Carbohydrate Research, 181 (1988) 1–12
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE NUCLEAR OVERHAUSER EFFECT AND STRUCTURAL FACTORS DETERMINING THE CONFORMATIONS OF DISACCHARIDE GLYCOSIDES

GRIGORY M. LIPKIND, ALEXANDER S. SHASHKOV, SUREN S. MAMYAN, AND NIKOLAY K. KOCHETKOV
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)

(Received November 17th, 1987; accepted for publication, January 25th, 1988)

ABSTRACT

Conformations of (1→2)-, (1→3)-, and (1→4)-linked disaccharide methyl glycosides involving aldohexopyranose residues have been studied on the basis of the inter-unit n.O.e. and theoretical calculations using atom–atom potential functions. The preferred conformations and the n.O.e. values are determined by the absolute configurations of constituent residues, the configuration and position of the glycosidic linkage, and the orientation of the protons at the aglycon carbon associated with the glycosidic linkage and the adjacent carbons.

INTRODUCTION

A knowledge of the conformation of the glycosidic linkage in disaccharides in aqueous medium is necessary for an understanding of the spatial organization of various biologically important oligo- and poly-saccharides.

We have studied, both experimentally and theoretically, the conformations in aqueous solution of (1→2)-, (1→3)-, and (1→4)-linked methyl disaccharide glycosides[1–8], involving residues with the *gluco, manno, galacto,* and *xylo* configuration, and also (1→3)-linked compounds the glycosylated monosaccharide of which has the *talo* configuration. These studies have enabled an analysis of those structural and stereochemical factors which determine the conformation of the glycosidic linkage, and this analysis is now reported.

RESULTS AND DISCUSSION

For the structures discussed (see Tables I–III), the n.O.e.'s were measured after pre-irradiation of the anomeric protons of the glycosidic linkages (and also of the other protons, if necessary). The n.O.e. is defined as the ratio of the intensities of the signals for nuclei d and s on saturation of nucleus s. The n.O.e. value f_s^d in a system composed of j protons is defined by the expression[9].

$$f_s^d = r_{ds}^{-6}/2 \sum_{j \neq d} r_{dj}^{-6} - \sum_{j \neq d,s} r_{dj}^{-6} \cdot f_s^j/2 \sum_{j \neq d} r_{dj}^{-6}.$$

The n.O.e. is determined by the distance r_{ds} between the protons s and d.

0008-6215/88/$ 03.50

TABLE I

N.O.E. (%) ON PRE-IRRADIATION OF H-1′ FOR COMPOUNDS IN WHICH THE AGLYCON HAD ONE EQUATORIAL PROTON AT THE β-CARBON[5–8]

Glycoside	*Structural factors*	*Observed protons*	
		H-2	*H-1*
α-D-Glc-(1→2)-α-L-Rha-OMe	α, D-L, n_{ev}, S_a, H_{n-1}	7.4	7.5
β-L-Rha-(1→2)-α-L-Rha-OMe	β, L-L, n_{ev}, S_a, H_{n-1}	10.4	8.4
α-D-Glc-(1→2)-α-D-Glc-OMe	α, D-D, n_{ev}, S_e, H_{n-1}	3.3	2.6
		H-3	*H-2*
α-D-Glc-(1→3)-α-L-Rha-OMe	α, D-L, n_{od}, S_e, H_{n-1}	4.5	4.5
β-L-Rha-(1→3)-α-L-Rha-OMe	β, L-L, n_{od}, S_e, H_{n-1}	11.0	8.5
		H-3	*H-4*
α-D-Glc-(1→3)-β-D-Gal-OMe	α, D-D, n_{od}, S_e, H_{n+1}	2.0	4.5
α-D-Man-(1→3)-β-D-Gal-OMe	α, D-D, n_{od}, S_e, H_{n+1}	3.8	8.1
β-L-Rha-(1→3)-β-D-Gal-OMe	β, L-D, n_{od}, S_e, H_{n+1}	4.8	4.5
		H-4	*H-5*e
β-D-Glc-(1→4)-β-D-Xyl-OMe	β, D-D, n_{ev}, S_e, H_{n+1}	9.5	4.0
		H-2	*H-1*
β-D-Glc-(1→2)-α-L-Rha-OMe	β, D-L, n_{ev}, S_a, H_{n-1}	10.5	1.0
α-L-Rha-(1→2)-α-L-Rha-OMe	α, L-L, n_{ev}, S_a, H_{n-1}	8.6	
β-D-Glc-(1→2)-α-D-Glc-OMe	β, D-D, n_{ev}, S_e, H_{n-1}	6.8	
		H-3	*H-2*
β-D-Glc-(1→3)-α-L-Rha-OMe	β, D-L, n_{od}, S_e, H_{n-1}	4.6	
α-L-Rha-(1→3)-α-L-Rha-OMe	α, L-L, n_{od}, S_e, H_{n-1}	11.0	
α-D-Man-(1→3)-α-D-Man-OMe	α, D-D, n_{od}, S_e, H_{n-1}	11.0	
		H-3	*H-4*
β-D-Glc-(1→3)-β-D-Gal-OMe	β, D-D, n_{od}, S_e, H_{n+1}	7.1	0.7
β-D-Man-(1→3)-β-D-Gal-OMe	β, D-D, n_{od}, S_e, H_{n+1}	5.9	0.7
α-L-Rha-(1→3)-β-D-Gal-OMe	α, L-D, n_{od}, S_e, H_{n+1}	6.6	0.6
		H-4	*H-5*e
α-D-Glc-(1→4)-β-D-Xyl-OMe	α, D-D, n_{ev}, S_e, H_{n+1}	16.0	1.0

The n.O.e. values for disaccharides have an important bearing on the conformations of oligosaccharides and polysaccharides. Comparison of the n.O.e. values for disaccharides and disaccharide units of polysaccharides may give a general idea of the conformations of polysaccharide chains in solution.

TABLE II

N.O.E. (%) IN GLYCOSYL-(1→3)-TALOSIDES

Glycoside	Structural factors	Observed protons		
		H-3	H-4	H-2
α-D-Man-(1→3)-β-D-Tal-OMe	α, D-D, n_{od}, S_e	3.1	5.2	[a]
β-L-Rha-(1→3)-β-D-Tal-OMe	β, L-D, n_{od}, S_e	4.1	4.3	0.5
β-D-Man-(1→3)-β-D-Tal-OMe	β, D-D, n_{od}, S_e	5.3	[b]	4.7
α-L-Rha-(1→3)-β-D-Tal-OMe	α, L-D, n_{od}, S_e	5.7	0.7	6.3

[a] Chemical shifts for H-2 and H-4 of the talose residue coincide. [b] Chemical shifts for H-4 of the talose residue and for H-2 of the mannose residue coincide.

TABLE III

N.O.E. (%) IN DISACCHARIDES WITH BULKY SUBSTITUENTS AT BOTH β-CARBONS OF THE AGLYCON[2–4,7]

Disaccharide	Structural factors	Observed protons	
		H-4	H-6
β-D-Glc-(1→4)-α-D-Glc-O-PO_3	β, D-D, n_{ev}, S_e	8.8	~2
α-D-Glc-(1→4)-β-D-Gal-OMe	α, D-D, n_{ev}, S_a	8.4	3.7
α-D-Glc-(1→4)-α-L-Rha-OMe	α, D-L, n_{ev}, S_e	9.5	2
α-D-Glc-(1→4)-β-D-Glc-OMe	α, D-D, n_{ev}, S_e	5.9	
β-D-Glc-(1→4)-β-D-Gal-OMe	β, D-D, n_{ev}, S_a	6.5	
β-D-Glc-(1→4)-α-L-Rha-OMe	β, D-L, n_{ev}, S_e	8.2	

The conformations of the disaccharide glycosides, which are optimal as regards the non-bonded interactions, were calculated using atom–atom potential functions. In aqueous solutions of disaccharides, the 6-12-type potentials are of "medium force" and they lead to a reasonable account of the conformational equilibrium[1]. The average values $\langle f_s^d \rangle$ of the n.O.e. can be calculated by the above formula for f_s^d if the potential energy surface of the disaccharide is known, *i.e.*, its conformational map ϕ–ψ where ϕ and ψ are the angles of rotation around the glycosidic bonds* (Fig. 1). Comparison of the observed and calculated values of the n.O.e. allows the conformational equilibria in aqueous solutions of disaccharides to be defined.

The preferred conformations of disaccharides are determined mainly by the non-bonded interactions associated with C-1′, H-1′, and O-5′. The presence of 2′-substituents makes the location of the aglycon near C-2′ unfavourable both in

---

*ϕ is the angle of rotation around the C-1′–O bond and ψ is the angle of rotation around the O–C(n) bond, where C(n) (n = 2–4) is the aglycon carbon involved in the glycosidic linkage. Primed numbers refer to atoms of the glycosylating residue. ϕ is zero when the C-1′–H-1′ and O–C(n) bonds are *cis*, and ψ is zero when the C-1′–O and C(n)–H(n) bonds are *cis*.

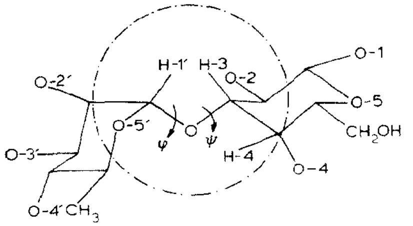

Fig. 1. Molecular model for the α-L-Rha-(1→3)-D-Gal unit. The moieties which determine the conformational states of the unit are encircled.

energy and in entropy[2-7]. Therefore, the aglycon preferentially adopts a *gauche* orientation with respect to the C-1'–O-5' bond. For the glycosylated residue, the most important non-bonded interaction involves H(n) attached to the substituted carbon C(n) (α-carbon), as well as the equatorial substituents belonging to the β-carbons (encircled in Fig. 1).

The conformational properties of disaccharides depend essentially on whether protons or more bulky groups (*e.g.*, a hydroxymethyl group) are the equatorial substituents at the β-carbons. Consequently, the disaccharides can be classified into three groups with (*a*) an equatorial proton at one of the β-carbons of the aglycon, (*b*) equatorial protons at both β-carbons of the aglycon, and (*c*) bulky equatorial substituents at both these carbons. In the sequel, the connection between the structure of disaccharide glycosides [except those which are (1→6)-linked] and the n.O.e. values for each stereochemical group will be analysed.

The data[1-8] show that the main structural factors which determine n.O.e. in disaccharides composed of aldohexopyranose residues are (*1*) the absolute configurations (D-D, L-L, D-L, L-D)* of the constituent residues, (*2*) the configuration (α or β) of the linkage, (*3*) the position of the α-carbon in the carbohydrate residue of the aglycon [whether its number is even, n_{ev} (C-2 or C-4), or odd, n_{od} (C-3)], and (*4*) the axial (S_a) or equatorial (S_e) orientation of the glycosylating residue at the α-carbon. Disaccharides of group (*a*) are characterized by an additional factor: (*5*) the position of the β-carbon carrying the equatorial proton (in the sense that its number is larger, H_{n+1}, or smaller, H_{n-1}, than that of the α-carbon of the aglycon). The physical meaning of the structural factors *3*–*5* is clarified below.

Aglycon with an equatorial proton at one β-carbon. — Of the numerous representatives of this class, n.O.e. data are available for (1→3)-linked disaccharides[5-7] with the *manno* or *galacto* configuration of the glycosylated residue and for a series of (1→2)- and (1→4)-linked disaccharide glycosides[6,8] (see Table I).

The disaccharide glycosides considered can be divided into two series according to the characteristic features in the n.O.e. spectra. For the first series, listed in the upper half of Table I, pre-irradiation of H-1' causes a comparable n.O.e. on the signals of the proton on the α-carbon of the aglycon and the adjacent equatorial

---

*D-Aldohexopyranoses exist usually as $^4C_1$ conformers, and L-aldohexopyranoses as $^1C_4$ conformers[10].

proton on the β-carbon. In contrast, for the second series (see the lower half of Table I), the n.O.e. on the signal for the equatorial proton either vanished or was an order of magnitude lower than that for H(*n*).

A comparison of the structures in the upper and lower halves of Table I which have an identical mode of substitution and configuration of the aglycon (*i.e.*, with fixed structural factors *3*–*5*), for example, the glycosyl-(1→3)-galactosides[7], shows that the disaccharide glycosides in these groups differ in either the configuration of the glycosidic linkage, *e.g.*, α-D-Glc-(1→3)-β-D-Gal-OMe and β-D-Glc-(1→3)-β-D-Gal-OMe, or the absolute configuration of one of the monosaccharide residues, *e.g.*, β-L-Rha-(1→3)-β-D-Gal-OMe and β-D-Man-(1→3)-β-D-Gal-OMe. Thus, the n.O.e. values are determined by the structural factors *1* and *2*, and simultaneous inversion of the absolute configuration of one of the residues and the configuration of the glycosidic linkage leads to small changes in the n.O.e. ratio for H-3 and H-4, and, hence, does not move the disaccharide glycoside from one series to the other, as occurs with β-D-Man-(1→3)-β-D-Gal-OMe and α-L-Rha-(1→3)-β-D-Gal-OMe (Table I).

Theoretical calculations show that a change in the structural factors *1* or *2* alters the spatial structure of the disaccharides. Hence, for example, the potential energy surface for α-D-Glc-(1→3)-β-D-Gal-OMe (Fig. 2a of ref. 7) contains a local minimum corresponding to a low-energy conformer with angles ϕ and ψ of $-60°$ and $-50°$, in which the distance between H-1′ and H-4 is merely 2.1 Å. Moreover, in ~90% of all the conformers admissible as regards non-bonded interactions, H-1′ and H-4 are in van der Waals contact, which results in a noticeable n.O.e. for H-4 (Table I). In contrast, for all possible conformations of β-D-Glc-(1→3)-β-D-Gal-OMe, the distance between H-1′ and H-4 is >3–4 Å, and the only protons in proximity (2.1–2.3 Å) are H-1′ and H-3. As a result, a noticeable n.O.e. is observed for H-3, whereas that for H-4 is an order of magnitude lower (Table I).

A typical arrangement of H-1′ and H-4 in the admissible conformations of α-D-Glc-(1→3)-β-D-Gal-OMe and β-D-Glc-(1→3)-β-D-Gal-OMe is shown in Fig. 2, which gives the projections of the disaccharide fragments on to the plane perpendicular to that of the glycosidic linkage, with the angle ϕ(C-1′–O) corresponding to the *gauche* orientation of the algycon with respect to the C-1′–O-5′ bond. In α-D-Glc-(1→3)-β-D-Gal-OMe, H-1′ and H-4 are *cis* with respect to the plane C-1′–O–C-3 (Fig. 2a), whereas they are *trans* (Fig. 2b) in β-D-Glc-(1→3)-β-D-Gal-OMe. A simultaneous change of both structural factors *1* and *2* does not alter the relative arrangement of H-1′ and H-4 or H-1′ and H-3, and, as a consequence, the relative n.O.e. values are retained.

Glycosyl-(1→4)-xylosides[8] with H-5 of the aglycon equatorial also belong to the series of disaccharide glycosides being considered and, for β-D-Glc-(1→4)-β-D-Xyl-OMe and α-D-Glc-(1→4)-β-D-Xyl-OMe, the ratios of n.O.e. for H-5*e* and H-4 of the xylose moiety, caused by pre-irradiation of H-1′, are 1:2 and 1:15, respectively (Table I in ref. 8).

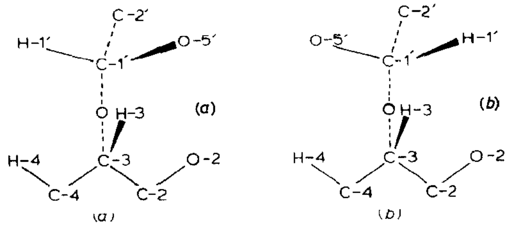

Fig. 2. Spatial arrangement of some moieties in (*a*) α-D-Glc-(1→3)-β-D-Gal-OMe and (*b*) β-D-Glc-(1→3)-β-D-Gal-OMe.

There is a certain similarity in the observed n.O.e. for glycosyl-(1→2)-α-rhamnosides[6] and glycosyl-(1→3)-rhamnosides[5,6] with identical structural factors *1* and *2* (Table I). Thus, the ratio of n.O.e. for H-2 and H-1 in the (1→2)-linked disaccharide glycosides and that for H-3 and H-2 in the (1→3)-linked compounds are similar, as for α-D-Glc-(1→2)-α-L-Rha-OMe and α-D-Glc-(1→3)-α-L-Rha-OMe. The distinctions between these disaccharide glycosides are the position of the α-carbon of the aglycon [even (C-2) in the first disaccharide and odd (C-3) in the second, *i.e.*, structural factor *3*] and the orientation of the glycosylating residue at this carbon (axial in the first disaccharide and equatorial in the second, *i.e.*, structural factor *4*). However, despite these distinctions, the conformational maps ϕ–ψ and the energies of the respective optimal conformations are practically the same (Figs. 3a and 3b). These facts, together with the n.O.e. data, show that each of the structural factors *3* and *4* should be considered along with the structural factors *1* and *2* as being mainly responsible for determining the conformation of the disaccharide unit.

Comparison of the conformational maps for α-D-Glc-(1→2)-α-L-Rha-OMe and α-D-Glc-(1→3)-α-L-Rha-OMe (Fig. 3) clearly demonstrates that the inversion of an even number of structural factors does not alter the shape of the potential energy surface and, as a consequence, the observed regularities in the n.O.e. spectra. The close connection between the n.O.e. values and the parity of the number for the α-carbon of the aglycon can be demonstrated by other examples. Thus, the alteration of the parity of this atom number in going from α-D-Glc-(1→2)-α-D-Glc-OMe to α-D-Man-(1→3)-α-D-Man-OMe, with the remaining structural factors being identical, leads to inversion of the regularities in the n.O.e. spectra (Table I). In fact, both these disaccharide glycosides have substituents in place of the adjacent equatorial hydroxyl groups of the glycosylated residue, which are directed towards different sides of the plane of the pyranose ring. As a result, the equatorial substituent at position $(n - 1)$ of the aglycon ring [in these examples $H(n - 1)$] may be in close proximity to the glycosylating residue or, alternatively, the equatorial substituent at position $(n + 1)$ [$O(n + 1)$], *i.e.*, the result is equivalent to changing the orientation of the substituent at the α-carbon.

For the structural factor *5*, the number $(n - 1$ or $n + 1)$ for the β-carbon carrying the equatorial proton with respect to n, the number for the α-carbon of the aglycon, is proposed. Replacement of the aglycon with the axial substituent at C-4

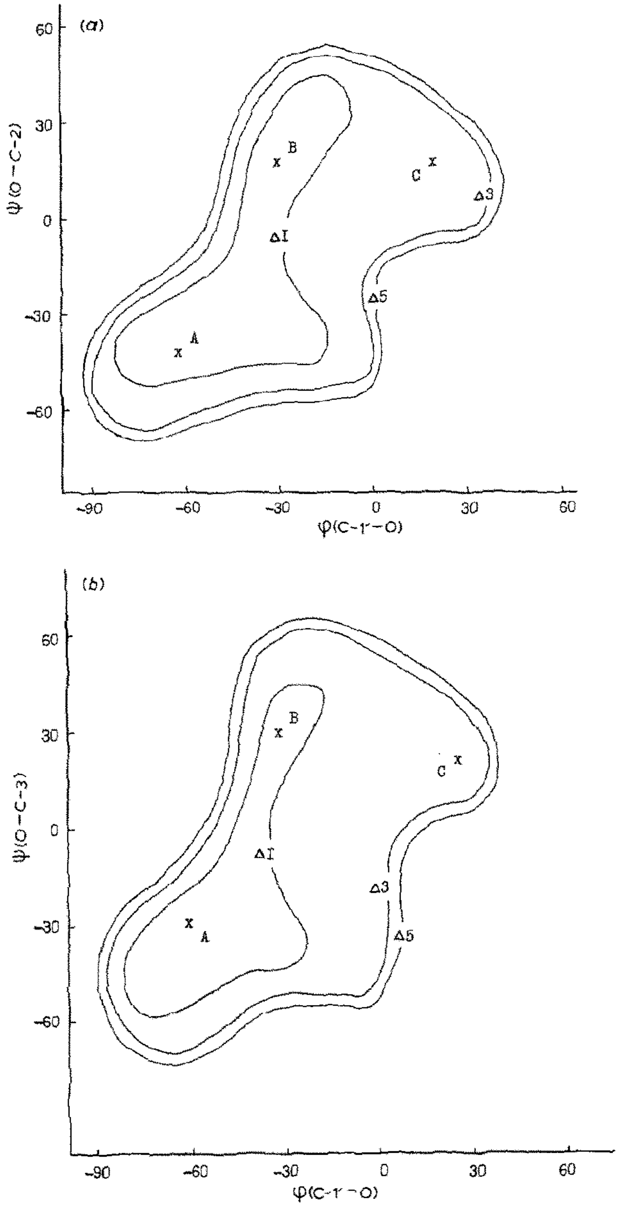

Fig. 3. Conformational maps ϕ-ψ for (*a*) α-D-Glc-(1→2)-α-L-Rha-OMe and (*b*) α-D-Glc-(1→3)-α-L-Rha-OMe. Iso-energy contour lines (kcal/mol) were calculated using the potential functions[11]: × denotes local minima.

(galactopyranose) by the aglycon with the axial substituent at C-2 (mannopyranose) leads to inversion of the n.O.e. regularities, which is demonstrated by comparing the data for α-D-Man-(1→3)-β-D-Gal-OMe and α-D-Man-(1→3)-α-D-Man-OMe (Table I). Thus, a change of the number for the β-carbon carrying the equatorial proton, from a larger to a smaller value with respect to the number for the α-carbon, causes the same effect on the n.O.e. as a change of any of the other structural factors. As a consequence, simultaneous alteration of the structural factor *5* (*e.g.*, replacement of H_{n-1} by H_{n+1} or H_{n+1} by H_{n-1}) and of any other factor, *e.g.*, the absolute configuration of one of the residues, gives a disaccharide glycoside belonging to the same conformational series as the initial compound. This situation is exemplified by the following pairs from Table I: β-L-Rha-(1→3)-α-L-Rha-OMe/β-L-Rha-(1→3)-β-D-Gal-OMe and α-L-Rha-(1→3)-α-L-Rha-OMe/α-L-Rha-(1→3)-β-D-Gal-OMe for which the conformational maps are similar (*cf.*, Figs. 1 and 2 in ref. 5 and Figs. 3 and 4 in ref. 6). The ratio of the n.O.e. values for the equatorial proton at the β-carbon and for H(*n*) also proved to be similar for each pair, ~1:1 for the first pair and ~1:10 for the second pair (Table I).

Finally, it is instructive to compare the data (Table I) for β-L-Rha-(1→2)-α-L-Rha-OMe and β-D-Glc-(1→4)-β-D-Xyl-OMe (see refs. 6 and 8), which have an identical structural factor *3* despite the different type of linkage. These compounds differ in the structural factors *4* and *5*; nevertheless, the observed regularities in the n.O.e. spectra are similar.

Thus, the disaccharides of the first series (upper half of Table I) are characterized by the following set of determining structural factors

$$\alpha,\ \text{D-D},\ n_{\text{od}},\ H_{n+1},\ S_e,$$

or by a set that differs from this by an even number of structural factors. The disaccharides of the second series (lower half of Table I) are associated with sets that differ from the above set by an odd number of structural factors.

Common regularities in the n.O.e. spectra for the disaccharide glycosides of the first and second series (Table I) are supported by the values of other parameters in the ^{13}C-n.m.r. spectra, *e.g.*, by the β-effects of glycosylation (*i.e.*, the difference between the chemical shifts of the resonance for the carbon which is β to that involved in glycosidic linkage, and the same carbon in the unsubstituted sugar). For all the disaccharide glycosides of the first series, the β-effects of glycosylation for carbons carrying equatorial protons are −2 to −5 p.p.m., *i.e.*, these carbons resonate at stronger fields in comparison with those in the corresponding free sugars, whereas, for the disaccharides of the second series, for which the n.O.e. for the equatorial protons in the β-position is small, the β-effects of glycosylation are negligible[12]. For example[7], for β-L-Rha-(1→3)-β-D-Gal-OMe, the β-effect of glycosylation for C-4 of the galactose residue is −3.1 p.p.m., whereas for α-L-Rha-(1→3)-β-D-Gal-OMe it is −0.7 p.p.m. The similar variation of the values of the β-effects of glycosylation and n.O.e. for equatorial protons supports the suggestion[12] that they result from the "*γ-gauche*" interaction of protons, *i.e.*, between H-1′ and H-4 in glycosyl-(1→3)-galactosides and between H-1′ and H-2 in glycosyl-(1→3)-mannosides [glycosyl-(1→3)-rhamnosides]. Thus, the β-effects of glycosylation in the disaccharide fragments are also determined by the structural factors *1–5*.

Aglycon with equatorial protons at both β-carbons. — Table II shows the data for several disaccharide glycosides with the *talo* configuration of the glycosylated residue. Pre-irradiation of H-1′ causes a n.O.e. of the signals for H-3 and a considerable effect on one of the equatorial protons (H-2 or H-4) of the talose residue (Table II).

The n.O.e. values for the glycosyl-(1→3)-talosides, glycosyl-(1→3)-galactosides, and glycosyl-(1→3)-mannosides [glycosyl-(1→3)-rhamnosides], provided that the absolute configurations of the residues and configurations of the glycosidic linkages are identical, shows that the situation for α-D-Man-(1→3)-β-D-Tal-OMe and β-L-Rha-(1→3)-β-D-Tal-OMe is similar to that for the corresponding glycosyl-galactosides, whereas for the second pair, β-D-Man-(1→3)-β-D-Tal-OMe and α-L-Rha-(1→3)-β-D-Tal-OMe, the situation is similar to that for the corresponding glycosyl mannosides. On the whole, the n.O.e. picture for glycosyl-(1→3)-talosides appears to be the sum of the effects associated with the two types of disaccharide glycosides with an equatorial proton at one of the β-carbons. This situation is a manifestation of the generic connection of the disaccharide glycosides of the two stereochemical groups (*a*) and (*b*). In fact, for α-D-Man-(1→3)-β-D-Gal-OMe, the n.O.e. observed for H-4 has approximately double the magnitude of that for H-3. For α-D-Man-(1→3)-α-D-Man-OMe, a n.O.e. is observed for H-3 but not for H-2 (Table I). For α-D-Man-(1→3)-β-D-Tal-OMe, the n.O.e. are observed for H-3 and H-4, with the latter being essentially larger (Table II). In contrast, merely a small n.O.e. was observed on H-4 of α-L-Rha-(1→3)-β-D-Gal-OMe (Table I); likewise, in the case of α-L-Rha-(1→3)-β-D-Tal-OMe, the n.O.e. on H-4 was an order of magnitude lower than that on H-2 and H-3.

A typical conformational situation for the glycosyl-(1→3)-talosides is demonstrated in Fig. 4 (*cf.* Fig. 2), which gives the projections of the disaccharide fragments on to the plane perpendicular to the plane of the glycosidic linkage. In most of the admissible conformers of α-D-Man-(1→3)-β-D-Tal-OMe and β-L-Rha-(1→3)-β-D-Tal-OMe, H-1′ and H-4 are *cis* with respect to the glycosidic linkage plane and in van der Waals contact with each other (Fig. 4a), whereas in β-D-Man-(1→3)-β-D-Tal-OMe and α-L-Rha-(1→3)-β-D-Tal-OMe, it is H-1′ and H-2 that are

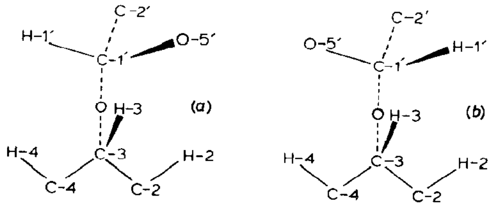

Fig. 4. Spatial arrangement of some moieties in the two series of glycosyl-(1→3)-talosides (Table II).

in the *cis* position and H-1′ and H-4 are remote (Fig. 4b).

A change of any of the structural factors *1–4* alters the arrangement of fragments in the projections to the opposite one, and, as a consequence, the n.O.e. picture is interchanged, *i.e.*, for the first pair of structures, a considerable n.O.e. is observed for H-4 of the talose residue, whereas for the second pair it is observed for H-2 (Table II). In each pair, the disaccharide glycosides differ by an even number of structural factors.

The shape of the potential energy surface for the glycosyl-(1→3)-talosides, with effective dispersion interactions of H-1′ with H-3 and H-4, is similar to that of the corresponding glycosyl-(1→3)-galactosides in which H-1′ and H-4 are in proximity. Hence, the favoured conformers of α-D-Man-(1→3)-β-D-Tal-OMe and α-D-Man-(1→3)-β-D-Gal-OMe are identical (ϕ and $\psi \sim -50°$). In contrast, the glycosyl-(1→3)-talosides, the structures of which are stabilized by van der Waals interactions of H-1′ with H-3 and H-2, are similar, as regards the conformations, to the corresponding glycosyl-(1→3)-mannosides, *e.g.*, β-D-Man-(1→3)-β-D-Tal-OMe and β-L-Rha-(1→3)-α-L-Rha-OMe.

Aglycon with bulky equatorial substituents at both β-carbons. — Since, in these compounds, there are no equatorial protons on the β-carbons, a marked n.O.e. is observed[2–4,7] only for the anomeric proton H(*n*) (Table III).

Nevertheless, the connection between n.O.e. and the structural factors *1–5* can be demonstrated. Thus, in the (1→4)-linked disaccharide glycosides, a n.O.e. is observed not only for H-4 but in some compounds for the protons of a CH_2OH or CH_3 group at position 5 of the aglycon. Comparison of the structural factors specified for each disaccharide glycoside in Table III shows that those compounds in which a n.O.e. is observed both for H-4 and H-6 differ by an even number of the factors, as, for example, in α-D-Glc-(1→4)-L-Rha-OMe and α-D-Glc-(1→4)-β-D-Gal-OMe. These disaccharide glycosides differ from those with no n.O.e. for H-6 by an odd number of factors, as, for example, in α-D-Glc-(1→4)-β-D-Gal-OMe and β-D-Glc-(1→4)-β-D-Gal-OMe (Table III).

The similarity of regularity in the n.O.e. spectra of (1→4)-linked disaccharide glycosides which differ by an even number of structural factors results also from the resemblance of the spatial structures of the corresponding molecules. This can be seen by comparison of the conformational maps ϕ–ψ, for example, for β-D-Glc-(1→4)-α-L-Rha-OMe and β-D-Glc-(1→4)-β-D-Gal-OMe. These disaccharide glycosides differ in both the absolute configuration of one of the residues and the orientation of the 4-substituent of the aglycon. Nevertheless, the respective conformational maps are strikingly similar (Fig. 5), which explains why the n.O.e. is observed for both H-4 and H-6 in each compound (Table III).

In the case of β-D-Glc-(1→4)-α-L-Rha-OMe, the occurrence, in aqueous solution, of the conformer with a rotation angle ϕ of ~180° should be emphasized. The statistical weight of the conformer is ~30%. This follows from the n.O.e. on H-3 and H-5 arising on pre-irradiation of H-1′ (~3%, Table III in ref. 4). The same conformer has been found for cellobiose[2]. Thus, the spatial structure of a disacchar-

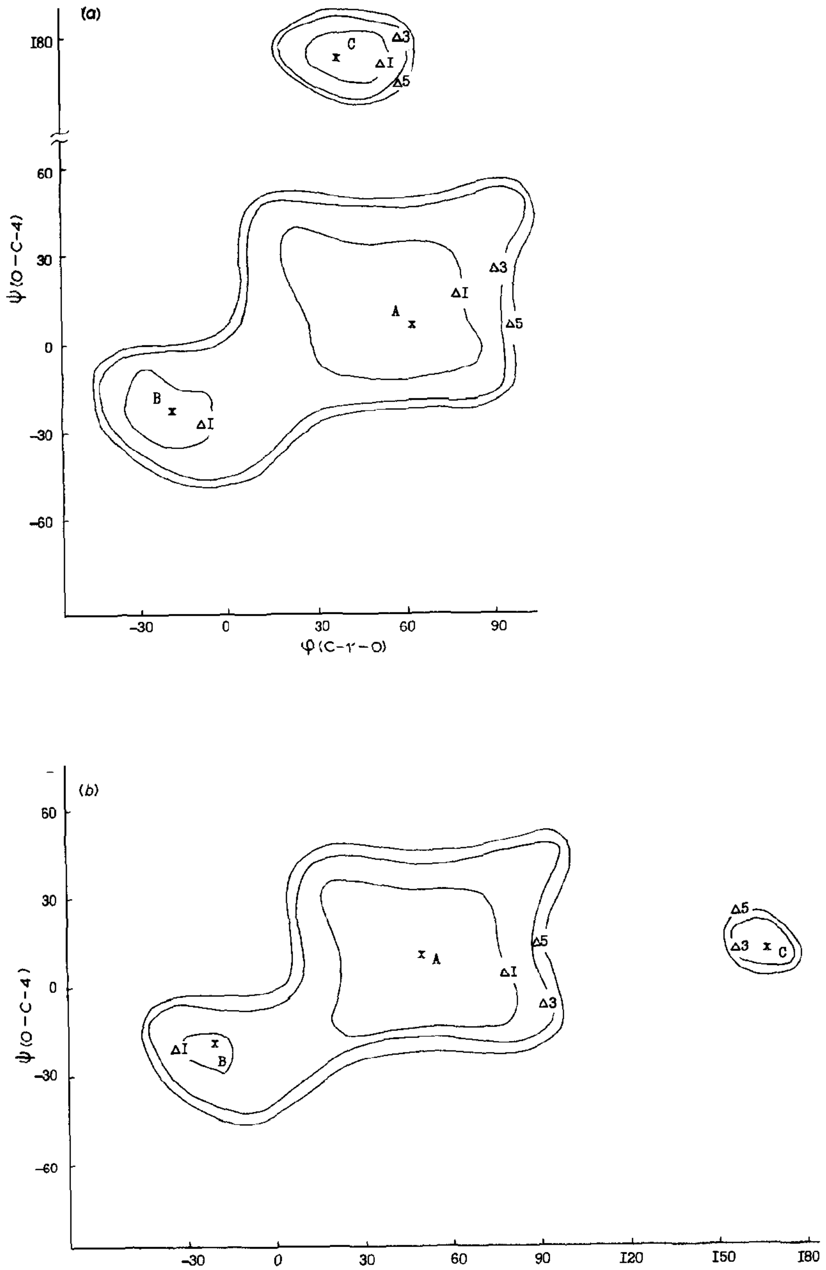

Fig. 5. Conformational maps ϕ–ψ for (*a*) β-D-Glc-(1→4)-α-L-Rha-OMe and (*b*) β-D-Glc-(1→4)-β-D-Gal-OMe. See caption for Fig. 3.

ide is described generally by the whole potential surface ϕ–ψ and not by a single rigid conformer.

Thus, the structural factors *1–5* are established for disaccharide fragments and determine their conformational properties and typical features in their ^{1}H- and ^{13}C-n.m.r. spectra. Disaccharide glycosides characterized by an identical set of the structural factors *1–5*, or differing by an even number of the factors, have similar spatial structures. This conclusion allows the division of the whole diversity of disaccharide fragments into a small number of stereochemical classes and permits assessment of the conformational properties of any disaccharide fragment (and also its n.O.e. spectra and the β-effects of glycosylation in the ^{13}C-n.m.r. spectra) on the basis of the data available for structurally related disaccharides.

EXPERIMENTAL

^{1}H-n.m.r. spectra were recorded with a Bruker AM-300 instrument for 3% solutions of the disaccharides in D_2O. N.O.e.'s were measured after pre-irradiation of H-1′ within the t.O.e. technique[13]. N.O.e.'s are expressed as ratios of the integrated intensities of the observed and presaturated protons in the difference n.O.e. spectrum.

REFERENCES

1 G. M. LIPKIND, V. E. VEROVSKY, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 133 (1984) 1–13.
2 G. M. LIPKIND, A. S. SHASHKOV, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 141 (1985) 191–197.
3 A. S. SHASHKOV, G. M. LIPKIND, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 147 (1986) 175–182.
4 G. M. LIPKIND, A. S. SHASHKOV, A. V. NIKOLAEV, S. S. MAMYAN, AND N. K. KOCHETKOV, *Bioorg. Khim.*, 13 (1987) 1081–1092.
5 G. M. LIPKIND, A. S. SHASHKOV, YU. A. KNIREL, AND N. K. KOCHETKOV, *Bioorg. Khim.*, 12 (1986) 771–779.
6 S. S. MAMYAN, G. M. LIPKIND, A. S. SHASHKOV, N. E. BAYRAMOVA, A. V. NIKOLAEV, AND N. K. KOCHETKOV, *Bioorg. Khim.*, 14 (1988) 205–215.
7 G. M. LIPKIND, S. S. MAMYAN, A. S. SHASHKOV, O. A. NECHAEV, V. I. TORGOV, V. N. SHIBAEV, AND N. K. KOCHETKOV, *Bioorg. Khim.*, 14 (1988) 340–351.
8 S. S. MAMYAN, G. M. LIPKIND, A. S. SHASHKOV, N. E. NIFANT'EV, AND N. K. KOCHETKOV, *Bioorg. Khim.*, 12 (1986) 1101–1110.
9 J. H. NOGGLE AND R. E. SCHIRMER, *The Nuclear Overhauser Effect*, Academic Press, New York, 1971.
10 J. F. STODDART, *Stereochemistry of Carbohydrates*, Wiley Interscience, New York, 1971.
11 R. A. SCOTT AND H. A. SCHERAGA, *J. Chem. Phys.*, 44 (1966) 3054–3068.
12 N. K. KOCHETKOV, O. S. CHIZHOV, AND A. S. SHASHKOV, *Carbohydr. Res.*, 133 (1984) 173–185.
13 G. WAGNER AND K. WÜTHRICH, *J. Magn. Reson.*, 33 (1979) 675–680.