ORGANIC LETTERS

2005
Vol. 7, No. 4
661−664

$^2J_{C,H}$ Index: A Nondestructive NMR Method for Differentiation of Aldohexopyranosyl Residues

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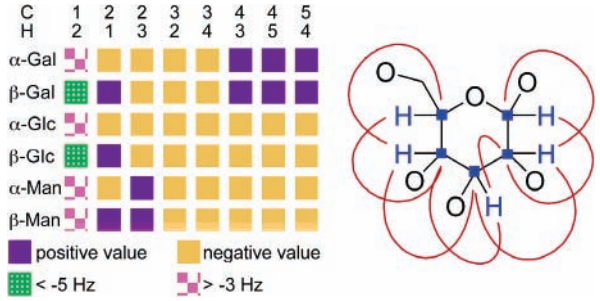
Received December 23, 2004

ABSTRACT



A new $^2J_{C,H}$ index method is described for identification of aldohexopyranose. This method is based on a fact that $^2J_{C,H}$ values reflect the stereochemistry for glycol connectivity. Based on the observed $^2J_{C,H}$ values for galactose, glucose, and mannose, $^2J_{C,H}$ profiles for other aldohexopyranoses are proposed. A combination of $^2J_{C,H}$ values was found to be useful for identification of aldohexopyranosyl residues in glycans.

Rapid and unambiguous identification methods for saccharide components in glycans are of growing importance since carbohydrates have a variety of important biological functions. Among several methods,[1] NMR is the most advantageous because the sample used can be recovered intact after the experiment. Conventional NMR studies for saccharide component analysis are mainly based on three-bond $^1H-^1H$ coupling constants ($^3J_{H,H}$) of vicinal protons and NOEs, by which the works are quite often complicated because of severe signal overlapping at oxymethine region.

We therefore turned our attention to possible eight $^2J_{C,H}$ values on a pyranose ring not only as a complementary method but also as an alternative one for differentiation of aldohexopyranoses. Since $^2J_{C2,H1}$ value was first reported by Casu and Perlin in 1969 on D-glucose,[2] absolute values of $^2J_{C,H}$ have been often appeared in the literature especially on $^2J_{C1,H2}$.[3] Complete sets of absolute $^2J_{C,H}$ values for methyl 2,3,4,6-tetra-*O*-acetylaldohexopyranosides were reported in a systematic manner by Morat et al. in 1988.[4] Until 1990s, the (±)-sign of $^2J_{C,H}$ value was not of significant interest except for the work made by Perlin et al.,[5] Bock et al.,[6] and

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10.1021/ol047358a CCC: $30.25
Published on Web 01/22/2005

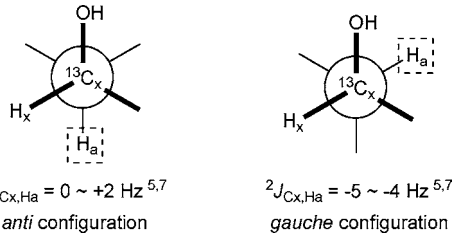

Figure 1. Two possible relative arrangements for Ha and the hydroxy group.

Serianni et al.,[7] where signs of $^2J_{C,H}$ values of some aldohexopyranose derivatives were reported partially. This situation is apparently due to the difficulties to determine the (±)-signs of the values.[5a] Differentiation of aldohexopyranoses on the basis of $^2J_{C,H}$ values thus has never been attempted in a systematic manner.[8]

In this paper we wish to report an empirical rule for identification of aldohexopyranoses based on *a combination of eight* $^2J_{C,H}$ *values* around all carbons in a pyranosyl ring. The work described here was evolved from two considerations. First, $^2J_{C,H}$ value including (±)-sign depends on the relative spatial arrangement of the proton and the electronegative substituent on the particular carbon, as originally proposed by Perlin et al. in 1975[5a] and confirmed partly by Serianni et al. later;[7] when Ha takes an *anti* position against an electronegative hydroxy group, the $^2J_{Cx,Ha}$ value ranges from 0 to +2 Hz, whereas the value is from −5 to −4 Hz in a *gauche* arrangement (Figure 1). Second, continuous glycol connectivity in carbohydrates would be suitable for structural analysis based on such $^2J_{C,H}$ values. In the present study, eight $^2J_{C,H}$ values of the respective α- and *β*-anomers of galactose, glucose, and mannose $^4C_1$ conformers (Figure 2) were fully determined at first in a systematic manner. Individual values as well as their whole profiles were compared each other as a characteristic of each aldohexopyranose. NMR analysis was further carried out on two disaccharides, lactose and sucrose, to prove that $^2J_{C,H}$ values are not substantially affected by long-range nonbonding interactions with another saccharide residue.[9]

HETLOC techniques[10] were used in the present study since it is the most simple method to determine $^2J_{C,H}$ values with (±)-sign among the methods so far known.[4,5,7,11] For selective three-bond magnetization transfer, mixing time of 15−40

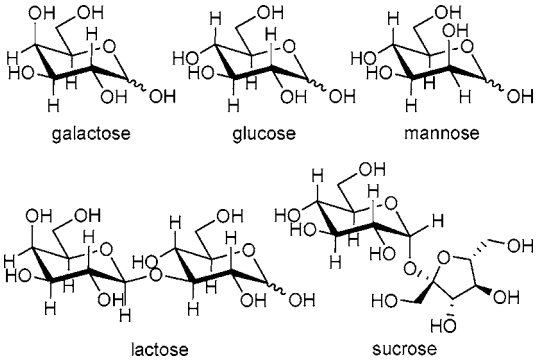

Figure 2. Carbohydrates used in the NMR study.

ms was applied for HETLOC measurements. The spectra were collected at 4K (*F*2) × 512 (*F*1) and 2-fold zero-fillings were applied in both dimensions. In all spectra the resolutions were satisfactorily less than 0.7 Hz. Details with regard to experimental are summarized in Supporting Information.

HETLOC spectra were analyzed on the basis of the assignments of proton signals. $^2J_{Cx,Ha}$ values were determined from the split HETLOC peaks as shown in Figure 3. In

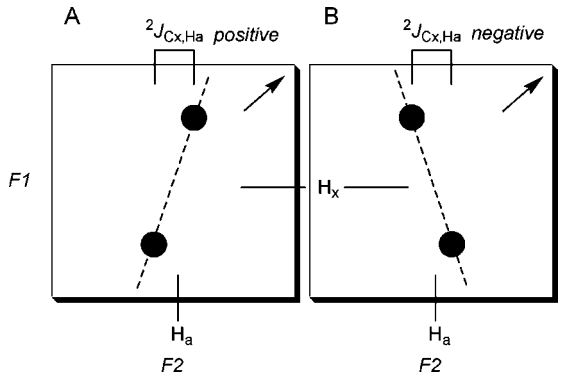

Figure 3. Appearance of HETLOC peaks against diagonal ones indicated by an arrow: (A) *anti* configuration shown in Figure 1 has positive $^2J_{Cx,Ha}$ value; (B) *gauche* configuration shown in Figure 1 has negative $^2J_{Cx,Ha}$ value.

general, the $^2J_{Cx,Ha}$ values were evaluated from a chemical shift difference of the two Hx(*F*1)−Ha(*F*2) peaks split in the *F*2 dimension. The (±)-sign of $^2J_{Cx,Ha}$ was determined

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Table 1. Eight $^2J_{C,H}$ Values of Three Aldohexopyranoses in D_2O at 303 K[a]

	anomeric config	C1/H2[b]	C2/H1	C2/H3	C3/H2	C3/H4	C4/H3	C4/H5	C5/H4
galactose	α	−0.4	−1.0	−5.4	−6.2	−4.6	+1.2	+5.6	+1.5
	β	−6.0	+0.4	−4.2	−4.4	−5.2	+1.8	+3.0	+0.7
glucose	α	−1.1	−1.7	−4.1	−4.0	−4.6	−4.6	−2.9	−3.7
	β	−6.4	+1.3	−4.7	−4.4	−5.0	−5.6	−4.2	−4.1
mannose	α	−1.1	−1.5	+1.3	−3.9	−3.3	−3.7	−1.8	−3.0
	β	−1.7	+8.0	+1.6	−4.3	−5.4	−3.9	−2.1	−3.1

[a] $^2J_{C,H}$ values were determined from HETLOC spectra collected on 500 or 600 MHz spectrometers. [b] Values are given in Hz with an experimental error of less than 0.7 Hz.

from the slope of the linked line between the split peaks against the slope of the diagonal peaks; the $^2J_{Cx,Ha}$ value is positive if the coefficients for the slope of both lines take the same (±)-signs (Figure 3A), whereas the value is negative if they have different signs of coefficients (Figure 3B). All pairs of HETLOC correlations required for determination of eight $^2J_{C,H}$ values on pyranose rings were observed, while some of them unfortunately overlap with other signals such as diagonal peaks. In such cases, evaluation was carried out by 2-fold multiplication of the difference value (Hz) of one remaining peak of a pair of HETLOC peaks from the correct chemical shift of the proton, which is shown in 1D NMR spectrum and should be the center of the split HETLOC signal (Figure 4).

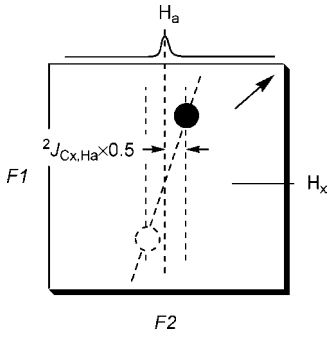

Figure 4. Determination of $^2J_{C,H}$ value in the case where one of HETLOC cross-peaks is hidden. For details, see text.

The $^2J_{C,H}$ values of galactose, glucose, and mannose are summarized in Table 1. All $^2J_{C1,H2}$ values are negative. The respective values for β-galactose and β-glucose were found to be −6.0 and −6.4 Hz, which were consistent with the data reported[5a,12] with an error of 0.7 Hz, to indicate the reliability of the analysis using HETLOC spectra. These values were particularly smaller than the corresponding values for other hexoses shown in Table 1. $^2J_{C2,H1}$ values relate with the configuration of anomeric position; α- and β-pyranoses have negative and positive values, respectively.[6,7] Exceptionally large $^2J_{C2,H1}$ value (+8.0 Hz) was observed for β-mannose in the present study as have been also stated by Serianni et al.[7] $^2J_{C2,H3}$ values reflect the difference of the three hexoses examined. Thus, galactose and glucose have negative values whereas the value of mannose is positive. In all three sugar residues in Table 1, minus signs are observed for both $^2J_{C3,H2}$ and $^2J_{C3,H4}$ values. This fact is due to the identical configuration of the C3-hydroxy groups. The (±)-signs of $^2J_{C4,H3}$, $^2J_{C4,H5}$, and $^2J_{C5,H4}$ values are also diagnostic for the three hexoses as are the $^2J_{C2,H3}$ value; glucose and mannose have negative values, whereas galactose has positive values for these three *J*-coupling constants.

From the results shown above, whole profiles[13] of eight $^2J_{C,H}$ values were expected to be a useful method for differentiation of aldohexopyranoses generally. To prove this hypothesis, HETLOC measurements were next carried out on two disaccharides, lactose and sucrose, to obtain $^2J_{C,H}$ values of galactose and glucose residues included therein. The results are summarized in Table 2. Nineteen $^2J_{C,H}$ values out of 40 entries listed in Table 2 were found to be coincident well within 0.7 Hz of spectrum resolution with the values listed in Table 1. Differences ranging 0.7−1.3 and 1.4−2.0 Hz were observed in 14 and 6 entries, respectively. The $^2J_{C3,H2}$ value (−7.2 Hz) in the β-galactosyl residue of β-lactose exhibits the largest fluctuation. Differences in these 21 cases are assumed to come from the slight conformational changes brought by the presence of the other saccharide residues. Such conformational changes, however, never alter the relative spatial relation between the protons and vicinal oxygen functions of interest which determine the sign of $^2J_{C,H}$ values (Figure 1).[14] In fact, the whole profile of $^2J_{C,H}$ values allows a proper assignment of the galactose and glucose residues in lactose and sucrose by an overall comparison with the profiles of monosaccharide residues shown in Table 1.[13] From these results, we concluded that the $^2J_{C,H}$ profiles can be utilized for differentiation of the α- and β-anomers of galactose, glucose, and mannose residues of $^4C_1$ conformations irrespective of their forms (as a monosaccharide or a part of glycan) and location (nonreducing to reducing end) in glycans.[15,16]

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(13) The profiles represented by graphs are included in Supporting Information.

(14) A quantitative interpretation for these discrepancies is under investigation using quantum mechanical calculations; see: Bifulco, G.; Bassarello, C.; Riccio, R.; Gomez-Paloma, L. *Org. Lett.* **2004**, *6*, 1025−1028.

Table 2. Eight $^2J_{C,H}$ Values of Galactose and Glucose Residues in Lactose and Sucrose[a]

	C1/H2b	C2/H1	C2/H3	C3/H2	C3/H4	C4/H3	C4/H5	C5/H4
α-lactose								
β-D-Gal*p*-(1→4)-α-D-Glc*p*	-6.1	+1.6	-5.3	-6.4	-4.3	+0.9	+5.0	+1.2
β-D-Gal*p*-(1→4)-α-D-Glc*p*	-0.8	-0.6	-4.7	-4.7	-4.2	-4.4	-4.8	-4.0
β-lactose								
β-D-Gal*p*-(1→4)-*β*-D-Glc*p*	-6.1	+0.2	-5.7	-7.2	-4.3	+1.3	+5.0	+1.2
β-D-Gal*p*-(1→4)-*β*-D-Glc*p*	-6.3	+0.5	-4.2	-5.1	-6.2	-5.0	-4.9	-5.5
sucrose								
β-D-Fru*f*-(2↔1)-α-D-Glc*p*	0	-2.2	-4.2	-4.0	-4.1	-4.5	-4.0	-3.0

[a] HETLOC measurements were carried out by 500 or 600 MHz NMR spectrometers to evaluate $^2J_{C,H}$ in D_2O at 303 K. [b] Values are given in Hz with an experimental error of less than 0.7 Hz.

We have thus confirmed for the first time in a systematic manner, by using α- and *β*-anomeric isomers of typical aldohexopyranoses, galactose, glucose, and mannose, that the $^2J_{C,H}$ values including (±)-signs of aldohexopyranoses evaluated by means of HETLOC spectra follow the rule proposed by Perlin et al.[5] The whole pattern of $^2J_{C,H}$ values was found to be a characteristic for each aldohexopyranose tested. This was also the case for *β*-galactose, α-glucose, and *β*-glucose residues in lactose and sucrose. It was thus concluded that the whole $^2J_{C,H}$ profiles are useful criteria for distinguishing aldohexopyranoses. We propose, however, that rough classification of each value (except for $^2J_{C1,H2}$) simply by (±)-sign should be enough to discriminate these hexoses (Figure 5). Other aldohexopyranoses such as allose, altrose, idose, and talose would also have their characteristic $^2J_{C,H}$ profiles as speculated in Figure 5.[15,16] Structural identification of unknown aldohexopyranoses will be generally possible by comparing the profile of $^2J_{C,H}$ values with authentic ones: we propose to name this the $^2J_{C,H}$ index method.

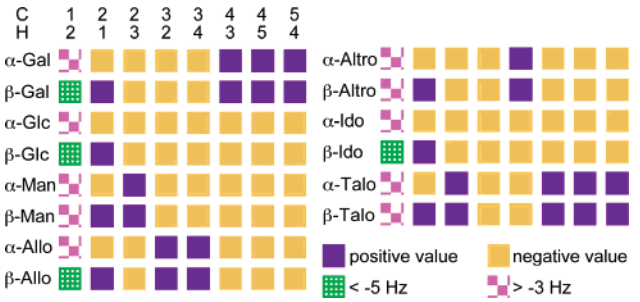

Figure 5. $^2J_{C,H}$ index of aldohexopyranoses ($^4C_1$ conformer) known. Indexes are characteristic to each saccharide except for glucose and idose. The (±)-sign was speculated from the work by Perlin[5] and by ourselves.

Although HETLOC is more sensitive than other NMR sequences used for determination of $^nJ_{C,H}$ values,[4,5a,7a] it still requires a larger amount of samples (nearly 10 mM or more) than that used for two-dimensional $^1H-^1H$ spectroscopy such as COSY and TOCSY. However, it should be emphasized here again the inherent advantage of nondestructive NMR analysis that allows recovery of the sample intact. In addition, structural analysis of saccharide residues would be possible by HETLOC spectrum alone since it contains TOCSY information as well. Preparation of authentic $^2J_{C,H}$ profiles for other carbohydrates is continued in our laboratory.

Supporting Information Available: The sample preparation, $^2J_{C,H}$ profiles represented by graphs, and HETLOC spectra for all aldohexopyranoses. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The applicability of this method to glycan has been also proved in our successful mannosyl residue analysis in a complex bacterial polysaccharide[16] composed of a repeating unit, →3)-*β*-D-Glc*p*-(1→6)[α-D-Gal*p*-NAc−(1→2)-*β*-D-Gal*p*-(1→4)]*β*-D-Glc*p*NAc−(1→3)-α-D-Gal*p*-(1→4)-*β*-D-Man*p*-(1→. Full account on this work will be published soon.

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