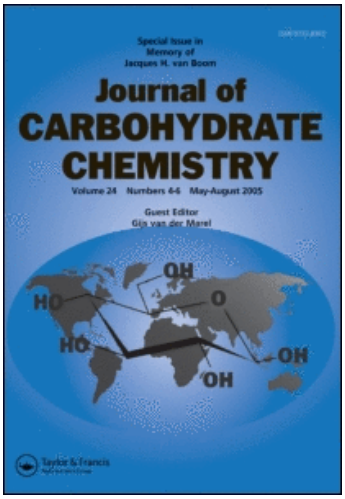

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IDENTIFICATION OF *O*-METHYL SUBSTITUTED GLYCOPYRANOSES AND THEIR METHYL GLYCOSIDES BY HMBC, ^{1}H AND ^{13}C NMR CHEMICAL SHIFT INCREMENTS AND THE $^{3}J(^{13}C,^{1}H)$ COUPLING CONSTANT ABOUT THE $\underline{C}H_3O\text{-}C\underline{H}$ BOND.

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ABSTRACT

The substitution site of partially *O*-methylated glycopyranoses is identified by HBMC. Their ^{1}H and ^{13}C NMR chemical shifts are evaluated by considering chemical shift increments. The $^{3}J(^{13}C,^{1}H)$ coupling constants are evaluated. Interesting applications of chemical shift increments are illustrated.

INTRODUCTION

Correct assignment of the resonances in ^{1}H and ^{13}C NMR spectra of carbohydrates can be obtained by 2D NMR experiments. Direct assignment of the ^{1}H chemical shifts is obtained by following the connectivities in a COSY experiment. By applying $^{13}C,^{1}H$ heteronuclear correlated experiment (if there are no collapses in the proton spectrum) the ^{13}C NMR chemical shifts are then assigned. From the knowledge of the resonances for the ring protons, with $^{13}C,^{1}H$ LR heteronuclear correlated experiments it should be possible to assign the

substitution site in *O*-methylated carbohydrates.[1-5] (The OCH_3 carbon resonances are correlated with the resonances for the ring protons). These possibilities have been reviewed by Van Halbeek.[6] Recently more sophisticated techniques for this aim were proposed.[7-10]

Although these techniques have been applied to the anomeric site of methyl glycosides, to the best of my knowledge there is no publication revealing such correlation at the other sites in the pyranose ring. The reason may be that because of averaging of the rotamers, the signals are too weak to be detected. I have now succesfully obtained such correlation by application of the sequence proposed by Bax and Summers[11] for 2-*O*-methyl carbohydrate pyranoses.

A correct assignment of the NMR chemical shifts and knowledge of the mutual correlation between the chemical shifts and structural features of the molecule is very important for the elucidation of a structure. The starting point of this work asks the question can the chemical shift increments caused by methylation be used for the identification of the substitution site in partially methylated derivatives? Knowledge of the rotameric distribution about the C(ring)-O bond and systematized data of such effects in model compounds is necessary. In this respect we also wonder if the heteronuclear coupling constants may give access to estimation of the rotameric distribution.

RESULTS AND DISCUSSION

I first studied the NMR parameters for 2-OMe-α-and -β-D-gluco- and -xylopyranose as well as for their methyl glycosides. The ^1H NMR chemical shifts, the ^{13}C NMR chemical shifts and the $^3J(C\underline{H}\text{-}O\underline{C}H_3)$ coupling constants for are given in **Tables 1, 2 and 3** respectively.

For the indication of an effect in this study the pathway of the torsion angles (g = gauche, a = antiperiplanar, + = clockwise), from the group causing the effect (*e.g.*, OH, CH_3) to the proton (or carbon) experiencing the effect is followed. For a *syn*-axial interaction between a hydroxyl group and a proton we write $\gamma OH(g^+g^-)H$.

Table 1. ^{1}H NMR Chemical Shifts of D-xylose, 2-OMe-D-xylose, D-glucose and 2-OMe-D-glucose in D_2O Solution

	H-1	H-2	H-3	H-4	H-5	H-5'
α-D-xylopyranose[a]	5.19	3.52				
2-OMe-α-D-xylopyranose	5.44(+0.25)	3.26(-0.26)	~3.65	~3.65	~3.68	~3.68
β-D-xylopyranose[a]	4.57	3.23	3.42	3.63	3.93	3.32
2-OMe-β-D-xylopyranose	4.63(+0.06)	2.99(-0.24)	3.48(+0.06)	3.65(+0.02)	3.92(-0.01)	3.31(-0.01)

	H-1	H-2	H-3	H-4	H-5	H-6	H-6'	OMe-2[c]	OMe-1
α-D-glucopyranose[b]	5.22	3.54	3.73	3.35	3.82	3.84	3.75		
2-OMe-α-D-glucopyranose	5.45(+0.23)	3.24(-0.30)	3.72(-0.01)	3.40	3.81	3.86	3.75	3.47	
Me 2-OMe-α-D-glucopyranoside	5.08	3.33	3.73	3.47	3.66	3.93	3.81	3.45	3.51
β-D-glucopyranose[b]	4.63	3.25	3.45	3.36	3.46	3.89	3.72		
2-OMe-β-D-glucopyranose	4.67(+0.04)	2.96(-0.29)	3.52(+0.07)	~3.42	~3.42	3.82	3.72	3.60	
Me 2-OMe-β-D-glucopyranoside	4.39	2.98	3.52	3.38	3.42	3.90	3.67	3.55	3.55

[a] Taken from Reference 43.
[b] Taken from Reference 40.
[c] Assigned by difference in integration shortly after solution.

Table 2. ^{13}C NMR Chemical Shifts of 2-Ome-α- and ß-D-xylopyranose and 2-OMe-α- and ß-D-glucopyranose in D_2O Solution

	C-1	C-2	C-3	C-4	C-5			
α-D-xylopyranose[a]	92.4	71.8	73.2	69.7	61.2			
2-OMe-α-D-xylopyranose	90.0(-2.4)	81.1(+9.3)	72.5(-0.7)	69.8	61.2			
β-D-xylopyranose[a]	97.0	74.6	76.3	69.7	65.6			
2-OMe-β-D-xylopyranose	97.0(-)	84.1(+9.5)	75.8(-0.5)	70.0	65.6			
	C-1	C-2	C-3	C-4	C-5	C-6	OMe-2	OMe-1
α-D-glucopyranose[a]	92.9	72.5	73.8	70.6	72.3	61.6		
2-OMe-α-D-glucopyranose	90.1(-2.8)	81.3(+9.2)	72.7(-1.1)	70.4	72.0	61.4	58.3	
Me 2-OMe-α-D-glucopyranoside	97.3	81.0	73.1	70.4	72.1	61.3	58.6	55.5
β-D-glucopyranose[a]	96.7	75.1	76.7	70.6	76.8	61.7		
2-OMe-β-D-glucopyranose	96.5(-0.02)	84.4(+9.3)	76.0(-0.7)	70.5	76.6	61.5	60.8	
Me 2-OMe-β-D-glucopyranoside	103.9	83.4	76.0	70.4	76.6	61.5	60.8	57.9

[a] Taken from reference 17. For comparative reasons the ^{13}C chemical shifts of α-D-xylopyranose were lowered by 0.7 ppm and of β-D-xylopyranose by 0.5 ppm.

Table 3. The $^{3}JC_{(2)}\underline{H}\text{-}O\underline{C}H_3$ coupling constants in 2-OMe-α,β-D-glucopyranose and Me 2-OMe-α,β-D-glucopyranoside.

compound	$\delta^{13}C$ (2OMe) ppm	J(H2-OMe) Hz	$\delta^{13}C$ (1OMe) ppm	J(H1-OMe) Hz
2-OMe-α-D-glucopyranose	58.4	4.23	--	--
2-OMe-β-D-glucopyranose	60.9	5.71	--	--
Me 2-OMe-α-D-glucopyranoside	58.6	4.19	55.6	3.51
Me 2-OMe-β-D-glucopyranoside	60.9	5.65	57.9	4.53

Assignment of the substitution site by HBMC.

The site of substitution could be unequivocally assigned by heteronuclear multiple-bond correlation experiments (HMBC).[11] The ^{1}H NMR resonances of the ring protons were obtained from a COSY experiment. The ^{13}C NMR resonances of the *O*-methyl carbons could be indicated from the approximate integration. A HMBC experiment reveals a correlation between each of these ^{13}C NMR *O*-methyl resonances and the resonances of the ring protons on the site of the substitution. Since we are certain about the assignment, we can now evaluate the parameters involved.

The $^{3}J(CH_3O\text{-}CH)$ Coupling Constants

The value found for these coupling constants at the glycosidic site is greater for β-D-glucopyranoses than for the α isomers (see **Table 3**), in agreement with the values found by Lemieux and Koto.[12]

This finding is in agreement with the Karplus curve type equation of vicinal proton-carbon coupling constants for $\underline{C}\text{-}O\text{-}C\text{-}\underline{H}$ derived by Hamer and coworkers on one hand and Tvaroska and coworkers on the other hand.[13,14]

We now find that the coupling constant between the carbon of a OMe-group and the ring proton on the carbon on which the substitution occurs is larger for 2-OMe-β-D-glucopyranose and its methyl glucoside than for the α-forms. Taking into consideration the Karplus-like curve established by Tvaroska,[14]

this means that the torsion angle is smaller for the β form than the α form when for both the averaged torsion angle is smaller than 90°. However other possibilities must be considered, *e.g.* the reverse holds when both are larger than 90° and smaller than 180°. We can only attribute a physical meaning to the values of the coupling constants when from the conformational aspects deduced from other data, as *e.g.* ^{1}H and ^{13}C NMR chemical shifts, a range for the torsion angles can be suggested. Such deduction is now discussed.

The ^{13}C NMR chemical shift increments

The complete assignment of the ^{13}C NMR chemical shifts of an anomeric mixture of 2-OMe-α- and -β-D-xylopyranose in D_2O solution is not straightforward. We have first analyzed the ^{1}H NMR spectrum using a COSY experiment (see **Table 1**). For the β modification, assignment of the ^{1}H NMR chemical shifts was readily achieved from consideration of the coupling constants. The resonances for H-3, -4, -5 and -5' of the α modification almost collapse so that only the resonances for H-1 and H-2 can be assigned with certainty. The ^{13}C NMR resonances for 2-OMe-β-D-xylopyranose could readily be assigned from an ^{13}C,^{1}H heteronuclear correlated experiment, as well as those for C-1 and C-2 of the α modification. From the high field position of C-5 at δ 61.5, the resonances for H-5 and H-5' of the α form could be assigned in the ^{1}H NMR spectrum from this HETCOR experiment. Knowing the increments on C-3 and C-4 caused by the introduction of an OMe on C-2 in 2-OMe-α-D-glucopyranose, application of these increments on the resonances for C-3 and C-4 of the parent sugar allows us to assign next the resonances with certainty of C-3 and C-4 in 2-OMe-α-D-xylopyranose at δ 72.5 and δ 69.8 respectively. In order to evaluate the ^{13}C NMR chemical shift increments we first have looked to the ^{13}C NMR chemical shift increments caused by methylation of an hydroxyl group on a six-membered ring.[15,16] The chemical shift values of α,β-D-glucopyranose compiled by Bock and Pedersen are used as basic values.[17] Lemieux and coworkers studied the favored orientation of the *O*-methyl group in methyl glycopyranosides in order to estimate the molar rotation in pyranoid carbohydrates.[18] Their conclusions are substantiated by

$^{13}C,^{1}H$ coupling data. When the exo-anomeric effect is taken into consideration in the case of the methyl glycopyranosides, there is no doubt about the favoured orientation of the substituent at the anomeric site. During our consideration of rotamers we shall use Lemieux' energy proposals, where the unfavored energetic interaction between two opposing axial substituents is essential in our estimation of the population of the rotamers,[19] and is in good agreement with the results from molecular mechanics calculations by Macromodel V3.O. We apply this findings for the data for 2-OMe-α- and -β-D-glucopyranose. The major rotamers are given in **Figures 1 and 2.**

Applying the lowest energy approach of Lemieux, only one rotamer must be considered for 2-OMe-α-D-glucopyranose (see **Figure 1**). In accord with ^{13}C NMR chemical shift increments outlined by Angyal and coworkers, besides a downfield shift of ca. 10 ppm for C-2, an upfield shift of -2.8 ppm for C-1 {a γCH_3(g)C effect} and a minor upfield increment (-1.1 ppm) for C-3 (a γCH_3(a)C effect) was observed (see **Table 2**).

Unfortunately, such a straightforward conclusion is not possible for the β-isomer. The three possible rotamers are represented in **Figure 2**. From ^{1}H NMR chemical shift increment considerations, rotamer 2c can virtually be excluded (see further). The ^{13}C NMR chemical shifts of 2-OMe-α- and -β-D-glucopyranose have been published by Bock and Pedersen on one hand and by Usui and coworkers on the other.[20,21] For the β modification Bock did not differentiate between C-3 and C-5 although a difference of 0.5 ppm is seen. Because certainty was necessary for this study we remeasured the ^{13}C NMR chemical shifts as obtained from a $^{13}C,^{1}H$ heteronuclear correlated experiment[22] in order to confirm the assigment of C-3 and C-5 by Usui. Although a difference of -0.2/-0.7 ppm is found for the increment of C-1 and C-3 (see **Table 2**) these values are within the range -0.3/-1.1 usually found in model compounds for a βCH_3(a)C arrangement. The involvement of a βCH_3(g)C arrangement (-2.4/-2.8 ppm) cannot be evaluated, nor the proportion of rotamers 2a and 2b, and consequently also not the averaged torsion angle. Therefore, establishing the ^{1}H NMR chemical shift increments become important to solve this problem.

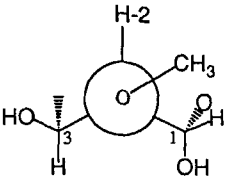
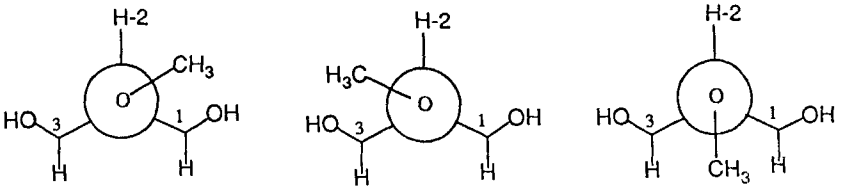


Figure 1. Lowest energy rotamer for 2-OMe-α-D-glucopyranose



2a 2b 2c

Figure 2. The possible romaters for 2-OMe-β-D-glucopyranose

The ^{1}H NMR chemical shift increments

The ^{1}H NMR chemical shifts for 2-OMe-α,β-D-glucopyranose and their methyl glucosides are given in **Table 1.**

The effect found when a proton is replaced by a substituent is considered as a chemical shift increment and was first proposed during a study of the effects of methyl groups in dioxanes[23] as well as during a study of methyl cyclohexanes and cyclohexanols.[24]

In **Table 4** we have gathered the ^{1}H NMR chemical shifts of the carbohydrates considered in the present study. In **Table 5** we have given the ^{1}H chemical shift increments for the same pathway in different classes of compounds.

In order to estimate the effect of an *O*-methyl group in a pyranose ring we have considered the changes of the chemical shifts of the ring protons of the two anomers of D-xylopyranoses, D-lyxopyranoses and L-arabinopyranoses on one hand and the anomers of D-quinovopyranose, L-rhamnopyranose and L-fucopyranose respectively on the other hand (see first and last column in **Table 5**).

Because of the typical structures and conformations in pyranose sugars, this set of increments is incomplete.

In order to estimate the validity when applying data measured for one class of compounds to another, we have compared the chemical shift increments found for carbohydrates with those found in the Me-cyclohexanes and in Me-1,4-dioxanes. Moreover, chemical shift increments may be different, *e.g.*, when on the pathway a methylene group is replaced by an oxygen, because of branching on the pathway, and if the proton on which the effect is operating belongs to an oxymethylene unit (-CH_2O-) or a hydroxymethine (CHOH-) unit of the ring (in **Table 5** indicated by "on CH_2" and "on CHOH", respectively).

The β Increments

In the two cases for the β increment of a torsion angle of 60° between the methyl group and the proton, a difference of 0.70 ppm between $\beta Me_{eq}(g)H_{ax}$ and $\beta Me_{eq}(g)H_{eq}$ is observed (see **Table 5**). The corresponding data for the Me-cyclohexanes and Me-dioxanes show the same important difference between $Me_{eq}(g)Ha_{ax}$ and $Me_{ax}(g)H_{eq}$.

Gorrichon[25] has systematically studied all possible β configurations in dioxanes and found some regularities governing the β increments. In comparison with a hypothetical dioxane ring in the same conformation, he found for the Me-dioxanes that: (1) if a ring oxygen occurs in the β position, no effect is experienced on the equatorial proton, but a downfield shift of +0.11 ppm occurs with the axial proton; (2) that if there is a ring oxygen or an exocyclic oxygen in the α position, both the axial and equatorial proton suffer from an upfield shift of -0.09 ppm; (3) an equatorial proton is found at -0.35 ppm in the higher field region in comparison with the axial partner. From these data we can give a quantitative rationale for the difference found for the H-4 chemical shifts in the two examples in the present study (see **Figure 3**).

In both examples there is an oxygen in the α position (-0.09ppm). For 3b we expect an increment of +0.11 ppm for the axial proton β to the ring oxygen. Finally an upfield shift of -0.35 ppm is calculated for the equatorial proton in 1a. When using this information, (-0.09) + (-0.35) = -0.44 ppm is expected for H-4 in

Table 4. ^{1}H NMR Chemical Shifts of the Monosaccharides considered in D_2O Solution (vs TSP as internal standard)

	H-1_{eq}	H-1_{ax}	H-2_{eq}	H-2_{ax}	H-3_{eq}	H-3_{ax}	H-4_{eq}	H-4_{ax}	H-5_{eq}	H-5_{ax}	H-6	H-6'	Me	Reference
1,5-anhydroxylitol	3.95	3.24	-	3.58	-	3.40	-	3.58	3.95	3.24	-	-	-	537
Aldopentopyranoses and Me derivatives														
α-D-xylopyranose	5.19			3.52		3.63		3.63	3.68	3.67				•
β-D-xylopyranose		4.57		3.23		3.42		3.63	3.93	3.32				•
α-D-lyxopyranose	5.01		3.81			3.88		3.82	3.81	3.69				**
β-D-lyxopyranose		4.87	3.93			3.64		3.89	3.96	3.26				**
β-L-arabinopyranose	5.24			3.82		3.89	4.01		3.66	4.01				33
α-L-arabinopyranose		4.52		3.51		3.66	3.95		3.91	3.67				33
4-O-methyl-β-L-arabinopyranose	5.22			3.76		3.93	3.65		3.84	3.91				33
4-O-methyl-α-L-arabinopyranose		4.51		3.45		3.70	3.60		4.11	3.53				33
6-deoxy-aldohexopyranoses														
α-D-quinovopyranose	5.18			3.54		3.66		3.14		3.90			1.26	*
β-D-quinovopyranose		4.63		3.25		3.43		3.16		3.49			1.29	*
α-L-rhamnopyranose	5.11		3.92			3.80		3.44		3.87			1.29	38
β-L-rhamnopyranose		4.86	3.94			3.60		3.36		3.40			1.29	38
α-L-fucopyranose	5.20			3.76		3.86	3.81			4.20			1.21	39
β-D-fucopyranose		4.55		3.45		3.64	3.75			3.80			1.25	39

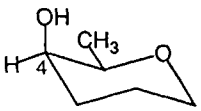
Aldohexopyranoses and Me derivatives											
α-D-glucopyranose	5.22		3.54		3.73		3.35	3.82	3.84	3.75	*
β-D-glucopyranose		4.63	3.25		3.45		3.36	3.46	3.89	3.72	*
β-D-gulopyranose		4.88	3.63	4.07		3.82		4.00	3.75	3.74	40
Me α-D-gluco-pyranoside	4.81		3.56		3.68		3.41	3.64	3.87	3.76	3.39	41
Me β-D-gluco-pyranoside		4.37	3.28		3.50		3.40	3.46	3.92	3.74	3.57	41
2-OMe α-D-glucopyranoside	5.45		3.24		3.72		3.40	3.81	3.86	3.75	3.41	33
2-OMe β-D-glucopyranoside		4.67	2.96		3.52		~3.42	~3.42	3.82	3.72	3.47	
3-O-Me β-D-gulopyranoside		4.81	3.65	3.67		4.03		3.92	3.74	3.74		33

* Remeasured at 500 MHz for this article.
** Taken from reference 33 and adapted to reference TSP

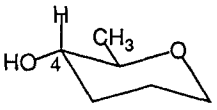
Table 5. Increments (ppm) caused by a Methyl Group on ß and γ Protons

	6-deoxy sugars* ON CHOH	Me-cyclo-hexanes[42] on CH_2	Me-dioxanes[25] on CH_2	spirodioxanes[25] C-CH_2 on CH_2	spirodioxanes[25] O-CH_2 on CH_2	cyclohexanol[24] CH_2-OH on CH_2	OMe ethers* in pyranoses on CH_2O	OMe ethers* in pyranoses on>CH-OH
β-increments								
β $Me_{eq}(g)H_{ax}$	-0.47/-0.53	-0.37	-0.46					
β $Me_{ax}(g)H_{eq}$		-0.14/-0.20	-0.10/-0.32					
β $Me_{eq}(g)H_{eq}$	+0.13/+0.19	-0.02	+0.09/+0.11					
β $Me_{ax}(a)H_{ax}$		+0.18	+0.17/+0.20					
γ-increments								
γ$CH_3(ag)H$	+0.04/-0.04	-0.01	+0.01/+0.03	+0.23/+0.30	+0.17/+0.19	+0.02	-	-0.06/+0.02
γ$CH_3(aa)H$	+0.04	-0.02	-0.02/-0.05	+0.20	+0.27	+0.01	-	+0.05
γ$CH_3(ag^-)H$		+0.23	+0.11/+0.25	+0.20/+0.40	+0.52/+0.60	+0.46	+0.20	+0.28
γ$CH_3(ga)H$		-0.26	-0.16/-0.22	-0.25/-0.33	-0.20	-0.27	-0.10/-0.14	-
γ$CH_3(g^+g^+)H$		-0.13**	-	-0.30	+0.28/+0.30	-	-	~+0.11

* Extracted from the data in Table 4.
** Measured in tert-butylcyclohexane



3a



3b

Figure 3. Examples of the influence of β-Methyl and β-ring oxygen on the position of H-4 in a pyranose system.

3a and (-0.09) + (+0.11) = +0.02 ppm for H-4 in 1b (see **Table 5** first column). The experimental increment value for $\beta Me_{eq}(g)H_{eq}$ is due.

The γ Increments

The two most striking γ increments are recognized, namely $\gamma CH_3(g^+g^-)H$[26,27] and $\gamma CH_3(ga)H$,[28] which show an important downfield and upfield shift respectively. Although the downfield effect of the former has been rationalized, so far no rationale has been given for the latter. Gorrichon suggested that the negative value for $\gamma CH_3(ga)H$ could originate from the orientation of one of the lobes of the ring oxygen.[25] In **Table 5** we see that the same effect exists in the cyclohexanes, although no ring oxygen is present.

From consideration of the data from **Table 5** it follows that increments found in spirodioxanes deviate much from those in simple systems. Even in simple monocyclic systems the values found for $\gamma CH_3(g^+g^+)H$ are not reliable. By considering the rotamers around the C-C$(CH_3)_3$ bond in *tert*-butylcyclohexane Gorrichon derived a value of -0.13 ppm for this increment.[25,29,30] A negative value (-0.06 ppm) was also found by Tavernier during his study of decaline derivatives.[31] In spirodioxanes important positive values were found for this increment.[30,32] Consequently, application of increments extracted from different classes of compounds, as we reported in a previous study, may lead to erroneous conclusions.[33] Therefore, in this study we have chosen to extract the increments from a few selected compounds with closely related structures and conformations.

Extraction of the ^{1}H NMR chemical shift increments for pyranose systems. Correlation between the ^{1}H NMR chemical shifts and the heteronuclear ^{3}J(CH_3O-CH) coupling constant.

The ^{1}H NMR chemical shift increments listed in the last column of **Table 5** are extracted from Me α- and -β-D-glucopyranose. Taking into consideration the exo-anomeric effect, only one rotamer must be considered when the anomeric hydroxyl is methylated. Two rotamers around the C_1H-OMe glycosidic bond are represented in 4a and 4b in **Figure 4** respectively.

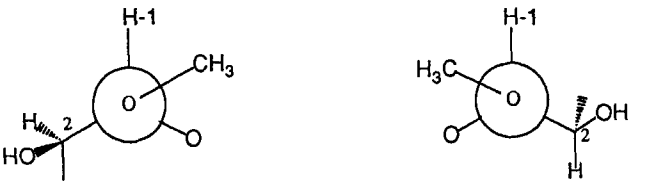
The β effect is -0.41 ppm and -0.27 ppm in the α and β modification respectively. In the α modification there is a γCH_3(aa)H effect operating on H-2 of +0.02 ppm while in the β modification there is a γCH_3(ag)H effect found of +0.03 ppm.

For comparative reasons and because we are interested in knowing the extent of such effects on methylene protons, we have measured the ^{1}H NMR chemical shift increments of 4-OMe-α-L-arabinopyranose. Because of the unfavorable *syn*-axial effect between OCH_3 and C_3-OH in one of the rotamers and the negative β effect, only one rotamer must be considered, as represented in **Figure 5.**

A smaller β effect on H-4 than found for H-1 in α-D-glucopyranose is explained by the fact that in the glucoside two α oxygens are operative and in the arabinose only one. There is a γCH_3(aa)H increment of -0.05 ppm on a >CHOH proton as well as a γCH_3(ga)H increment of -0.14 ppm and a $\gamma CH_3(g^+g^-)$H increment of +0.20 ppm on the -CH_2O- protons (see **Table 5**).

For the present study we remeasured the ^{1}H NMR data of 2-OMe-α- and -β-D-glucopyranose at 500 MHz. They were published earlier, as assigned by homo-INDOR experiments at 300 MHz.[33] The chemical shifts for the ring protons were assigned from a COSY experiment. The new study showed that only the value for the chemical shift of H-5 must be reconsidered, δ 3.82 instead of δ 3.68.

For 2-OMe-α-D-glucopyranose only one rotamer is possible when it is taken into consideration that in the other rotamers an unfavorable *syn*-axial interaction should exist between OCH_3 and C-3-OH on one hand and that the β effect is found to be negative. The most probable rotamer is represented in **Figure 1.** On H-1 a $\gamma CH_3(g^+g^-)$H effect (synaxial) of + 0.28 ppm is observed,



4a 4b

Figure 4. Two rotamers for Me α-D-glucopyranoside (4a) and Me β-D-glucopyranoside (4b).

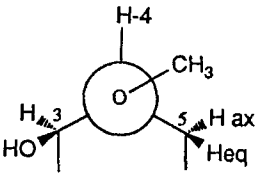


Figure 5. Rotamer of 4-OMe-α-L-arabinopyranose

while on H-3 there is no γCH_3(ag)H. These values are indeed expected for such γ effects (see **Table 5**).

For the β analogues the situation is more complex. The three relevant rotamers around C-2-OMe are represented in **Figure 2.**

Since the β-effect must cause an upfield shift to correspond with the experimental values, rotamer 2c can virtually be excluded. Moreover, for 2c a γ *syn*-axial effect would be operative on both H-1 and H-3 for which increments of +0.28 ppm are expected. This does not at all agree with the experimental values. Unfortunately, in both 5a and 5b we encounter each time an unfavorable OCH_3,COH interaction. In 2a there is a $\gamma CH_3(g^+,g^+)H$ increment for H-1 and a γCH_3(ag)H increment for H-3. Except for spirodioxanes, in all the classes of compounds γCH_3(ag)H an increment close to 0 ppm (see **Table 5**) is seen. For H-3 we find an increment of +0.05/0.07 ppm, a value too large for such a γCH_3(ag)H increment. For 2b we expect a γCH_3(ag)H increment for H-1. Experimental values of +0.03/0.04 ppm can be considered in agreement with such an arrangement. The small differences found for H-1 and H-3 lead us to assume that

rotamer 2a is slightly less populated than rotamer 2b. This implies that $\gamma CH_3(g^+,g^+)H$ should have a positive value. This finding is in contradiction with the values proposed for such an increment by Gorrichon[25] or Tavernier,[31] but it is in agreement with the results of our study on ^1H NMR information of the glycosidic bond.[34] That means that there is an averaging between rotamers 5a and 5b so that the averaged torsion angle between OCH_3 and C-H-2 must be smaller for a β form than an α form, although we cannot derive if it is in the positive or negative direction from the eclipsed position. The observation is in agreement with the values measured for the coupling constants $^3J(CH_3O\text{-}CH)$ (greater for β than for α, and consequently - as both are between 0° and 90°, or one between 0° and 90°, and the other between 300° and 0° - a smaller torsion angle for β than for α).

Applications of the proposed chemical shift increments for the identification of 3-OMe-β-D-gulopyranose

The following is an example of how the proposed chemical shift increments might be used in distinguishing 3-O-Me-β-D-gulopyranose form the 2-O-Me and 4-O-Me isomers. With a HMBC experiment such identification can be achieved when a correlation between a resonance for a ring proton and a ^{13}C NMR methyl carbon resonance can be found. With the ^1H NMR chemical shift increments proposed in this study and/or the ^{13}C NMR increments proposed by Angyal such identification is readily achieved.[15]

For 3-OMe-β-D-gulopyranose only one rotamer must be considered (because of the O-Me, C_2-OH *syn-axial* interaction in the other rotamer) as represented in **Figure 6.**

In 6a a $\gamma CH_3(g^-g^+)H$ effect is operating on H-4 for which a value of +0.28 ppm is expected and a $\gamma CH_3(aa)H$ effect on H-2 for which a small down-field shift is expected. For H-1 and H-5 a δ effect must be considered.[33] A comparison between the calculated and the experimental values is given in **Table 6.** The values expected are in good agreement with the experimental values (smaller than 0.10 ppm).

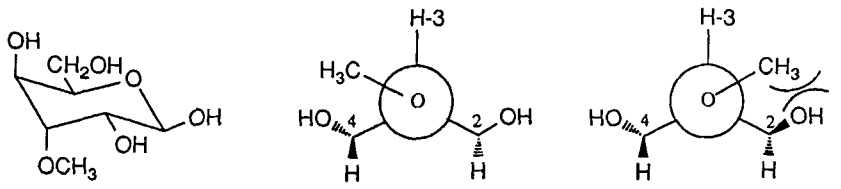


Figure 6. Two rotamers of 3-OMe-β-D-gulopyranose

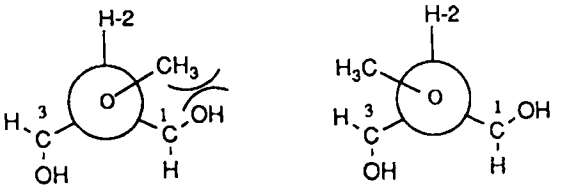
TABLE 6. Comparison between the calculated chemical shifts for 3-OMe-β-D-gulopyranose and the experimental data for 3-OMe-β-D-gulopyranose

	H-1	H-2	H-3	H-4	H-5
Chemical shifts of β-D-gulopyranose	4.88	3.63	4.07	3.82	4.00
Increments	-0.05	+0.02	-0.30	+0.28	-0.05
Expected values for 3-OMe-β-D-gulopyranose	4.83	3.65	3.77	4.10	3.95
Experimental values	4.81	3.65	3.67	4.03	3.92

For 2-OMe-β-D-gulopyranose (Figure 7) the calculated values would not agree with the experimental data from the 3-OMe derviative. Rotamer 7a can be excluded because of the *syn*-axial interaction between OCH_3 and C-1-OH.

In 7b there is a $\gamma CH_3(g^+g^-)H$ increment for H-3 and a $\gamma CH_3(aa)H$ increment for H-1. We have represented the calculated and experimental values in **Table 7.** There is no agreement at all between both sets of values.

Likewise a discrepancy between the calculated values for the 4-OMe dervative and the experimental values for the 3-Me derivative is found. The possible rotamers are represented in **Figure 8.** In this case only rotamer 8b must be considered, because in 8a there is a *syn*-axial interaction between OCH_3 and CH_2OH.

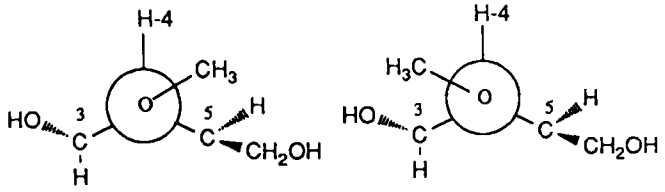


7a 7b

Figure 7. Two rotamers for 2-OMe-β-D-gulopyranose

TABLE 7. Comparison between the calculated chemical shifts for 2-OMe-β-D-gulopyranose and the experimental data for 3-OMe-β-D-gulopyranose

Chemical shifts of β-D-gulopyranose	4.88	3.63	4.07	3.82	4.00
Increments	+0.02	-0.30	+0.28	-0.05	-
Expected values for 2-OMe-β-D-gulopyranose	4.90	3.33	4.35	3.77	4.00
Experimental values	4.81	3.65	3.67	4.03	3.92



8a 8b

Figure 8. Two rotamers for 4-O-Me-β-D-gulopyranose

In 8b we must consider a $\gamma CH_3(g^+g^-)H$ increment for H-3 and a $\gamma CH_3(aa)H$ increment for H-5. The expected and experimental values are represented in **Table 8.** For β $Me_{eq}(g)H_{ax}$ the averaged value -0.30 ppm was used.

A previous study on the ^1H NMR chemical shifts increments concluded that effects causing the increments are cumulative.[33] Recently Vogt and co-workers published the ^1H and ^{13}C NMR data of some partially O-methylated α- and β-D-galactopyranose derivatives and of their methyl glycosides in D_2O solution.[35] Application of cumulative chemical shift increments applied on the chemical shifts of the protons of these compounds are found in agreement with the experimental values.

EXPERIMENTAL

The ^1H NMR spectrum at 20 °C was obtained with a Bruker AM-500 spectrometer operating at at 500.12 MHz, using a pulse angle of 19° and a resolution of 0.33 Hz/point. The ^{13}C NMR experiment was run on a Bruker WH-360 spectrometer operating at 90.556 MHz, with a pulse angle of 18° and a resolution of 1.327 Hz/point.

One-dimensional ^1H-coupled ^{13}C NMR spectra and two-dimensional HMBC spectra were recorded at the Complex Carbohydrate Research Center (University of Georgia, Athens, GA, USA) on Bruker AM-500 and AM-250 spectrometers, respectively, at 23 °C. ^1H Chemical shifts (δ) are expressed in ppm downfield from internal 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) with an accuracy of 0.01 ppm; ^{13}C chemical shifts are expressed in ppm downfield from internal DSS with an accuracy of 0.02 ppm. The accuracy of the coupling constants (J) expressed in Hz has an accuracy of 0.1 Hz.

The COSY 45 experiment involved the sequence $90°(^1H)\text{-}t_1\text{-}45°(^1H)\text{-}t_2$[36]. The $90(^1H)$ pulse was 4.8 μs. A $\pi/2$ shifted sine-bell function was used in each dimension. A 1 x 0.5 K matrix was obtained using 16 scans per t_1 increment.

The $^{13}C,^1H$ heteronuclear correlated experiment involved the Bax-Morris sequence $90°(^1H)\text{-}t_{1/2}\text{-}180(^{13}C)\text{-}t_{1/2}\text{-}\Delta_1\text{-}90°(^1H)90°(^{13}C)\text{-}\Delta_2\text{-}t_2$[22]. The $90°(^1H)$ pulse was 8.20 μs, the $90°(^{13}C)$ pulse was 10.20 μs. Δ_1 was 3.2 ms and Δ_2 was 2.1 ms. The

TABLE 8. Comparison between the calculated chemical shifts for 4-OMe-β-D-gulopyranose and the experimental data for 3-OMe-β-D-gulopyranose

Chemical shifts of β-D-gulopyranose	4.88	3.63	4.07	3.82	4.00
Increments	-	-0.05	+0.28	-0.30	+0.05
Expected values for 4-OMe-β-D-gulopyranose	4.88	3.58	4.35	3.52	4.05
Experimental values	4.81	3.65	3.67	4.03	3.92

spectral width for ^{13}C was 20000.0 Hz and 2000.0 Hz for ^{1}H. A maxtrix of 4 x 1 K data points was obtained using 88 scans per t_1 increment.

Two-dimensional HMBC experiments[11] were performed in absolute-value mode, using a 5-mm dual-frequency probe with conventional geometry. The ^{1}H spectral width was set to 1000 Hz (at 250 MHz) and the carrier placed at the center of the spectrum (δ 3.75) 128 FIDs of 1024 complex points were acquired with 256 scans per FID. The spectral width in the ^{13}C dimension was set to 2525 Hz (40.1 ppm at 62.9 MHz) with the carrier at δ64, based on internal DSS. Time intervals were set as follows: relaxation delay, 1.2 s; the delay for the evolution of long range couplings, 60 ms; acquisition times, 512 and 12.672 ms in the t_2 and t_1 dimensions, respectively. Data were processed with sine-bell weighting functions applied in the t_2 and t_1 domains, and zero-filling to 1024 x 256 points before Fourier transformation.

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