ELSEVIER

Carbohydrate Research 258 (1994) 27–33

CARBOHYDRATE RESEARCH

NMR assignments for the aldopentoses

Alan J. Benesi [a], Christopher J. Falzone [a], Soojay Banerjee [b], Gregory K. Farber [b,*]

[a] *Department of Chemistry, and* [b] *Department of Chemistry and Center for Biomolecular Structure and Function, 152 Davey Laboratory, The Pennsylvania State University, University Park, PA 16802-6300, USA*

(Received October 19th, 1993; accepted in revised form December 19th, 1993)

Abstract

The ^{1}H and ^{13}C NMR assignments for the simple aldopentopyranoses in $^{2}H_2O$ were made using high field 2D NMR spectroscopy. The furanose forms of ribose were also tentatively assigned. The 2D techniques used to assign the resonances include the HMQC, HMBC, 2QF-COSY, TOCSY, 2Q, and INADEQUATE experiments. Selective 1D TOCSY experiments were also necessary to assign the crowded proton spectrum of ribose.

1. Introduction

In light of their importance in biological systems, it is surprising that the ^{1}H NMR signal assignments for the simple aldopentoses in aqueous solution have not been made. In this paper, we have used ^{13}C, ^{1}H, 2D, 1D, and selective NMR techniques to assign the 500-MHz ^{1}H NMR spectra of the pyranose forms of L-lyxose, L-arabinose, D-xylose, and D-ribose in $^{2}H_2O$. We have also made tentative assignments for the furanose forms of D-ribose. As discussed below, these assignments are complicated by strong ^{1}H scalar (J) coupling [1].

The ^{13}C chemical shifts of the aldopentoses in aqueous solution are known [2], so as much as possible we have used the heteronuclear multiple quantum coherence 2D experiment (HMQC [3]) to assign directly attached protons. We have also used the homonuclear 2D ^{13}C–^{13}C INADEQUATE experiment [4] to trace the carbon skeletons of the sugars, because for some of the sugars the ^{13}C spectrum is quite crowded. In some of the INADEQUATE spectra, strong ^{13}C scalar (J) coupling is observed, as discussed below. For this reason, heteronuclear 2D HMBC

* Corresponding author.

SSDI 0008-6215(94)00004-Y

[3] and ^1H homonuclear 2D 2QF-COSY [5], TOCSY [6], 2Q [7], and 1D selective TOCSY experiments were used to make some of the assignments.

2. Experimental

D-Xylose, D-ribose, L-arabinose, and L-lyxose were purchased from Aldrich Chemical Co. and used without further purification. Both 0.10 and 2.0 M solutions were prepared in 99.9% ^2H_2O (MSD Isotopes). Solutions were allowed to stand at room temperature for at least 24 before spectra were collected. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used as an internal reference (δ ^1H 0.00 ppm; δ ^{13}C 0.0 ppm).

All NMR experiments were performed at 500.13 MHz (^1H) and 125.78 MHz (^{13}C) on a Bruker AM-500 spectrometer operating in the quadrature mode at 25°C. One-dimensional ^1H and ^{13}C spectra were acquired for each sugar solution. For the 0.10 M solutions, two dimensional ^1H phase-sensitive double-quantum-filtered COSY spectra [5], TOCSY spectra [6], and double-quantum spectra [7] were also obtained. 2QF-COSY data were obtained with a spectral width of 2000 Hz in both dimensions except for L-arabinose, which had a spectral width of 2202 Hz in both dimensions. Each 2QF-COSY spectrum contained 4K complex points in the F2 dimension, and 8 transients were collected for each of the 1024 t_1 values. TOCSY spectra were acquired with 4K complex points and a spectral width of 6250 Hz in F2, with 512 experiments and a spectral width of 4000 Hz in the F1 dimension. The mixing time in the TOCSY experiments was 46 ms using DIPSI-2 for spin-locking [8]. ^1H 2Q spectra of D-ribose and L-arabinose were measured with a spectral width of 2000 Hz in the F2 dimension and 4000 Hz in the F1 dimension with 1024 experiments being collected in the latter dimension. The mixing time for the ^1H 2Q experiments was 25 ms. A total of 16 transients were collected per t_1 increment for both the TOCSY and ^1H 2Q data. Final data matrices were either 2K × 1K or 2K × 2K real points.

Direct ^1H–^{13}C two-dimensional correlation information was obtained for the 0.10 M solutions by applying a modified HMQC experiment [3] in which a BIRD sequence [9] was used to facilitate suppression of protons not coupled to ^{13}C. GARP-1 decoupling of ^{13}C was used during acquisition [10]. The spectral widths for the HMQC experiment were 2000 Hz (F2, ^1H) and 10000 Hz (F1, ^{13}C) with 256 experiments in the ^{13}C dimension. For the 0.10 M ribose solution, selective 1D TOCSY experiments were run in which a selective purged half Gaussian 90° pulse [11] replaced the initial hard 90° pulse of the TOCSY sequence. This experiment was used to determine protons which were scalar coupled to each of the resolved anomeric protons of D-ribose. Mixing times of 50–200 ms were used. For the same sample, an HMBC experiment was also run to establish long range ^{13}C–^1H connectivity [3].

Direct ^{13}C–^{13}C two dimensional correlation information was obtained for the 2.0 M solutions with the INADEQUATE experiment [7]. The spectral widths in the F1 and F2 dimensions for these experiments were 6024 and 12048 Hz,

respectively, with 128 experiments of 2K complex data points and 768 scans each zero-filled to 512W in t_1 prior to Fourier transformation. Due to fast relaxation of the viscous 2.0 M solutions, it was possible to use a short relaxation delay of 1.2 s between scans. The $^1J_{CC}$ coupling constants were observed to be ~ 40 Hz, so a $1/(4J)$ value of 6.25 ms was used in the INADEQUATE experiments.

3. Results and discussion

The INADEQUATE spectrum of 2.0 M D-xylopyranose in ^2H_2O obtained at 11.747 Tesla and 25°C, is shown in Fig. 1, which allows direct ^{13}C resonance assignments to be made (Table 1). For xylose and the other aldopendoses, some differences in ^{13}C and ^1H shifts, usually less than 0.3 ppm for ^{13}C and 0.1 ppm for ^1H, were observed for the 0.10 and 2.0 M sugar solutions; the sequence of shifts was unchanged except for one case with ribose discussed below.

The shifts reported in Table 1 are those of the 0.10 M solutions. Xylose does not yield significant quantities of furanose forms under these conditions. The only closely spaced resonances in the 1D ^{13}C spectrum are those corresponding to the 4α and 4β carbons, but these are easily differentiated in 1D slices from the INADEQUATE spectrum, and by the greater signal intensity for the β anomer.

After making the ^{13}C assignments, the directly attached non-exchangeable hydrogen atoms may be assigned from the HMQC spectrum (Fig. 2). The ^1H assignments are given in Table 1, and are further verified by comparison with 2QF-COSY and TOCSY spectra. No correction of the ^1H chemical shifts for strong scalar coupling has been made in Table 1, because the largest $^3J_{H,H}$ observed for aqueous sugar solutions is 10 Hz [12] and the minimum ^1H shift difference observed in the sugar solutions we studied was 15–20 Hz. In these

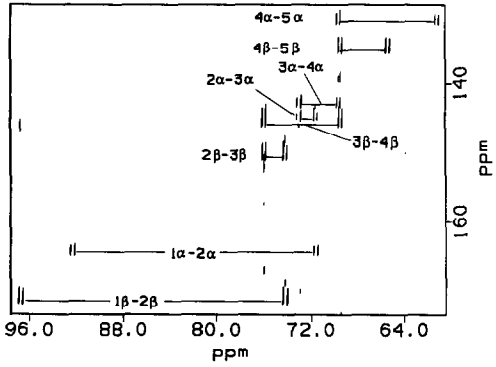

Fig. 1. 2D magnitude-mode ^{13}C INADEQUATE [7] spectrum obtained at 125.78 MHz for 2.0 M D-xylose in ^2H_2O at 25°C. Experimental details are described in the text. Adjacent coupled ^{13}C nuclei in the sugar skeleton appear as separated doublets at the chemical shifts of the respective carbons in the rows of the spectrum. The F1 frequency for a row is the sum of the chemical shifts of the coupled carbons. The identities of the coupled ^{13}C pairs are indicated.

Table 1
The ^{1}H and ^{13}C signal assignments for the aldopentopyranoses [a] in $^{2}H_2O$

Sugar	Proton	α Anomer	β Anomer	Carbon	α Anomer	β Anomer
L-*Arabino*	1	4.51	5.23	1	99.2	95.0
	2	3.50	3.81	2	74.4	71.0
	3	3.65	3.87	3	75.0	71.1
	4	3.93	3.99	4	71.1	71.3
	5a	3.67	3.64	5	69.0	65.1
	5b	3.89	4.02			
L-*lyxo*	1	5.01	4.86	1	96.7	96.9
	2	3.81	3.93	2	72.9	73.0
	3	3.90	3.64	3	73.3	75.5
	4	3.86	3.83	4	70.3	69.3
	5a	3.83	3.27	5	65.8	67.1
	5b	3.70	3.96			
D-*ribo*	1	4.87	4.93	1	96.3	96.7
	2	3.82	3.53	2	73.0	73.9
	3	3.93	4.11	3	72.2	71.9
	4	3.87	3.90	4	70.1	70.2
	5a	3.62	3.70	5	65.8	65.7
	5b	3.93	3.84			
D-*xylo*	1	5.18	4.57	1	95.0	99.4
	2	3.51	3.22	2	74.2	76.7
	3	3.65	3.42	3	75.7	78.6
	4	3.61	3.63	4	72.1	72.1
	5a	3.68	3.92	5	63.7	68.0
	5b	3.68	3.31			

[a] 0.1 M solutions of L-arabinose, L-lyxose, D-ribose, and D-xylose.

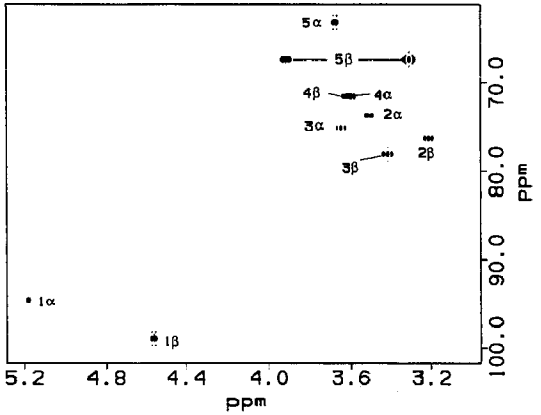

Fig. 2. 2D phase-sensitive ^{1}H–^{13}C HMQC [3] spectrum of 0.1 M D-xylose in $^{2}H_2O$ at 25°C. Experimental details are given in the text. The cross-peak frequencies are those of a given hydrogen (F2) and its directly bonded carbon (F1). The identities of the ^{13}C–^{1}H pairs are indicated.

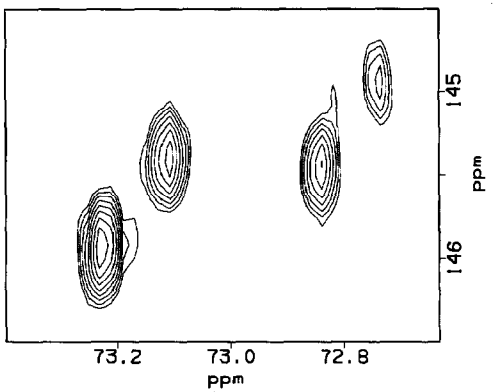

Fig. 3. Expansion from the 2D INADEQUATE spectrum of 2.0 M L-lyxose in ^2H_2O at 25°C showing the strong $^1J_{C,C}$ between C-2 and C-3 of the α-pyranose. In the weak coupling limit (Fig. 1), the two doublets would appear on the same row of the plot. ^{13}C chemical shifts for C-2 and C-3 for the 2.0 M solution are slightly different than those observed for the 0.1 M solution in Table 1. These changes are due partly to concentration effects and partly to strong coupling.

extreme cases, the actual ^1H chemical shifts are slightly different (an error of < 0.03 ppm).

Lyxose also exists primarily in pyranose forms in aqueous solutions. The ^{13}C chemical shifts for the 1α and 1β carbons differ by only 0.2 ppm, but these carbons are easily assigned by the greater intensity of the α anomer, and confirmed by comparison with the HMQC spectrum and the 1D ^1H spectrum, where the larger $^3J_{H\text{-}1,H\text{-}2}$ in the α anomer is readily observed [12]. The remaining ^{13}C assignments for L-lyxopyranose follow directly from the INADEQUATE spectrum (Table 1), with an interesting twist brought about by the strong ^{13}C–^{13}C scalar (J) coupling observed between C-2 and C-3 of the α anomer (Fig. 3). In the 2D intensity map the intensities of the strong coupling multiplet components are found to vary from row to row. Thus, the maximum ^{13}C connectivity intensity is no longer confined to a single row as expected for weak scalar coupling, but spread over several rows. Nevertheless, the ^1H assignments (Table 1) are clear and follow directly from the HMQC spectrum. These assignments are also consistent with the 2QF-COSY and TOCSY data.

The INADEQUATE spectrum for L-arabinopyranose in ^2H_2O reveals strong coupling and severe signal intensity loss for C-2, C-3, and C-4 of the less abundant β anomer, and strong coupling with some intensity loss between C-2 and C-3 of the α anomer. For the β anomer, the carbon skeleton could not be definitively assigned on the basis of the INADEQUATE spectrum, and the ^1H 2Q experiment was used to assist with the ^1H assignments in this case (Fig. 4). For arabinose the furanose forms are barely observable in aqueous solution, the ^1H resonances of the pyranose anomers are sufficiently dispersed that definitive assignments can be made, and the ^{13}C assignments follow immediately from the HMQC spectrum.

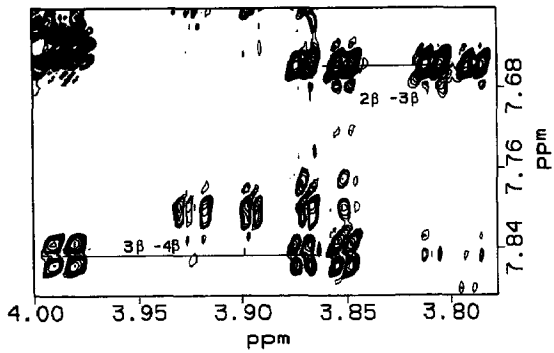

Fig. 4. Expansion from the phase-sensitive 2D 25 ms ^{1}H-2Q experiment [7] for 0.1 M L-arabinose in $^{2}H_2O$ at 25°C. Experimental details are described in the text. $^{3}J_{H,H}$ and $^{2}J_{H,H}$ values between pairs of protons (and to a lesser extent coupling to other protons) appear as separated multiplets at the chemical shifts of the respective protons in rows from the spectrum. The F1 frequency for a row is given by the sum of the chemical shifts of the coupled protons. The correlations between the H-2–H-3 and H-3–H-4 of the β-pyranose are shown. Positive and negative cross-peaks are plotted without distinction.

Again, the ^{1}H assignments are consistent with the 2QF-COSY and TOCSY spectra.

All spectra obtained for D-ribose in $^{2}H_2O$ solution are complicated by the presence of significant quantities of both furanose anomers, which are found in concentrations nearly as high as those arising from the less abundant α-pyranose form. Although the signal-to-noise of the INADEQUATE spectrum for ribose was sufficient to make the ^{13}C assignments (and hence the ^{1}H assignments) of the β-pyranose anomer, it was not sufficient to make ^{13}C assignments of the α-pyranose and the furanose-anomers. Strong coupling between C-2, C-3, and C-4 of the α-pyranose caused severe signal loss, and the signal intensity for the furanose anomers was insufficient on the basis of their low concentrations. To overcome this problem, we performed a selective TOCSY experiment in which H-1 of the α-pyranose was irradiated with a selective 90° pulse. By varying the mixing time (spin-lock time) from 50 to 200 ms, it was possible to assign H-2, H-3, and H-4 of the α-pyranose, and also one of the two C-5 protons. Using HMQC data, the carbons and the other C-5 proton could also be assigned. The ^{1}H assignments for the β-pyranose were verified with the selective TOCSY experiment, but this experiment did not yield good data for the furanose forms, possibly because of their increased conformational mobility [12]. However, we were able to make tentative assignments for the furanose anomers by identifying the H-12 and H-13 on the basis of $^{3}J_{H\text{-}1,H\text{-}2}$ [13], by examining the proton connectivity as revealed in the 2D TOCSY and ^{1}H 2Q experiments, and through their long-range connectivity to ^{13}C as revealed in a 2D HMBC experiment. These data, when used in combination with the HMQC data, led to the tentative assignments for the furanose forms shown in Table 2. Except for C-5 and its attached protons, the ^{1}H and ^{13}C assignments in Table 2 agree with those reported for 5-*O*-methyl-

Table 2
Tentative ^{1}H and ^{13}C signal assignments for D-ribofuranose [a] in $^{2}H_2O$

Proton	α Anomer	β Anomer	Carbon	α Anomer	β Anomer
1	5.39	5.25	1	99.2	103.9
2	4.10[1]	4.00[2]	2	72.8[3]	78.1[4]
3	4.11[1]	4.22	3	73.8[3]	73.3
4	4.14[1]	3.99[2]	4	85.9[3]	85.3[4]
5a	3.65	3.67	5	64.1	65.2
5b	3.76	3.82			

[a] The tentative assignments in each of the four groups (labeled with superscripts 1–4) may be interchanged.

ribofuranoses [13]. The ^{13}C chemical shifts of the furanose forms of ribose were quite concentration dependent. Large differences in ^{13}C shifts (up to 2 ppm) were observed for the 2.0 and 0.1 M ribose solutions, particularly for C-5 of both anomers.

Acknowledgments

This work was supported by the Petroleum Research Fund of the American Chemical Society and by the Searle Scholars Program.

References

[1] L.D. Hall, *Adv. Carbohydr. Chem.*, 29 (1974) 11–40.
[2] M.J. King-Morris and A.S. Serianni, *J. Am. Chem. Soc.*, 109 (1987) 3501–3508.
[3] M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, 108 (1983) 4285–4294.
[4] A. Bax, R. Freeman, and T. Frenkiel, *J. Am. Chem. Soc.*, 103 (1981) 2102–2104.
[5] M. Rance, O.W. SØrensen, G. Bodenhausen, G. Wagner, R.R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 117 (1983) 479–485.
[6] M. Rance, *J. Magn. Reson.*, 74 (1987) 557–564.
[7] L. Braunschweiler, G. Bodenhausen, and R.R. Ernst, *Mol. Phys.*, 48 (1983) 535–560.
[8] A.J. Shaka, C.J. Lee, and A. Pines, *J. Magn. Reson.* 77 (1988) 274–293.
[9] J.R. Garbow, D.P. Weitekamp, and A. Pines, *Chem. Phys. Lett.*, 93 (1982) 504–509.
[10] A.J. Shaka, P.B. Barker and, R. Freeman, *J. Magn. Reson.*, 64 (1985) 547–552.
[11] H. Kessler, S. Mronga, and G. Gemmecker, *Magn. Reson. Chem.*, 29 (1991) 527–557.
[12] C. Jones, *Adv. Carbohydr. Anal.*, 1 (1991) 145–194.
[13] J.R. Snyder and A.S. Serianni, *Carbohydr. Res.*, 163 (1987) 169–188.