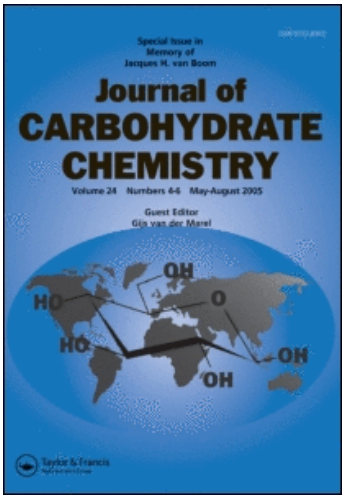

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TRIMETHYLSILYLATION - AID IN NMR ANALYSIS OF OLIGOSACCHARIDES. ASSIGMENT OF ^{29}Si AND ^{13}C NMR SPECTRA OF TRIMETHYLSILYLATED METHYL β-D-XYLOBIOSIDES BY 2D NMR

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ABSTRACT

The ^{1}H, ^{13}C and ^{29}Si NMR spectra of methyl β-D-xylopyranoside and three methyl β-D-xylopyranosyl-β-D-xylopyranosides have been measured and assigned by two-dimensional NMR spectroscopy. According to the determined proton-proton coupling constants, the ring conformer ratio is essentially the same in the studied compounds. The assigned ^{13}C chemical shifts provide correct substituent chemical shifts for assignments in the spectra of higher trimethylsilylated xylo-oligosaccharides. Heteronuclear chemical shift correlated 2D NMR spectroscopy is proven to be a usable experimental method for ^{29}Si NMR line assignment in carbohydrates. The assigned silicon shifts identify the site of glycosidation.

INTRODUCTION

Trimethylsilylation is a well-established derivatization method in carbohydrate chemistry.[1] It is used not only for chromatographic

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separation purposes but also for NMR determination of hydroxyl groups. Traditional ^{1}H NMR determinations of OH groups utilize the nine-fold increase in signal intensity as one OH proton is replaced by nine $Si(CH_3)_3$ protons. Thus, the accuracy and sensitivity of the determination are increased.[2] However, the ^{1}H NMR lines of $OSi(CH_3)_3$ groups are difficult to assign and are usually not too well separated as the chemical shifts are not very sensitive to the structure of the rest of the molecule.

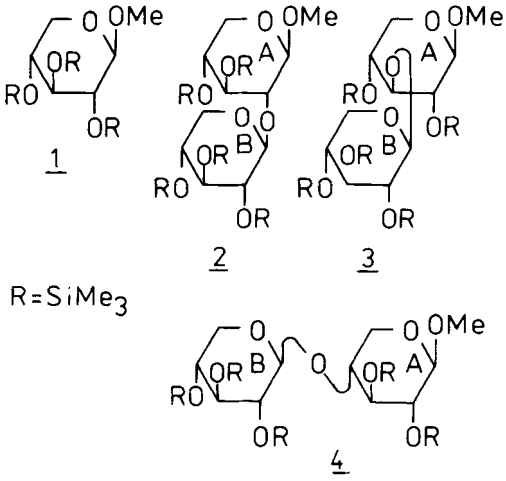
In contrast, ^{29}Si NMR chemical shifts of the $OSi(CH_3)_3$ groups are very susceptible to molecular structure,[3] but silicon is not very sensitive to NMR detection.[4] Fortunately, the recent progress in NMR instrumentation and the introduction of polarization transfer techniques like INEPT[5] or DEPT[6] have dramatically enhanced the sensitivity of ^{29}Si NMR spectroscopy. Although these experiments require relatively large quantities of material, the trimethylsilylation reaction conditions allow the recovery of the intact oligosaccharides for additional experimental purposes.

^{29}Si NMR spectra of trimethylsilyl derivatives are now routinely measured[7] and the spectral data can be used for the determination of different OH groups originally present in the sample. More detailed interpretation requires assignment of ^{29}Si chemical shifts. Several tedious methods have been developed for this purpose.[8-10] Surprisingly, simple heteronuclear ^{1}H - ^{29}Si chemical shift correlated two-dimensional NMR spectroscopy[11] has not yet been explored, even though it is especially advantageous for carbohydrate applications. In this paper, we present the first results of such experiments carried out on trimethylsilylated methyl β-D-xylopyranoside derivatives **1** - **4**.

RESULTS AND DISCUSSION

^{1}H NMR Spectra.

In any heteronuclear correlation assignment experiment, multiplets in ^{1}H NMR spectra must be assigned first. Among the available experimental techniques, two-dimensional homonuclear shift correlation spectroscopy is preferred for reasons stated elsewhere[11] and these homonuclear two-dimensional spectra greatly facilitate interpretation of heteronuclear spectra to be analysed.[12]



For the assignment of line multiplets in ^{1}H NMR spectra of trimethylsilylated methyl β-D-xylobiosides, we have employed the standard Jeener (or COSY 90°) experiment.[11] A contour plot of a typical two-dimensional Jenner spectrum of a disaccharide is shown in Fig. 1. Though some protons are strongly coupled and some multiplets overlap, the Jeener spectra of disaccharides are easily analysed and the multiplets assigned along the lines described in the literature.[11,12]

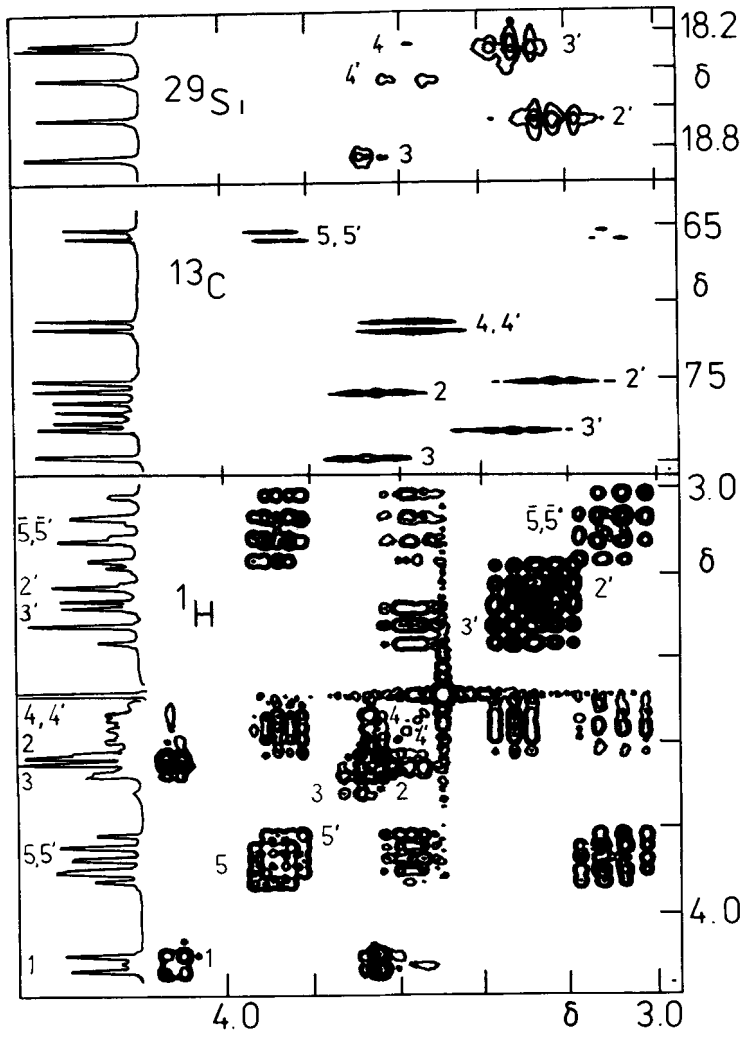


Fig. 1. Partial ^{1}H, ^{13}C and ^{29}Si NMR spectra and their correlations as contour plots (compd.2). Primed are nuclei of B ring. The proton on C-5 carbon resonating at a higher field is denoted by $\bar{5}$.

We have not attempted to analyse the assigned spectra in terms of proton chemical shifts and coupling constants. Only the vicinal couplings between H-1 and H-2 protons, $^{3}J(1,2)$, have been extracted as they provide information on ring conformation and can be read directly from the spectra. As is apparent from Table 1, the coupling constant

TABLE 1

NMR Parameters of Methyl β-D-Xylopyranoside Derivatives 1 - 4

Compd	Ring	Coupling[a]	^{29}Si Chemical Shifts[b]			^{13}C Chemical shifts[b]				
		$^{3}J(1,2)$	Si-2	Si-3	Si-4	C-1	C-2	C-3	C-4	C-5
1	A	7.32	19.14	18.57	18.85	105.11	75.73	78.42	71.31	66.07
2	A	7.28	-	18.88	18.28[c]	103.17	75.83[d]	79.81[d]	72.07[c]	66.42[c]
	B	7.31	18.62	18.31	18.49[c]	101.56	75.19	78.19	71.51[c]	65.90[c]
3	A	7.28	19.42[d]	-	19.18[d]	105.05	76.13	80.21	69.06	66.45
	B	7.42	18.33	18.42	18.20[d]	102.38	75.27	78.59	71.86	66.19
4	A	7.43	19.59	19.15	-	105.29	75.06	76.49	75.00	62.91
	B	7.10	19.08	18.60	18.44	101.56	75.10	78.69	71.49	66.10

[a] in Hz. [b] in δ scale, approximate error ± 0.04 ppm, silicon atoms numbered as the nearest skeletal carbon atoms. [c] assignment of the lines in the column may be interchanged. [d] assignment may be interchanged.

has essentially the same value in both A and B rings of the disaccharides and in all four compounds studied. One must therefore conclude that the pertrimethylsilylated methyl β-D-xylobiosides have about the same ratio of conformer populations in both rings and that this ratio is similar to that found in other trimethylsilylated methyl β-D-xylopyranoside derivatives ($^4C_1$:$^1C_4$ = 0.9).[13]

^{13}C NMR Spectra

Homonuclear ^1H - ^1H and heteronuclear ^1H - ^{13}C chemical shift correlated 2D NMR spectroscopy form a powerful synergistic combination for assignment of both ^1H and ^{13}C NMR spectra.[12] This is again demonstrated in the spectra of **2** shown in Fig. 1 which represents the most unfavourable case studied here. For example, C-5 carbon lines of glycopyranosides are readily identified by their chemical shifts. In the hereonuclear correlation spectra, these carbon lines exhibit correlations with both H-5 and H-5' protons and, thus, can be used to identify the two geminal protons' multiplets in the ^1H NMR spectrum. Due to the complete overlap of H-4 (ring A) and H'-4 (ring B) proton multiplets, the lines due to carbons C-4 and C-5 cannot be unambiguously assigned to the two rings. More significant, however, is the uncertainty due to strong coupling between H-2 and H-3 protons of ring A. At 200 MHz, the two protons are so strongly coupled that the lines in the proton multiplet cannot be assigned to individual protons. Hence, the assignment of the carbon lines that is indicated in Fig. 1 may be in error as it follows only from a juxtaposition of the maxima in homo- and heteronuclear correlation contour maps.

Trimethylsilylation of methyl β-D-xylopyranoside derivatives leads, through conformational homogenization of the series of

compounds, to the improved additivity of ^{13}C chemical shifts.[13] The above-demonstrated conformational homogeneity of trimethylsilylated methyl β-D-xylobiosides suggests that the same can be expected for higher trimethylsilylated xylo-oligosaccharides. Hence, the ^{13}C chemical shifts that are assigned here (Table 1) should yield the correct values of substituent chemical shifts (SCS) of xylopyranosyl groups for the prediction or assignment of the chemical shifts in higher trimethylsilylated xylo-oligosaccharides.

^{29}Si NMR Spectra

For the reasons explained elsewhere,[10,14] heteronuclear ^{1}H-^{29}Si correlation assignment experiments can utilize only long-range couplings of the silicon with protons attached to the skeletal carbon atoms, $^{3}J(^{29}Si\text{-}O\text{-}C\text{-}^{1}H)$. Since these couplings are small (3 - 4 Hz), the ^{1}H - ^{29}Si experiments can be set up in a similar way as the more common ^{1}H - ^{13}C chemical shift correlations through long-range couplings; i.e., long delays (cca 140 ms) must be used in the mixing period. Additional problems arise from the particular features of ^{29}Si NMR (slow relaxation, negative gyromagnetic ratio) and from the presence of nine equivalent methyl protons which are also coupled to the silicon. The former problems call for optimization of the equilibration delay, the latter require careful selection of proton carrier frequency and spectral width (t_1 increment) so that strong f_1 folded signal due to correlation with methyl protons cannot be mistaken for the sought correlation signal.

The correlation experiments with compounds **1** - **4** were conclusive, except for the few signals noted in Table 1, where the ambiguity was due to the almost complete overlap of multiplets of H-4 proton in the

two rings which were additionally overlapped with H-2 multiplet in compound **3**. Despite this, in all three disaccharides, the protons at the glycosidic linkage showed no correlation with ^{29}Si NMR signals and so clearly the site of linkage could be identified.

Though all the ^{29}Si lines are well resolved in the compounds studied, the chemical shift differences are too small to apply an empirical assignment rule.[15] This stresses the need for exact experimental assignment procedures. The above results prove that assignments can be achieved through heteronuclear chemical shift correlation 2D NMR experiments and that the sites of glycosidation can be localized through the use of assigned ^{29}Si NMR spectra.

EXPERIMENTAL

Synthesis

The trimethylsilyl derivatives **1** - **4** were prepared by trimethylsilylation of the corresponding β-**D**-xylopyranosyl derivatives of methyl β-**D**-xylopyranosides which were obtained as described by Kovac.[16] For trimethylsilylation, we have adopted the procedure proposed by Chambaz et al.[17,18] Approximately 100 - 150 mg of the xylobioside were placed in a dry small reaction vessel equipped with a teflon septum seal. A 200% stoichiometric excess of trimethylsilylating mixture [bis(trimethylsily)acetamide and trimethylchlorosilane in 5:1 molar ratio] was added through a syringe. The reaction mixture was vibrationally stirred and heated at 60-70°C for about 1 hour. In the preparation of **4**, dry pyridine had to be added in order to dissolve the parent compound. Excess reagents were distilled off in vacuo under dry nitrogen atmosphere. Purity of the compounds was checked by gas chromatography and NMR spectroscopy.

NMR Measurements

The samples were measured in deuteriochloroform solutions containing 2% (V/V) of hexamethyldisilane which served as a reference for ^{1}H (δ= 0.04) and ^{29}Si (δ= -19.79) NMR spectra. The data reported in Table 1 were obtained in 0.05M solutions, two-dimensional spectra were measured in 0.2 - 0.4M solutions.

All the spectra were measured on a Varian XL-200 spectrometer operating at 200.05 MHz for protons, at 50.3 MHz for carbons, and at 39.7 MHz for silicon spectra. Standard software provided by the manufacturer (H-2Z version) was employed. All the 2D spectra were stored at 1024 x 1024 data matrices.

The Jeener spectra were measured using HOMCOR pulse sequence (with 90° flip angles) which incorporates quadrature detection in both dimensions. Strong signals of trimethylsilyl protons were eliminated by a presaturation through equilibration period lasting 2s. Pseudo-echo shaping was used in both dimensions without zero-filling. Spectral width varied between 250 and 400 Hz, the spectra were symmetrized around the diagonal.

The hereonuclear shift correlated spectra were measured with HETCOR pulse sequence which was slightly modified for the measurement of ^{29}Si spectra. The refocusing delay in the mixing period was programmed as an explicit delay instead of its introduction as a receiver off period in the manufacturer-provided sequence. The ^{13}C NMR spectra were measured with the spectral width 2500 - 3000 Hz and with the same settings in the proton dimension that was used in the Jeener spectra. 512 FIDs were recorded with the refocusing delay corresponding to $J(^{13}C - ^{1}H)$ = 145 Hz. Exponential weighting corresponding to line-broadening of 2 Hz was used in both dimensions.

The ^{29}Si spectra were measured in a narrow width of 100 - 250 Hz, equilibration delay was 5s, refocusing delay was 142 ms. Usually about 64 FIDs were accumulated for each value of time t_1 and about 100 increments of t_1 were used with zero-filling to 512 data points in the interferograms. One-dimensional ^{29}Si NMR spectra were measured by routine INEPT technique.[7]

REFERENCES

1. A. E. Pierce, "Silylation of Organic Compounds", Pierce Chemical Company, Rockford, Illinois, 1979.
2. F. K. Schweighardt, H. L. Retcofsky, S. Friedman and M. Hough, Anal. Chem., 50, 368 (1973).
3. J. Schraml, J. Pola, H. Jancke, G. Engelhardt, M. Černy and V. Chvalovský, Collect. Czech. Chem. Commun., 43, 360 (1976).
4. C. Brevard and P. Granger, "Handbook of High Resolution Multinuclear NMR", Wiley, New York, New York, 1982, p. 100.
5. G. A. Morris and R. Freeman, J. Amer. Chem. Soc., 101, 760 (1979).
6. D. M. Doddrell, D. T. Pegg and M. R. Bendal, J. Magn. Resonance, 48, 323 (1982).
7. J. Schraml, Collect. Czech. Chem. Commun., 48, 3402 (1983).
8. A. H. Haines, R. K. Harris and R. C. Rao, Org. Magn. Resonance, 9, 432 (1977).
9. D. J. Gale, A. H. Haines and R. K. Harris, Org. Magn. Resonance, 7, 635 (1975).
10. J. Past, J. Puskar, J. Schraml and E. Lippmaa, Collect. Czech. Chem. Commun., in press.

11. A. Bax, "Two-Dimensional Nuclear Magnetic Resonance in Liquids", Delft Univ. Press, Delft, 1982, p. 50.

12. J. Schraml, J. Hirsch, E. Petráková, E. Krahé and C. Bliefert, Collect. Czech. Chem. Commun., 49, 1605 (1984).

13. J. Schraml, E. Petráková, O. Pihar, J. Hirsch and V. Chvalovský, Collect. Czech. Chem. Commun., 48, 1829 (1983).

14. J. Schraml, J. Magn. Resonance, 59, 515 (1984).

15. J. Schraml, E. Petráková, O. Pihar, J. Hirsch and V. Chvalovský, Org. Magn. Resonance, 21, 666 (1983).

16. P. Kováč, Collect. Czech. Chem. Commun., 45, 892 (1980).

17. E. M. Chambaz and E. C. Horning, Anal. Lett., 1, 201 (1967).

18. E. M. Chambaz, G. Maume, B. Maume and E. C. Horning, Anal. Lett., 1, 749 (1967).