

# Unveiling epimerization effects: a rotational study of $\alpha$ -D-galactose

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By studying its C<sub>4</sub> epimer  $\alpha$ -D-galactose, the effects of epimerization on the conformational behaviour of  $\alpha$ -D-glucose have been unveiled. Using laser ablation of crystalline samples, four conformers of  $\alpha$ -D-galactopyranose have been observed, for the first time, in a supersonic expansion by analyzing the Fourier transform rotational spectrum.

The conformational behaviour of the archetypal monosaccharide D-glucose has recently been revealed<sup>1</sup> using Fourier transform microwave techniques in conjunction with laser vaporization methods conducted in supersonic expansions.<sup>2</sup> Since rotational spectra are extremely sensitive to molecular geometry, four conformers of  $\alpha$ -D-glucopyranose and three of  $\beta$ -D-glucopyranose in the <sup>4</sup>C<sub>1</sub> ring configuration have been unequivocally identified.<sup>1</sup> Under isolation conditions of the gas phase, stereo-electronic hyperconjugative factors such as anomeric or gauche effects as well as the relative orientations (axial/equatorial) of the hydroxyl groups were found to be key factors controlling conformational preferences. D-Glucopyranose adapts its molecular shape to optimize the sequence of intramolecular hydrogen bonds in order to increase their strength by cooperativity.<sup>3–5</sup> Hence, the most abundant conformers, G<sup>+</sup>g<sup>+</sup>/cc/t and G<sup>+</sup>g<sup>-</sup>/cc/t (see the footnote of Table 1 for labelling), present a counterclockwise network of four cooperative hydrogen bonds, while the least abundant G<sup>-</sup>g<sup>+</sup>/cl/g<sup>-</sup> conformer presents a clockwise arrangement of three cooperative hydrogen bonds.

As does D-glucose, crystalline D-galactose exists only in the  $\alpha$ -pyranose form (Chart 1a) with a <sup>4</sup>C<sub>1</sub> chair configuration where the hydroxymethyl group is in the energetically favoured equatorial position (Chart 1b). The hydroxymethyl group contains an additional rotatable C5–C6 bond characterized by the C6–O6–C5–O5 torsional

angle associated with three staggered conformers labelled G<sup>+</sup>, G<sup>-</sup> and T which assume values of ca. 601, -601 and 1801, respectively (Chart 1c). Both in the solid<sup>6</sup> and solution phases,<sup>7</sup>  $\alpha$ -D-glucopyranosides display nearly equal proportions of G<sup>+</sup> and G<sup>-</sup> conformers with almost total absence of T forms. This predominance is known as the gauche effect.<sup>8</sup> In contrast, galactopyranosides present a different hydroxymethyl rotamer distribution displaying a large T component.<sup>7,9</sup> This has been rationalized in terms of the solvent effects<sup>10,11</sup> and/or unfavourable 1,3-diaxial interactions.<sup>12</sup>  $\alpha$ -D-Galactopyranose only differs from  $\alpha$ -D-glucopyranose in the hydroxyl group at the C<sub>4</sub> position (C<sub>4</sub> epimers, Chart 1a and b), which is in the axial orientation in  $\alpha$ -D-galactopyranose. What should the effect of epimerization on the already known conformational behaviour of the archetypal  $\alpha$ -D-glucose be? How does it affect the formation of the intramolecular hydrogen bonding networks?

All the above-mentioned results highlight the importance of unveiling the stable conformations of isolated  $\alpha$ -D-galactose in the gas phase, free from the bulk effects of the solvent, which modulates the epimerization effects. To the best of our knowledge, only the phenyl derivative of  $\beta$ -galactopyranose has been investigated in the gas phase through a combination of laser spectroscopic techniques and *ab initio* calculations,<sup>13</sup> with no structural data having been reported for free  $\alpha$ -D-galactose to date. With this aim, we transferred crystalline  $\alpha$ -D-galactose (m.p. = 170 °C) into the gas phase by laser ablation so as to describe its most abundant conformers in a supersonic expansion using Chirped Pulse Fourier Transform Microwave (CP-FTMW) spectroscopy. The details of the experimental setup are given elsewhere.<sup>14</sup> The congested broadband rotational spectrum from 6 to 12 GHz shown in Fig. 1 is full of unwanted photofragmentation lines found in previous studies of sugars.<sup>15</sup> Once these lines were identified and removed from the spectrum, a series of m<sub>a</sub>-type R-branch lines belonging to four different rotamers, labelled I, II, III and IV, were discovered. Following iterative fitting and prediction, new m<sub>a</sub>-type lines for rotamers I, II and IV and m<sub>b</sub>-type lines for rotamers II and III were assigned and measured. All lines collected in Tables S1 to S4 of the ESI† were analyzed using a rigid rotor analysis to give the sets of rotational

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Table 1 Experimental rotational parameters for the four observed rotamers of *a*-D-galactopyranose in comparison with those predicted by ab initio calculations<sup>a</sup>

Parameter	I	$G^-g^+/cl/g^-^h$	II	$G^+g^-/cc/t$	III	$Tg^+/cc/t$	IV	$G^-g^-/cc/t$
A <sup>b</sup>	1394.47809 (55) <sup>§</sup>	1402.6	1393.50250 (40)	1403.9	1424.41881 (51)	1439.2	1398.69258 (68)	1407.3
B	800.07740 (24)	803.0	756.83243 (22)	758.6	752.79300 (25)	759.3	785.47416 (40)	787.5
C	592.89948 (24)	595.9	551.86369 (12)	554.9	560.63145 (17)	564.9	584.48300 (22)	588.2
$m_a/m_b/m_c$	—	0.7/0.1/0.4	—	1.6/1.9/0.7	—	2.2/1.5/0.5	—	2.5/1.2/2.4
$s^c$	11.5	—	9.4	—	9.3	—	11.6	—
N <sup>d</sup>	48	—	80	—	49	—	35	—
DE <sup>e</sup> /cm <sup>-1</sup>	—	0	—	295	—	467	—	598
DG <sup>f</sup> /cm <sup>-1</sup>	—	0	—	94	—	362	—	490

<sup>a</sup> The most stable conformers resulting from full geometric optimization computed at the MP2/6-311++G(d,p) level of theory.<sup>17</sup> <sup>b</sup> A, B and C are the rotational constants in MHz;  $m_a$ ,  $m_b$  and  $m_c$  are the absolute values of the electric dipole moment components in D. <sup>c</sup> Rms deviation of the fit in kHz. <sup>d</sup> Number of transitions fitted. <sup>e</sup> MP2-6311++G(d,p) electronic energies in cm<sup>-1</sup>. <sup>f</sup> Gibbs energies at the same level of calculation in cm<sup>-1</sup>. <sup>§</sup> Standard error in parentheses in units of the last digit. <sup>h</sup> The symbols in capital letters, G<sup>+</sup>, G<sup>-</sup> and T, describe the O6-C6-C5-O5 torsional angle (see Chart 1c) of about 60°, -60° and 180°, respectively. The lower case symbols, g<sup>+</sup>, g<sup>-</sup>, and t, likewise describe the torsional angle, H6-O6-C6-C5. These symbols are followed by a slash and the symbol cl or cc describes, respectively, the clockwise or counterclockwise arrangement of the cooperative network of intramolecular hydrogen bonds. The last symbols, g<sup>+</sup>, g<sup>-</sup> and t, give the values of the H1-O1-C1-C2 torsional angle that describes the orientation of the anomeric hydroxyl group hydrogen atom.

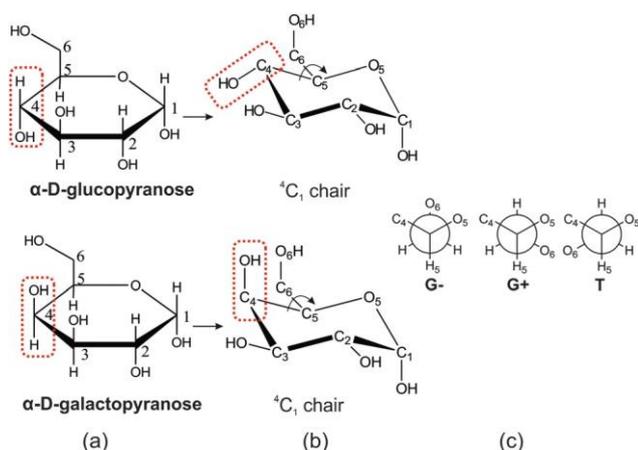


Chart 1 (a) Haworth projections of *a*-anomers of D-galactopyranose and D-glucopyranose. (b) <sup>4</sup>C<sub>1</sub> chair configurations. (c) Newman projections around the C5–C6 bond showing G<sup>-</sup>, G<sup>+</sup> and T configurations of the hydroxymethyl group.

constants given in Table 1. They were directly compared with those theoretically predicted for the most stable conformers for *a*-D-galactopyranose in its <sup>4</sup>C<sub>1</sub> ring configuration,<sup>16,17</sup> also listed in Table 1. Scale factors ranging from 1.002 up to 1.010 almost bring the ab initio values of theoretical rotational constants for the  $G^-g^+/cl/g^-$ ,  $G^+g^-/cc/t$ ,  $Tg^+/cc/t$  and  $G^-g^-/cc/t$  conformers in line with experimental values for rotamers I, II, III, and IV, respectively. The observed type spectra are also in accordance with the predicted dipole moment components. This is additionally supported by the conformational abundances roughly estimated from relative intensity measurements,  $G^-g^+/cl/g^-$  (85%),  $G^+g^-/cc/t$  (9%),  $Tg^+/cc/t$  (4%) and  $G^-g^-/cc/t$  (2%), in accordance with the predicted ab initio energies. This constitutes conclusive evidence for the existence of the aforementioned conformers of *a*-D-galactopyranose in supersonic expansion.

The four conformers of *a*-D-galactopyranose, depicted in Fig. 2 (see Cartesian coordinates in the ESI<sup>†</sup>), are found to be over-stabilised by cooperative networks of intramolecular hydrogen bonds between vicinal hydroxyl groups stretching throughout the whole

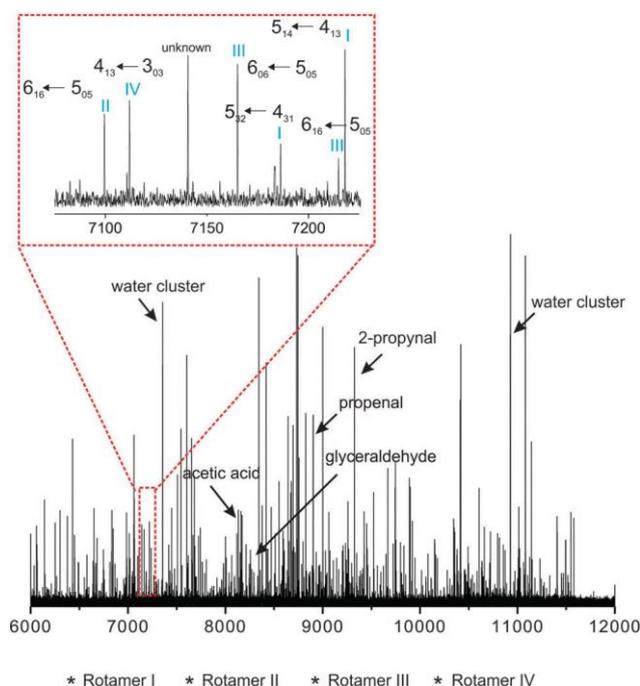


Fig. 1 Broadband CP-FTMW rotational spectrum of D-galactose in the 6–12 GHz frequency region indicating some lines of unwanted photo-fragmentation products (acetic acid, formaldehyde, glycerinaldehyde, propenal, 2-propenal, and water complexes). Top inset: details of the CP-FTMW spectrum showing rotational transitions ascribed to the four detected rotamers.

molecule. Hence, the most abundant conformer  $G^-g^+/cl/g^-$  is stabilised by the network  $OH_{(1)} \cdots OH_{(2)} \cdots OH_{(3)} \cdots OH_{(4)} \cdots OH_{(6)} \cdots O_{(5)}$  with a clockwise (cl) arrangement of the OH groups. The axial OH group at epimeric C<sub>4</sub> carbon favours an extra  $OH_{(4)} \cdots OH_{(6)}$  interaction which maximizes the number of cooperative H-bonds up to five. This most abundant conformer in *a*-D-galactopyranose is the least abundant in *a*-D-glucopyranose, and exhibits the same clockwise arrangement but with only three cooperative  $OH_{(1)} \cdots OH_{(2)} \cdots OH_{(3)} \cdots OH_{(4)}$  hydrogen bonds. In contrast, the least abundant  $G^-g^-/cc/t$  in *a*-D-galactopyranose species

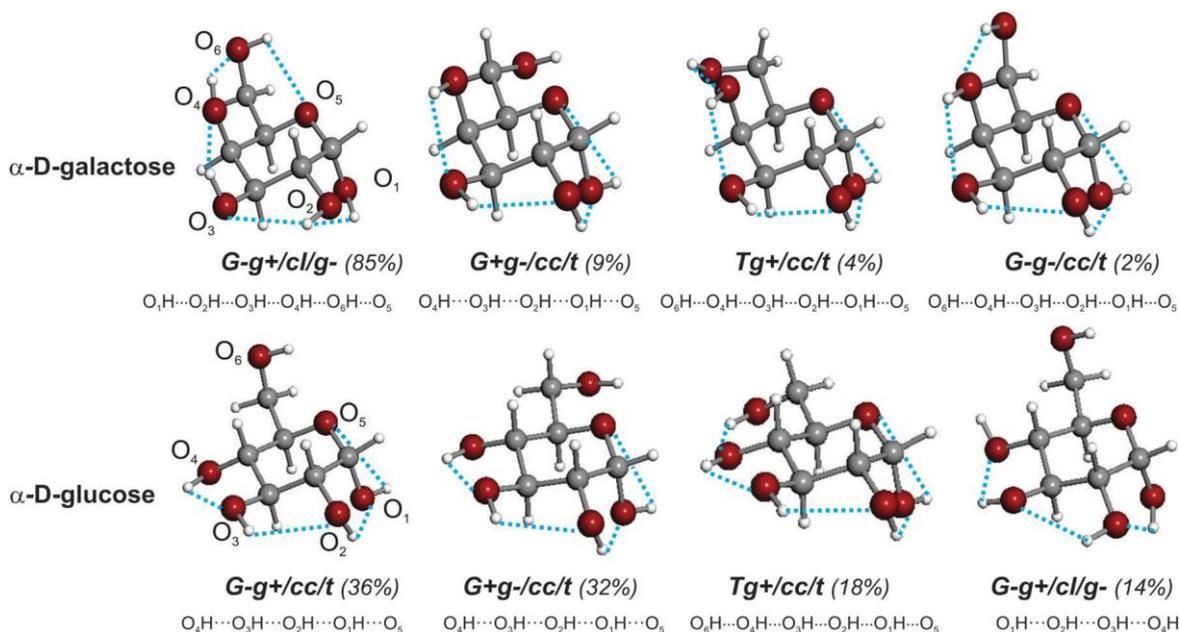


Fig. 2 Three dimensional structures of the four observed conformers of  $\alpha$ -D-galactose showing the cooperative intramolecular hydrogen bonding networks compared with those observed in  $\alpha$ -D-glucose. Their abundances are also indicated.

corresponds to the most abundant  $G^+g^-/cc/t$  form in  $\alpha$ -D-glucopyranose, each presenting networks of five and four cooperative H-bonds with counterclockwise (cc) arrangements. The middle two  $G^+g^-/cc/t$  and  $Tg^+/cc/t$  conformers of  $\alpha$ -D-galactopyranose correlate with those of  $\alpha$ -D-glucopyranose with the same counterclockwise arrangements, but relatively low abundances (see Fig. 2).

In summary, by analyzing its rotational spectrum, we provide the first experimental information on isolated  $\alpha$ -D-galactose. A comparison between the observed forms in  $\alpha$ -D-glucopyranose clearly shows how epimerization at  $C_4$  drastically changes the conformational behaviour observed in  $\alpha$ -D-glucose. Our gas phase experiment reveals a small proportion of T forms through the presence of the  $Tg^+/cc/t$  conformer. This conflicts with the large T component observed in condensed phases<sup>7,9</sup> ascribed to either solvent interactions and/or 1,3-diaxial interactions. The latter, caused by electronic and steric repulsion between  $O_4$  and  $O_6$  atoms in galactopyranosides, strongly contribute to destabilise the  $G^-$  conformers in favour of the T ones. Given that solvent effects are absent in our experiment, 1,3-diaxial interactions do not seem to play an important role since the conformational panorama of  $\alpha$ -D-galactopyranose is dominated by the  $G^+g^-/cl/g^-$  form. Solvent effects must be responsible for the large T component controlling the conformational distribution of  $\alpha$ -D-galactopyranose.<sup>10,11</sup>

In an effort to connect the present results in vacuo conditions with those in solution,<sup>9</sup> investigation is being undertaken to analyze how the observed conformational behaviour is altered by docking water molecules.

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## References

- 1 J. L. Alonso, M. A. Lozoya, I. Peña, J. C. López, C. Cabezas, S. Mata and S. Blanco, *Chem. Sci.*, 2014, 5, 515–522.
- 2 C. Bermúdez, S. Mata, C. Cabezas and J. L. Alonso, *Angew. Chem., Int. Ed.*, 2014, 53, 11015–11018.
- 3 (a) G. A. Jeffrey and W. Saenger, *Hydrogen Bonding in Biological Structures*, Springer-Verlag, New York, 1991; (b) G. A. Jeffrey, *Introduction to Hydrogen Bonding*, Oxford University Press, Oxford, 1997.
- 4 I. Peña, S. Mata, A. Martín, C. Cabezas, A. M. Daly and J. L. Alonso, *Phys. Chem. Chem. Phys.*, 2013, 15, 18243–18248.
- 5 I. Peña, L. Kolesniková, C. Cabezas, C. Bermúdez, M. Berdakin, A. Simão and J. L. Alonso, *Phys. Chem. Chem. Phys.*, 2014, 16, 23244–23250.
- 6 (a) G. A. Jeffrey, R. K. McMullan and S. Takagi, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1977, 33, 728–737; (b) R. H. Marchessault and S. Pérez, *Biopolymers*, 1979, 18, 2369–2374; (c) G. M. Brown and H. A. Levy, *Science*, 1965, 147, 1038–1039.
- 7 Y. Nishida, H. Ohrui and H. Meguro, *Tetrahedron Lett.*, 1984, 25, 1575–1578.
- 8 S. Wolfe, *Acc. Chem. Res.*, 1972, 5, 102–111.
- 9 (a) R. U. Lemieux and T. J. Brewer, *Conformational preferences of solvated hydroxymethyl groups in hexopyranose structures, in Carbohydrates in solution*, ed. R. Gould, American Chemical Society, Washington, DC, 1973, vol. 117, p. 121; (b) M. L. C. E. Kouwijzer, B. P. van Eijck, H. Kooijman and J. Kroon, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1955, 51, 209–220; (c) G. A. Jeffrey and R. Shiono, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1977, 33, 2700–2701.
- 10 K. N. Kirschner and R. J. Woods, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, 98, 10541–10545.
- 11 C. B. Barnett and K. J. Naidoo, *J. Phys. Chem. B*, 2008, 112, 15450–15459.
- 12 K. Bock and J. Ø. Duus, *J. Carbohydr. Chem.*, 1994, 13, 513–543.
- 13 (a) R. A. Jockusch, F. O. Talbot and J. P. Simons, *Phys. Chem. Chem. Phys.*, 2003, 5, 1502–1507; (b) P. Çarçabal, R. A. Jockusch, I. Hünig, L. C. Snoek, R. T. Kroemer, B. G. Davis, D. P. Gamblin, I. Compagnon,

- J. Oomens and J. P. Simons, *J. Am. Chem. Soc.*, 2005, 127, 11414–11425.
- 14 (a) S. Mata, I. Peñ̄a, C. Cabezas, J. C. López and J. L. Alonso, *J. Mol. Spectrosc.*, 2012, 280, 91–96; (b) G. G. Brown, B. C. Dian, K. O. Douglass, S. M. Geyer, S. T. Shipman and B. H. Pate, *Sci. Instrum.*, 2008, 79, 053103.
- 15 C. Bermúdez, I. Peñ̄a, C. Cabezas, A. M. Daly and J. L. Alonso, *ChemPhysChem*, 2013, 14, 893–895.
- 16 F. A. Momany, M. Appell, J. L. Willett, U. Schnupf and W. B. Bosma, *Carbohydr. Res.*, 2006, 341, 525–537.
- 17 M. J. Frisch, G. W. Trucks and H. B. Schlegel, et al., *Gaussian 09*, Revision B.01, Gaussian, Inc., Wallingford, CT, 2010.