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Structure of 2-C-(hydroxymethyl)-D-ribose (hamamelose) in the solid-state analyzed by CP MAS NMR and X-ray crystallography

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Abstract—D-Hamamelose, a branched-chain ribose (2-C-(hydroxymethyl)-D-ribose), has been synthesized and its solid-state structure analyzed by ^{13}C CP MAS NMR spectra and X-ray data. The presence of the complex pattern of resonances in the anomeric region, as well as in the ring carbon region, in ^{13}C CP MAS NMR spectrum indicated that the mixture of four cyclic forms, α- and β-furanoses, as well as both α- and β-pyranoses were present in the solid-state. X-ray analysis of crystals showed that D-hamamelose belongs to the monoclinic system with unit cell: $a = 4.790$ Å, $b = 8.671$ Å, $c = 8.880$ Å and $\beta = 98.89°$, space group $P2_1$. The furanose ring has the $_2E$ conformation.

Keywords: D-Hamamelose; Plant tannins; Solid-state; NMR; X-ray; Carboxylase/oxygenase regulators

1. Introduction

Branched-chain sugars are components of many natural biologically active compounds and have been subject of interest on their synthesis, structure and biological properties. D-Hamamelose, a branched-chain ribose (2-C-(hydroxymethyl)-D-ribose), is widely distributed among many plant species and found predominantly in their leaves.[1,2] D-Hamamelose, and especially its derivative hamamelitannin, is known as a potent scavenger of reactive oxygen species involved in degenerative diseases.[3,4] This branched-chain saccharide is currently intensively studied also due to its relationship to a regulator of the enzyme ribulose bisphosphate carboxylase/oxygenase.[5,6]

Methodologies for the stereo-controlled construction of the carbon–carbon bond in carbohydrate chemistry are very challenging. Synthesis of many of natural branched-chain sugars have therefore been approached by new synthetic techniques during the past decade.[7–9] These studies were aimed, not only at finding the most efficient way to synthesize these compounds, but to analyze their structure and biological properties. Recently, D-hamamelose was effectively prepared from D-fructose utilizing the catalytic effect of molybdate ions.[10,11] Other biologically active higher saccharides were also obtained by this method and allowed further studies of their structure and biological properties.[12,13] One of the sought after issues in glycobiology is the detailed understanding of the biosynthesis of higher saccharides and their role in intracellular communication that occurs in plants. This knowledge is only possible when the structural details of these saccharides are fully understood. The present report is aimed at detailed examination of structural properties of D-hamamelose by analysis of ^{13}C CP MAS NMR spectra and X-ray data.

2. Results and discussion

The presence of all four cyclic forms in aqueous solution, α,β-furanoses and α,β-pyranoses, were observed in high-resolution 1D and 2D NMR spectra in previous studies.[10,11,14] The integral intensities in ^1H NMR

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Table 1. 75.45 MHz ^{13}C NMR chemical shifts (δ, ppm) of D-hamamelose in aqueous solution and in solid-state

		C-1	C-2	C-3	C-4	C-5	C-2′
Solution[a]	α-Furanose	97.8	78.3	70.6	81.6	62.9	61.3
	β-Furanose	101.5	81.2	71.6	82.5	63.6	62.9
	α-Pyranose	94.8	75.3	66.9	68.9	66.5	61.1
	β-Pyranose	95.3	75.7	66.6	68.6	63.6	63.4
Solid-state	α-Furanose	94.2	[b]	71.0	81.1	65.2	62.7
	β-Furanose	100.6	[b]	72.9	82.6	65.2	65.2
	α-Pyranose	90.7	[b]	70.5	70.5	68.0	60.6
	β-Pyranose	90.7	[b]	68.7	70.5	68.0	62.7

[a] Taken from Refs. 11 and 14.
[b] Not assigned.

spectrum (not shown) indicated that the furanose form is more abundant (~67%) than the pyranose one and the values of chemical shifts based on former analyses are listed in Table 1.

Solid-state ^{13}C CP MAS NMR spectra of carbohydrates are mostly similar with high-resolution data where both the number of resonances and the values of chemical shifts are comparable. In some cases, however, the increased number of resonances indicates that either more than one chemically equivalent molecule is present in the crystal unit cell or various types of crystals are formed due to the different content of water (solvent) molecules in crystals.[15] Inspection of the ^{13}C CPMAS spectrum of crystalline D-hamamelose (Fig. 1A) reveals that several significant differences exist between the CP MAS and solution NMR data. Three resonances in the anomeric region were detected with chemical shifts 100.6, 94.2 and 90.7 ppm (Table 1). The values of the most deshielded resonance correspond well with that of the β-furanose anomeric signal in solution. The remaining two signals are shifted to higher fields than those in aqueous solution. The chemical shift of α-furanose is 94.2 ppm (compared to 97.8 ppm) and the signal at 90.7 ppm likely corresponds to the overlapped both α- and β-pyranoses (~95 ppm in solution). The effect of molybdate ions resulted in a more complicated CP MAS spectrum of the D-hamamelose–Mo(VI) complex, as expected. However, the anomeric region of the spectrum was found to be relatively well resolved indicating that all four forms of D-hamamelose are present in solid-state (Fig. 1B). The displacements of carbons (at the anomeric centres) chemical shifts to higher values (109.3, 105.4, 103.3 and 98.0 ppm) are due to the presence of Mo(VI) ions. The 'shoulder' (85–89 ppm) of the large signal corresponds to C-4 of furanose rings. The corresponding C-4 signals of pure D-hamamelose (82.6 and 81.1 ppm) match well with C-4 furanose resonances in solution (Table 1). C-4 resonances of both pyranose forms are more shielded and tentatively assigned to 70.5 ppm. However, strong overlap in the region 68–72 ppm prevented more precise analysis of CP MAS spectrum.

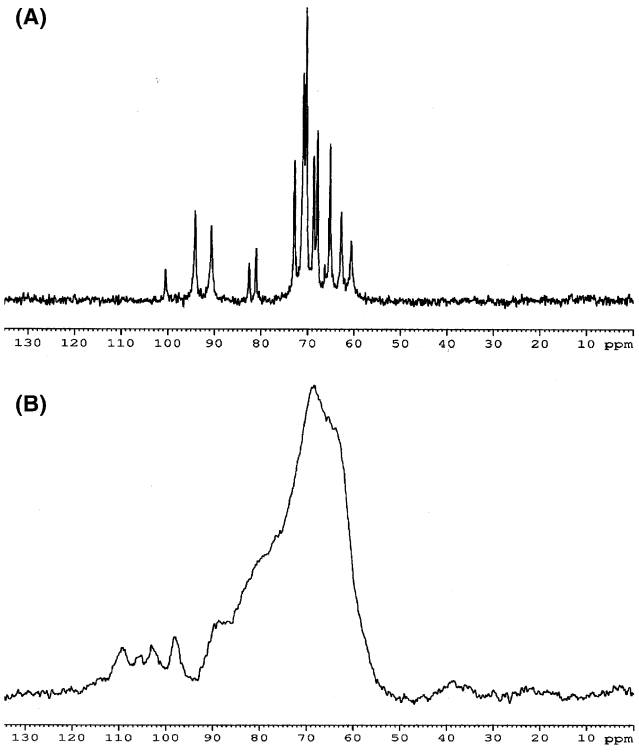

Figure 1. 75.45 MHz ^{13}C CPMAS NMR spectra of crystalline D-hamamelose (A) and the spectrum of the complex of D-hamamelose with molybdic acid (B).

D-Hamamelose has been further re-crystallized to obtain suitable crystals for X-ray study. The crystallographic analysis revealed that the studied molecule existed in the β-furanose form. Figure 2 shows a perspective view and the atom labelling of the X-ray structure of β-D-hamamelose. Examinations of bond lengths and valence angles demonstrate that the values conform to those observed in other furanose analogues.[16] The conformation of D-hamamelose is described by torsion angles reported in Table 2. The endocyclic dihedral angles are in the range of values observed in many structures containing a furanosyl moiety.[17] In particular, the furanose ring of D-hamamelose has a C-2-*exo* pucker. In terms of the conformational descriptors,[18] the pseudorotation phase angle P is of −22.6°, the maximum angle of pseudorotation τ is of 40.2°, and the $_2E$

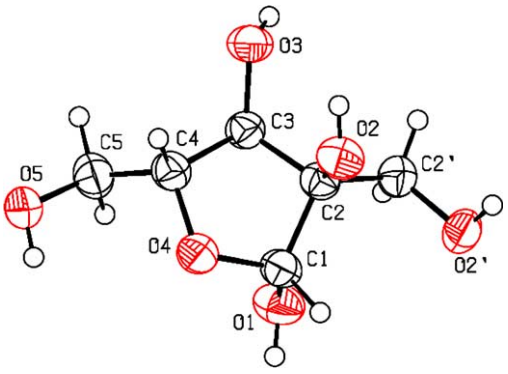

Figure 2. A perspective view of the X-ray crystal structure of β-D-hamamelose. Thermal ellipsoids are at 50% probability level.

Table 2. Torsion angles (°) of β-D-hamamelose

Endocyclic	
C-1–C-2–C-3–C-4	36.25
C-2–C-3–C-4–O-4	−21.69
C-3–C-4–O-4–C-1	−3.01
C-4–O-4–C-1–C-2	26.74
O-4–C-1–C-2–C-3	−39.07
Exocyclic	
O-1–C-1–O-4–C-4	−90.07
O-1–C-1–C-2–O-2	−164.96
O-4–C-1–C-2–O-2	76.72
O-1–C-1–C-2–C-3	79.25
O-1–C-1–C-2–C-2′	−43.77
O-4–C-1–C-2–C-2′	−162.09
O-2–C-2–C-3–O-3	42.77
C-2′–C-2–C-3–O-3	−82.48
O-2–C-2–C-3–C-4	−75.49
C-1–C-2–C-3–O-3	154.51
C-2′–C-2–C-3–C-4	159.27
O-3–C-3–C-4–O-4	−142.05
O-3–C-3–C-4–C-5	96.53
C-2–C-3–C-4–C-5	−143.11
C-1–O-4–C-4–C-5	120.11
Hydroxymethyl groups	
O-2–C-2–C-2′–O-2′	59.16
C-1–C-2–C-2′–O-2′	−59.60
C-3–C-2–C-2′–O-2′	−175.06
O-4–C-4–C-5–O-5	65.32
C-3–C-4–C-5–O-5	−175.17

conformation can be assigned. In this form, the atoms C-1, C-3, C-4, O-4 define a nearly perfect plane, with C-2 displaced out of plane by 0.593 Å on the opposite side of C-5. As mentioned, such 3D structure is comparable with other monosaccharide furanose rings.

Crystal packing involved hydrogen bonding, with the hydroxyl groups participating in every interaction. Six oxygen atoms were found in the asymmetric unit of the crystal and 20 short intra and intermolecular contacts were in the range between 2.7 and 3.2 Å[19] assuming the van der Waals radius $R_0 = 1.60$ Å. Among these short distances (all the hydrogen atoms were localized by ΔF Fourier syntheses), nine hydrogen bonds were recognisable, two of them being two centred.

In conclusion, the analysis of solid-state structure showed that the hydroxymethyl group have not brought about significant ring distorsions and the overall structural features of 2-C-(hydroxymethyl)-D-ribose remained comparable with non-substituted monosaccharides. High-resolution and solid-state NMR spectra confirmed that furanose forms were more stable than pyranose ones. The X-ray crystal structure showed that furanose ring of D-hamamelose has a C-2-*exo* pucker with $_2E$ conformation.

3. Experimental

D-Hamamelose was synthesized utilizing skeletal rearrangement in the molecule of D-fructose to the 2-C-(hydroxymethyl)-D-ribose.[10,11] The isomerisation took place in mild acid solution in the presence of catalytic amount of molybdic acid. The equilibrium mixture of the starting D-fructose and the product D-hamamelose was obtained within a short time. Purification of the reaction mixture into its components was achieved by column chromatography. The structure of the product was analyzed by high-resolution 1D and 2D NMR.

Cross-polarization magic angle spinning (CP MAS) NMR spectra of solid-state samples were measured at 7 T at room temperature on a DSX300 NMR spectrometer. The samples (100–150 mg) were spun in zirconia rotors at 4 kHz and 1000 scans were collected for each spectrum and the delay between pulses was 5 s.

In order to prepare crystals suitable for solid-state NMR and X-ray studies, syrupy D-hamamelose was dissolved in a small amount of water and MeOH was added. The solution was evaporated several times from MeOH. The mixture was left to stand at room temperature to crystallize. The molybdate complex of D-hamamelose was prepared by the reaction between saccharide and molybdic acid. Concentrated aqueous solution containing 2 equiv of molybdic acid (324 mg; 2 mmol) was added to a concentrated aqueous solution of D-hamamelose (180 mg; 1 mmol). The resulting mixture was stirred for 15 min. The solution was kept in a desiccator over sulfuric acid for several weeks until the complex crystallized.

Final crystals for X-ray analysis were prepared by recrystallization from dry MeOH. The structure of D-hamamelose was unambiguously confirmed by X-ray crystallographic analysis using synchrotron radiation data collected at the XRD-1 Beam Line at ELETTRA, Trieste, Italy ($\lambda = 0.8000$ Å). A marCCD detector (mar-USA Inc., USA) and focusing optics were employed for the measurements. Seventy images were collected at 298 K and a 5° oscillation range was used for all images, which roughly corresponds to a full sphere of data. The

degree of linear polarization was assumed to be 0.95 and the mosaic spread of the crystal was estimated to be 0.64. Raw data were indexed, integrated, scaled and reduced using the HKL[20] and the CCP4[21] packages. Crystals belong to the monoclinic system with unit cell: $a = 4.790$ Å, $b = 8.671$ Å, $c = 8.880$ Å and $\beta = 98.89°$, space group $P2_1$. The intensity data were merged to give 944 unique reflections, merging $R = 0.055$, of which 805 with $I \geqslant 2.0\sigma(I)$. The structure was solved by direct methods. All non-hydrogen atoms were refined with individual anisotropic thermal parameters. Difference Fourier syntheses, using only data with $\sin\theta/\lambda \leqslant 0.5$ Å^{-1}, showed all H atoms in configurationally feasible positions. H atoms were refined as riding atoms with the relative isotropic parameters. The final refinement, based on F^2 (all data), gave $R_1 = 0.0651$, $wR_2 = 0.1713$, $S = 1.081$, highest peak and deepest hole 0.29 and -0.26 e Å^{-3}. All calculations were performed with the SHELX-97 program package.[22] The illustration was made with the PLATON program.[23] Crystallographic data (excluding structure factors) for the structure D-hamamelose have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 236217. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1 EZ, UK [fax: +44(0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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References

1. Yoshimura, J. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 69–134.
2. Shafizadeh, F. *Adv. Carbohydr. Chem. Biochem.* **1956**, *11*, 263–283.
3. Masaki, H.; Atsumi, T.; Sakurai, H. *J. Dermatol. Sci.* **1995**, *10*, 25–34.
4. Roche, A. C.; Barzilay, M.; Midoux, P.; Junqua, S.; Sharron, N.; Monsigny, M. *J. Cell. Biochem.* **1983**, *22*, 131–140.
5. Andralojc, P. J.; Keys, A. J.; Martindale, W.; Dawson, G. W.; Parry, M. A. J. *J. Biol. Chem.* **1996**, *271*, 26803–26809.
6. Andralojc, P. J.; Keys, A. J.; Kossmann, J.; Parry, M. A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4742–4747.
7. Collins, P. M.; Ferrier, R. J. *Monosaccharides: Their Chemistry and their Roles in Natural Products*; John Wiley & Sons: Chichester, UK, 1996.
8. Sanki, A. K.; Suresh, C. G.; Falgune, U. D.; Pathak, T. *Org. Lett.* **2003**, *5*, 1235–1238.
9. Filho, J. R. F.; Srivastava, R. M.; Silva, W. J. P.; Cottier, L.; Sinou, D. *Carbohydr. Res.* **2003**, *338*, 673–680.
10. Hricovíniová, Z.; Hricovíni, M.; Petruš, L. *Chem. Pap.* **1998**, *52*, 692–698.
11. Hricovíniová-Bíliková, Z.; Hricovíni, M.; Petrušová, M.; Serianni, A. S.; Petruš, L. *Carbohydr. Res.* **1999**, *319*, 38–46.
12. Hricovíniová, Z. *Synthesis* **2001**, *5*, 751–754.
13. Hricovíniová, Z. *Tetrahedron: Asymmetry* **2002**, *13*, 1567–1571.
14. Schilling, G.; Keller, A. *Liebigs Ann. Chem.* **1977**, 1475–1479.
15. Taylor, M. G.; Marchessault, R. H.; Perez, S.; Stephenson, P. J.; Fyfe, C. A. *Can. J. Chem.* **1985**, *63*, 270–273.
16. Jeffrey, G. A. *Acta Cryst.* **1990**, *B46*, 89–103.
17. Swaminathan, P.; Anderson, L.; Sundaralingam, M. *Carbohydr. Res.* **1979**, *75*, 1–10.
18. Altona, C. L.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205–8212.
19. Ferraris, G.; Franchini-Angela, M. *Acta Cryst.* **1972**, *B28*, 3572–3583.
20. Otwinowski, Z.; Minor, W. In *Macromolecular Crystallography, Part A*; Carter, C. W., Jr., Sweet, R. M., Eds.; Methods in Enzymology; Academic: New York, 1997; 276, pp 307–326.
21. The CCP4 Suite: Programs for Protein Crystallography. *Acta Cryst.* **1994**, *D50*, 760–763.
22. Sheldrick, G. M. SHELX-97, Release 97-2, University of Göttingen, Germany, 1998.
23. Speck, A. L. *J. Appl. Cryst.* **2003**, *36*, 7–13.