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VIP Structure Determination

The Crystal Structure of D-Ribose—At Last!**

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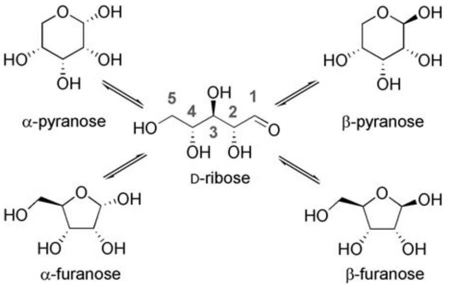
Among the half-million or so chemical compounds whose crystal structures have been determined by X-ray or neutron diffraction, the crystal structure of D-ribose is conspicuously absent.[1] Thus, although most modern chemistry textbooks and handbooks show the molecule of D-ribose in the β-furanose form, as present in countless biochemically important ribose derivatives, it has been known for more than forty years from early NMR observations[2–4] that D-ribose exists in aqueous solution as a mixture of α- and β-pyranoses and α- and β-furanoses with the β-pyranose form predominating (Scheme 1).



Scheme 1. Structural variations of D-ribose.

The structure of crystalline D-ribose has remained unknown up until now. In a 1956 X-ray study[5] the crystals were shown to be monoclinic, space group $P2_1, Z=4, Z'=2$, in other words, with two symmetry-independent molecules in the unit cell. At that time, more than fifty years ago, before the advent of direct methods, a full structure analysis of this crystal seemed out of the question, especially since the crystals obtained were described as being extremely small, twinned, and generally of poor quality. As far as we are aware, the crystal structure problem has remained dormant since that time, although the crystal structures of the other common pentoses and hexoses have all been determined and can be retrieved from the Cambridge Structural Database (CSD).

When we became aware that the crystal structure of D-ribose was still unknown, we felt a certain obligation to fill this gap. Indeed, the compound proved to be recalcitrant to crystallization. Hundreds of experiments involving cooling or evaporation of saturated solutions of commercial D-ribose (Sigma–Aldrich) in a variety of pure and mixed solvents produced exclusively polycrystalline solid materials. Attempts to grow single crystals from a capillary melt also proved unsuccessful. Despite these setbacks, the crystal structure of D-ribose has now been established, first from the powder diffraction pattern by the application of the direct-space global optimization[6] program FOX[7] combined with difference Fourier techniques,[8] and subsequently, in Essen, by single-crystal analysis of material obtained by advanced zone-melting recrystallization (ZMR) techniques.[9] With 20 main scattering centers in the asymmetric unit, the D-ribose crystal structure represents a formidable task for powder diffraction methods (see the Supporting Information for further details of the structure analyses). Gratifyingly, the two methods led to closely similar results.[10] The two-symmetry independent molecules in the crystal both have the pyranose ring structure but differ in the configuration at the anomeric carbon atom (Figure 1).

According to the powder analysis, the asymmetric unit of the crystal contains a β-pyranose molecule (equatorial OH group at C1) at one site and an α-pyranose molecule (axial OH group at C11) at the other site (ring conformation in both molecules: $^4C_1$). In contrast, the single-crystal analysis shows a disordered arrangement of the anomeric hydroxy group at the second site at C11 with roughly equal occupation factors (Figure 1). Disregarding the orientation of the hydroxy groups at C1 and C11, the two symmetry-independent molecules are related by an approximate noncrystallographic screw axis parallel to the *b* axis of the crystal.

Later, a second crystal form (Form II, space group $C2, Z=12, Z'=3$) was found.[12] Of the three independent sites in this crystal, two contain β-pyranose molecules while the third shows a disordered arrangement of the anomeric hydroxy group (Figure 2). Again, as in Form I, the three symmetry-independent molecules in Form II are related by approximate

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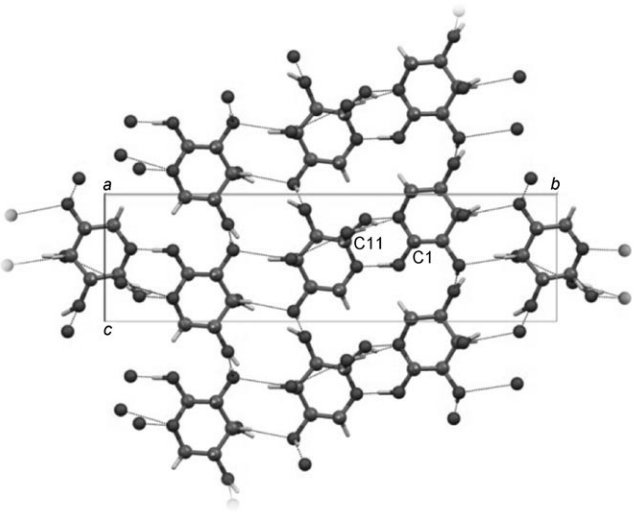

Figure 1. View of the D-ribose crystal structure (Form I) projected down the short *a* axis, according to the X-ray analysis of the crystal obtained by zone melting (drawn with Mercury[11]); light gray C, dark gray O, white H. Note the difference in the orientation of the hydroxy groups in the two molecules: equatorial at C1, equatorial and axial at C11 (disordered mixture).Intermolecular hydrogen bonds are indicated by dashed lines. The structure derived by analysis of the powder pattern is virtually superimposable but the relative occupancies of the hydroxy groups at C11 differ.

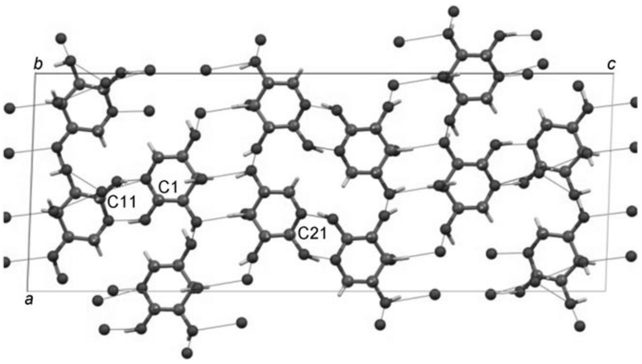

Figure 2. View of the D-ribose crystal structure (Form II polymorph) projected down the short *b* axis; light gray C, dark gray O, white H. Note the difference in the orientation of the hydroxy groups between the equatorial hydroxy groups at C1 and C21 and the equatorial and axial hydroxy groups at C11 (disordered mixture). Intermolecular hydrogen bonds are indicated by dashed lines.

noncrystallographic screw axes, each pointing nearly parallel to one of the crystal axes.

These findings require slight modification owing to the results of a subsequent independent solid-state ^{13}C MAS NMR study (Figure 3), according to which the ratio of β to α in the polycrystalline material is approximately 2:1 rather than 1:1, as from the X-ray analysis of the powder, or 3:1 as from the single-crystal analysis of Form I. The cross-polarization NMR spectrum of the polycrystalline sample (Figure 3a) shows two signals for the anomeric carbon atoms at $\delta = 94.9$ and 92.5 ppm with an integrated peak ratio of 2:1, which, on the base of the X-ray results, can be assigned to β- and α-pyranose, respectively (for details see the Supporting Information). On melting (m.p. ≈90 °C), an equilibrium mixture of the four anomers is formed (46 % β-pyranose, 25 % α-pyranose, 12 % α-furanose, 17 % β-furanose; Figure 3b). Cooling the melt leads to broad NMR signals indicative of a glassy state (Figure 3c). The sample was then kept at 4 °C for one month and then remeasured, and the features of the spectrum of the polycrystalline sample were recovered with a slightly altered ratio of 2.5:1 between the signals of the anomeric carbon atoms (Figure 3d).

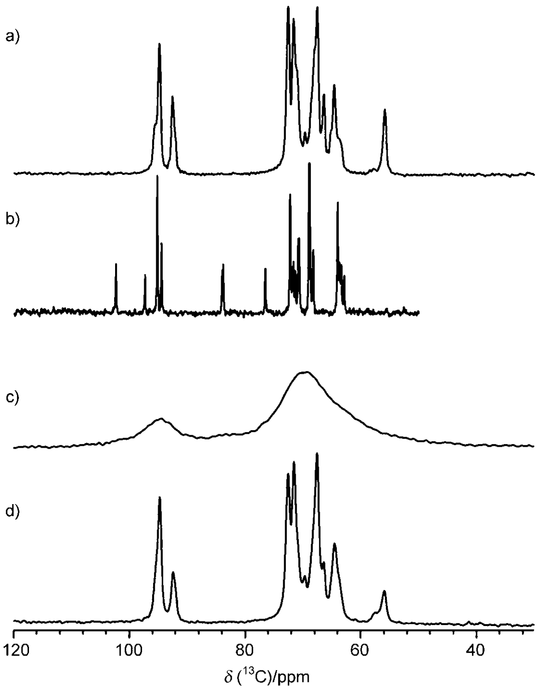

Figure 3. ^{13}C MAS NMR spectra of D-ribose. Spectrum (a) was measured at 20 °C, the initial polarization was generated by cross polarization (contact time 1 ms), and 240 scans were added. Spectrum (b) was measured at 90 °C by direct excitation of ^{13}C spins and is the sum of 80 acquisitions. Subsequently, the sample was cooled to 20 °C and spectrum (c), obtained by cross polarization with a contact time of 0.1 ms, was acquired by adding 2000 scans. Spectrum (d) was obtained at 20 °C from the sample after about one month at 4 °C, using the same experimental conditions as for spectrum (a). Spectra were collected at a MAS frequency of 10 kHz and were externally referenced to the peak of adamantane at $\delta = 38.48$ ppm relative to TMS.

With this knowledge from the NMR study, we reexamined the results of the X-ray powder diffraction analysis to see if α/β disorder is present at either of the two crystal sites of the polycrystalline material. Indeed, at the site previously identified as containing an α-pyranose molecule, a β/α ratio of 0.27:0.73 was estimated, while the β site remained essentially unchanged. Thus, the revised overall β/α ratio in the original polycrystalline sample material comes out at about 1.7:1, compared with the 2:1 ratio from the NMR study. Although it is difficult to estimate the reliability of these β/α ratios derived from different kinds of measurements, both methods agree that the proportion of the β anomer in the melted and then recrystallized sample seems to be somewhat higher than in the initial polycrystalline material. We need not be too surprised since the conditions under which the two kinds of material were formed differ. However, we do not know of any other system for which analogous behavior has been observed.

Our work has filled a gap in our knowledge about D-ribose and has raised questions about its behavior in the solid state. Why does D-ribose crystallize with two (or three) symmetry-independent molecules instead of by simple repetition of a single molecule by space group symmetry operations? Is it simply because the α- and β-pyranose molecules present in the solution and in the melt are almost superimposable except for the different positions of the anomeric hydroxy group? The difference in position does not seriously affect the hydrogen-bond pattern. Are all possible $Z'=1$ crystal structures energetically disfavored with respect to the observed structures with $Z'=2$ and $Z'=3$? These questions could be subjects of additional study using existing computer programs for generating virtual crystal structures and estimating their energies, but we do not intend to pursue them further. It would seem that the difference between the β/α ratio (0.64:0.36) of the anomers in the untreated solid according to NMR and powder analysis and the 0.75:0.25 ratio according to the single-crystal analysis is real. The two products were obtained from equilibrium mixtures containing different proportions of anomers. Unfortunately, we do not know what solvent and what conditions were used for the commercial preparation that served as the basis for the X-ray powder analysis and NMR study. In response to our enquiry, Sigma–Aldrich was unable to disclose specific details because of patent restrictions. However, it seems unlikely that the commercial product was obtained by cooling of the melted compound. Another uncertainty is that we do not yet know the proportion of α and β anomers in the crystalline material obtained by evaporation of solutions of different solvents[13,14] at different temperatures. There are still details to be settled.

Experimental Section

NMR measurements: ^{13}C NMR spectra were recorded with a Varian Infinity+ 500 spectrometer operating at a ^{13}C Larmor frequency of 125.67 MHz with a 4 mm Varian T3 double-resonance MAS probe. Cross-polarization (CP) ^{13}C spectra were acquired where the protons were excited with a 4.5 μs pulse and the polarization was subsequently transferred to ^{13}C using an adiabatic CP step with a spin-lock field centered at 50 kHz. During acquisition 60 kHz TPPM (two-pulse phase modulation) decoupling was applied with a 30° phase shift. The spectral width was 16.7 kHz, the acquisition time was 15.4 ms and a line broadening of 20 Hz was applied before Fourier transform. The ^{13}C NMR spectra in the melt were acquired using either direct excitation or refocused INEPT, and no significant differences were noted between the two methods. At 90 °C, the ^{13}C 90° pulse was 6 μs, the spectral width was 10 kHz, and during the 51.2 ms acquisition time 5 kHz continuous-wave ^{1}H decoupling was applied. The recycle delay was 60 s.

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[1] The half-million mark was passed on December 10, 2009 (http://www.ccdc.cam.ac.uk/500000.php).

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[6] W. I. F. David, K. Shankland, *ActaCrystallogr. Sect. A* **2008**, *64*, 52–64.

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[8] In direct-space methods, a trial structure is generated within the asymmetric unit using known chemical information (e.g. atomic connectivity, bond distances and angles), and a powder pattern is calculated and compared with the measured one to get an estimate of the structure's correctness. The model is then modified and the corresponding pattern calculated. If the fit of this new pattern is better, the "move" is accepted and the cycle is repeated (see Ref. [6] for more information on global optimization procedures). Of importance here is the fact that a starting structural model is required. We did not know which of the five possible structures (Scheme 1) ribose would adopt in the solid state, so different combinations of chains and rings were used as input to FOX. With two molecules in the asymmetric unit, the problem is a nontrivial one for powder methods. After many FOX runs, a ring structure seemed more likely, but it was not clear whether this was a five- or six-membered ring, and none of the structural models was significantly better than the others. A long and tedious process of generating difference electron density maps from various starting models eventually led to an approximate structure with one α- and one β-pyranose molecule in the asymmetric unit. Later, an alternative approach involving the combination of an approximate (but incorrect) model from FOX with the powder charge flipping algorithm (C. Baerlocher, L. B. McCusker, L. Palatinus, *Z. Kristallogr.* **2007**, *222*, 47–53) led to the structure in a more straightforward manner.

[9] R. Boese, M. Nussbaumer in *In situ Crystallization Techniques In Organic Crystal Chemistry* (Ed.: D. W. Jones), Oxford University Press, Oxford, **1994**, pp. 20—37; OHCD: www.sci.ohcd.e u.

[10] Crystallographic data for D-ribose (Form I): by powder diffractometry, $a=4.8205(4)$, $b=21.6435(1)$, $c=6.5069(5)$ Å, $\beta=111.0(1)°$ at room temperature, space group $P2_1$, $Z=4$, $Z'=2$; by zone melting $a=4.7860(13)$, $b=21.463(5)$, $c=6.4873(17)$ Å, $\beta=110.824(11)°$ at 123 K, space group $P2_1$, $Z=4$, $Z'=2$.[14]

[11] Mercury CSD 2.0—New Features for the Visualization and Investigation of Crystal Structures C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek, P. A. Wood, *J. Appl. Crystallogr.* **2008**, *41*, 466–470.

[12] Crystallographic data for D-ribose (Form II): $a=12.129(10)$, $b=4.7753(4)$, $c=32.116(3)$ Å, $\beta=92.103(5)°$ at 123 K, space group $C2$, $Z=12$, $Z'=3$.[14]

[13] Although both known polymorphs of crystalline D-ribose contain α- and β-pyranose molecules, they should not be described as co-crystals. Since α- and β-pyranose are in dynamic equilibrium (along with α- and β-furanose) in the melt and in the solutions from which D-ribose has been crystallized, only a single component is present in the crystalline materials.

[14] CCDC 768155 (Form I, powder diffraction), CCDC 768156 (Form I single crystal from zone melting), and CCDC 768157 (Form II) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.)