearly related to the "cavity surface area"[5], and to the free energy of transfer (from water to the hydrocarbon phase) for the corresponding alkanes[4] [ΔG^0(alk)]. Fig. 2 shows that, for derivatives having the branched-chain isopropyl structure, $\Delta G^0(K_i)$ is linearly related to the Hansch[6] hydrophobicity parameter π.

The natural substrate of β-D-xylosidase is β-D-xylobiose (4-*O*-β-D-xylopyrano-

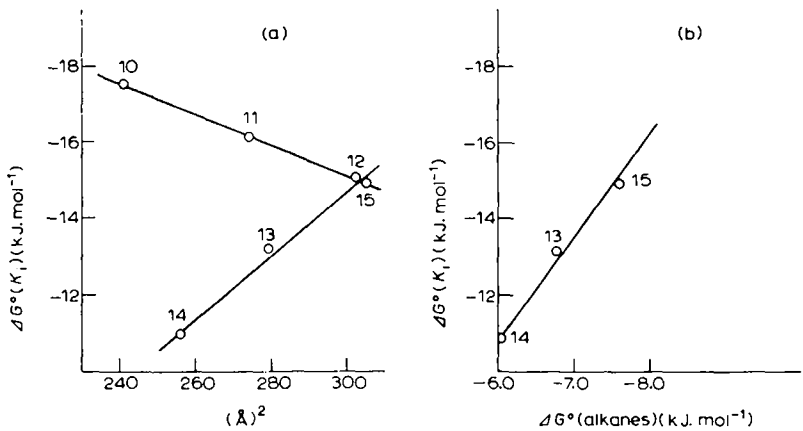

Fig. 1. (*a*) $\Delta G^\circ(K_i)$ *versus* cavity surface-area (Å)^2; (*b*) $\Delta G^\circ(K_i)$ *versus* ΔG° for transfer (water→hydrocarbon phase) of corresponding alkanes (Ref. 4).

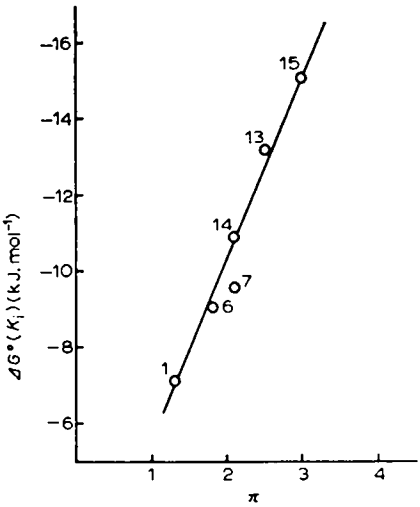

Fig. 2. $\Delta G^{\circ}(K_i)$ *versus* the Hansch hydrophobicity parameter π.

syl-D-xylopyranose)[7]. The "aglycon" group of this substrate is a D-xylopyranose residue, and it resembles the structure of the cyclic-alkyl groups. Thus, the structure of the enzyme is probably such that it can accept these cyclic structures. This possibility would explain the above-cited, simple relationships with hydrophobicity parameters.

For the transfer from water to the hydrocarbon phase, the free energy of transfer[4] (ΔG^0) is -25.1 kJ.mol^{-1} for cyclopentane, -28.2 kJ.mol^{-1} for cyclohexane, and -31.9 kJ.mol^{-1} for 3-methylcyclohexane. Since the free energy of binding for D-xylose is -7.7 kJ.mol^{-1}, the contribution of the cyclic aglycons to the binding of derivatives **13–15** is -5.49, -3.19, and -7.30 kJ.mol^{-1}, respectively. The first reason for these low contributions is the branched structure at C-1 (*cf.* isopropyl). The second reason is probably that desolvation of the rings is incomplete. This effect is indicated by the fact that the slope of the function line in the plot $\Delta G^0(K_i)$ *versus* ΔG^0(alk) is not unity but ~ 0.64, and the slope in the plot $\Delta G^0(K_i)$ *versus* (Å)^2 is 0.08 or 64% of the slope in a plot of ΔG^0(alk) *versus* (Å)^2. A further indication can be found when cyclohexyl and 3-methylcyclohexyl are compared. $\Delta\Delta G^0(K_i) = -1.8$ kJ.mol^{-1}, whereas for the alkane-model system, $\Delta\Delta G^0$(alk) $= -3.7$ kJ.mol^{-1}. Because of the regular increase (Fig. 2), conformational changes of the ring structures are less likely (although possible).

Phenyl-substituted alkyl β-D-xylopyranosides

The data in Table I and Fig. 1 show that, in this series, $\Delta G^0(K_i)$ is linearly related to the number of CH_2 spacer-groups, and to (Å)^2 for corresponding alkylbenzenes[5]. However, the binding becomes weaker with increasing hydrophobicity. Thus, the favourable effect of the aromatic ring decreases regularly when the ring is moved away from the exocyclic oxygen-atom. This effect is also clearly illustrated by the following examples:

methyl→benzyl $\Delta\Delta G^0 = -9.5$ kJ.mol^{-1};
ethyl→2-phenylethyl $\Delta\Delta G^0 = -2.5$ kJ.mol^{-1};
propyl→3-phenylpropyl $\Delta\Delta G^0 = -1.6$ kJ.mol^{-1};

or (replacement of methyl by phenyl),

ethyl→benzyl $\Delta\Delta G^0 = -7.7$ kJ.mol^{-1};
propyl→2-phenylethyl $\Delta\Delta G^0 = -2.7$ kJ.mol^{-1};
butyl→3-phenylpropyl $\Delta\Delta G^0 = +1.7$ kJ.mol^{-1}.

However, in such model systems as transfer from water to a hydrocarbon phase, replacement of a hydrogen atom or a methyl group by a phenyl ring yields a nearly constant increase in hydrophobicity (ΔG^0), as illustrated by following examples[4]:

methane→methylbenzene $\Delta\Delta G^0 = -10$ kJ.mol^{-1};
ethane→ethylbenzene $\Delta\Delta G^0 = -9$ kJ.mol^{-1};
propane→propylbenzene $\Delta\Delta G^0 = -9$ kJ.mol^{-1};

or, ethane→methylbenzene $\Delta\Delta G^0 = -6$ kJ.mol^{-1};
propane→ethylbenzene $\Delta\Delta G^0 = -5$ kJ.mol^{-1}.

Consequently, the decrease in K_i must be caused by the structure of the active site of the enzyme.

Because of the peculiar behaviour of phenyl-substituted alkyl β-D-xylopyranosides, we determined K_i for benzyl β-D-xylopyranoside and its 1-thio analogue at five temperatures, and calculated the thermodynamic equilibrium parameters (Table II). For benzyl β-D-xyloside, compared to D-xylose, $\Delta\Delta G^0 = -9.7$ kJ.mol^{-1}, $\Delta\Delta H^0 = +22.3$ kJ.mol^{-1}, and $\Delta\Delta S^0 = +107$ J.K^{-1}.mol^{-1}. Thus, the better binding of the benzyl derivative stems from the entropy factor, whereas the enthalpy has become less favourable. The large, positive value of $\Delta\Delta S^0$ clearly indicates hydrophobic binding, caused by the return of highly structured, water molecules around the benzyl group to the less-structured bulk-water. However, comparison with model systems reveals that the magnitude, and even the sign, of $\Delta\Delta H^0$ and $\Delta\Delta S^0$ cannot be explained merely by a hydrophobic contribution of the benzyl group to the overall binding-energy. For the transfer of ethylbenzene from water to an organic phase[4], $\Delta H^0 = -1.67$ kJ.mol^{-1} (*i.e.*, negative) and $\Delta S^0 = +79$ J.K^{-1}.mol^{-1}. In this case, desolvation is complete and yet ΔS^0 is smaller than $\Delta\Delta S^0$ for the benzyl xyloside. Theoretically, the benzyl aglycon can be formed by replacement of a hydrogen atom at C-1 of methyl β-D-xyloside (or of the methyl group in ethyl β-D-xyloside) by a phenyl group. Such a substitution would yield:

methyl→benzyl $\Delta\Delta G^0 = -9.65$ kJ.mol^{-1},
$\Delta\Delta H^0 = +0.3$ kJ.mol^{-1},
and $\Delta\Delta S = +32$ J.K^{-1}.mol^{-1};

and ethyl→benzyl $\Delta\Delta G^0 = -8.1$ kJ.mol^{-1},
$\Delta\Delta H^0 = -1.7$ kJ.mol^{-1},
and $\Delta\Delta S^0 = +19$ J.K^{-1}.mol^{-1}.

In model systems[4], the same substitution yields:

ethane→methylbenzene $\Delta\Delta G^0 = -6.4$ kJ.mol^{-1},
$\Delta\Delta H^0 = -11.9$ kJ.mol^{-1},
and $\Delta\Delta S^0 = -18.3$ J.K^{-1}.mol^{-1};
and propane→ethylbenzene $\Delta\Delta G^0 = -4.3$ kJ.mol^{-1},
$\Delta\Delta H^0 = 9.16$ kJ.mol^{-1},
and $\Delta\Delta S^0 = -16.5$ J.K^{-1}.mol^{-1}.

Thus, compared to the model systems, substitution by a phenyl group in the enzymic system yields a $\Delta\Delta S^0$ value that is positive and too favourable, whereas $\Delta\Delta H^0$, being ~ 0, is not favourable enough.

Benzyl 1-thio-β-D-xylopyranoside binds less than the oxygen analogue, although the intrinsic, relative hydrophobicity of thioglycosides is higher than that of their oxygen analogues[8]. Compared to D-xylose, $\Delta\Delta H^0 = +35$ kJ.mol^{-1} and $\Delta\Delta S^0 = +143$ J.K^{-1}.mol^{-1} (*cf.* ethylbenzene: $\Delta S^0 = +80$ J.K^{-1}.mol^{-1}). Compared to benzyl β-D-xylopyranoside, $\Delta\Delta H^0 = +13$ kJ.mol^{-1} and $\Delta\Delta S^0 = +36$ J.K^{-1}.mol^{-1}.

Replacement of a hydrogen atom or a methyl group by a phenyl ring yields, for the enzymic system,

methyl 1-thio→benzyl 1-thio: $\Delta\Delta G^0 = 3$ kJ.mol^{-1},
$\Delta\Delta H^0 = +19$ kJ.mol^{-1},
and $\Delta\Delta S^0 = +72$ J.K^{-1}.mol^{-1};
and ethyl 1-thio→benzyl 1-thio: $\Delta\Delta G^0 = -0.6$ kJ.mol^{-1},
$\Delta\Delta H^0 = +16$ kJ.mol^{-1},
and $\Delta\Delta S^0 = +59$ J.K^{-1}.mol^{-1}.

The same conclusions can be drawn as for the oxygen analogue. The disagreement with the model systems is even more pronounced. Although water–organic solvent systems are far from being perfect models for the enzymic system, the disagreement is too large to be ignored.

However, the afore-mentioned comparisons are valid only if all of the inhibitors used bind to the enzyme in the same way. For methyl and ethyl β-D-xylosides, the main part of the binding energy originates from the binding of the correctly positioned glycon. However, this is not necessarily true for phenyl-substituted alkyl xylosides. The overall, free energy of binding for D-xylose is $\Delta G^0 = -7.7$ kJ.mol^{-1}, whereas the free energy of transfer (water→organic phase) of methylbenzene is 10 kJ.mol^{-1}. It is possible that, for benzyl derivatives, the decrease in free energy resulting from correct positioning of the benzyl ring (maximal hydrophobic-binding) is greater than the decrease that would result from a correct fit of the glycon moiety (maximal glycon-binding) without maximal, hydrophobic interaction.

If such complexes are compared with "normal" complexes, the values of $\Delta\Delta G^0$, $\Delta\Delta H^0$, and $\Delta\Delta S^0$ will reflect more than differences in relative hydrophobicity, and will no longer be comparable with the values of the model systems.

Incorrect positioning of the xylose moiety of the inhibitor will result in a loss of binding enthalpy [ΔH^0(xylose) = -50 kJ.mol^{-1}], but at the same time the entropy [ΔS^0(xylose) = -142 J.K^{-1}.mol^{-1}] will become less negative. Thus, the afore-mentioned hypothesis would explain why comparisons with "normal" complexes and with model systems consistently yielded $\Delta\Delta H^0$ and $\Delta\Delta S^0$ values that were too large (positive). It would also explain why the value of K_i decreases with increasing number of spacer groups between the phenyl ring and the exocyclic oxygen-atom, if it is assumed that the addition of spacer groups removes the xylose moiety still further away from the correct binding-position. The increase in binding energy resulting from the enhanced hydrophobicity of the aglycon would then be more than cancelled by the loss of glycon-binding energy. From the partition coefficients in water–octanol[9], it follows that the difference in free energy of transfer ($\Delta\Delta G^0$) between benzene and nitrobenzene is 1.5 kJ.mol^{-1}; between anisole (methoxybenzene) and *p*-nitroanisole, $\Delta\Delta G^0$ is $\sim$0. Thus, substitution by a nitro group does not significantly change the relative hydrophobicity. Since benzyl and nitrobenzyl β-D-xylopyranoside have the same binding constant (Table I), both the benzyl and nitrobenzyl groups bind by hydrophobic forces only. More-direct interactions with enzyme groups would result in a larger difference between the K_i values.

In our previous paper[2], it was argued that, because of the change in bond angles and distances, the mode of binding of 1-thioxylosides was different from that of their oxygen analogues. This is probably also true for benzyl 1-thio-β-D-xylopyranoside. Comparison of its binding parameters with those of the oxygen analogue shows that the entropy factor has become more favourable, but that part of the binding enthalpy has been lost. The simplest explanation would be that the aglycon group is locked-up in the same position as for the oxygen analogue, but that the glycon-binding energy has further diminished, because of the altered bond-angles. However, since the total, free energy of binding has also diminished (the enzyme–inhibitor complex has become *less* stable) and since substitution by a nitro group significantly decreases K_i (Table I), it seems probable that the mode of binding has changed more drastically. The exact nature of this change is unknown.

CONCLUSIONS

From the foregoing discussion, it is evident that the aglycon part of the active site of β-D-xylosidase does not resemble a simple cleft or hydrophobic pocket in which the aglycon group is simply buried. Although the main aglycon-binding forces are undoubtedly of hydrophobic nature, there is no simple relationship between binding parameters and relative hydrophobicity. All of the evidence available indicates that the aglycon sub-site has a very specific, three-dimensional structure, which forces the atoms of the aglycon group to take up well-defined positions. The

supplementary energy-requirements resulting from the restrictions imposed on the aglycon seem to be the major reason for the irregular and unpredictable way in which the binding parameters depend on the aglycon structure.

EXPERIMENTAL

The following substrate and inhibitors were synthesised by literature procedures: *p*-nitrophenyl β-D-xylopyranoside[10], alkyl β-D-xylopyranosides[11,12], and alkyl 1-thio-β-D-xylopyranosides[13]. The isolation, purification, and standardisation of the β-D-xylosidase have been described[14]. The substrate used was *p*-nitrophenyl β-D-xylopyranoside, and its hydrolysis was followed continuously at 400 nm. As all of the xylopyranosides tested behaved as fully competitive inhibitors, the inhibition constant K_i (expressed as association constant) could be calculated by the classical formula[15,16]. Further experimental details were as described previously[2].

ACKNOWLEDGMENTS

This work was supported by a NATO Research Grant and by the Nationaal Fonds voor Wetenschappelijk Onderzoek.

REFERENCES

1 H. KERSTERS-HILDERSON, E. VAN DOORSLAER, AND C. K. DE BRUYNE, *Carbohydr. Res.*, 65 (1978) 219–227.
2 H. KERSTERS-HILDERSON, E. VAN DOORSLAER, AND C. K. DE BRUYNE, *Carbohydr. Res.*, 78 (1980) 163–172.
3 A. J. GORDON AND R. A. FORD, *The Chemist's Companion*, Wiley, New York, 1972, p. 109.
4 C. TANFORD, *The Hydrophobic Effect*, Wiley, New York, 1973, pp. 9–20.
5 R. B. HERMAN, *J. Phys. Chem.*, 76 (1972) 2754–2759.
6 A. C. HANSCH AND E. W. DEUTSCH, *Biochim. Biophys. Acta*, 126 (1966) 117–128.
7 H. KERSTERS-HILDERSON, E. VAN DOORSLAER, C. K. DE BRUYNE, AND KEI YAMANAKA, *Anal. Biochem.*, 80 (1977) 41–50.
8 C. K. DE BRUYNE AND M. YDE, *Carbohydr. Res.*, 56 (1977) 153–164.
9 A. LEO, C. HANSCH, AND C. CHURCH, *J. Med. Chem.*, 12 (1969) 766–771.
10 F. G. LOONTIENS AND C. K. DE BRUYNE, *Naturwissenschaften*, 51 (1964) 359.
11 C. K. DE BRUYNE AND G. VAN DER GROEN, *Carbohydr. Res.*, 2 (1966) 173–175.
12 C. K. DE BRUYNE AND G. VAN DER GROEN, *Carbohydr. Res.*, 5 (1967) 95–97.
13 M. YDE AND C. K. DE BRUYNE, *Carbohydr. Res.*, 30 (1973) 205–206.
14 H. KERSTERS-HILDERSON, F. G. LOONTIENS, M. CLAEYSSENS, AND C. K. DE BRUYNE, *Eur. J. Biochem.*, 7 (1969) 434–441.
15 M. DIXON AND E. C. WEBB, *Enzymes*, Longmans Green, London, 2nd edition, 1964, pp. 120–150.
16 K. J. LAIDLER, *The Chemical Kinetics of Enzyme Action*, Clarendon Press, Oxford, 1958, pp. 117–143.