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Structure determination of the stable anhydrous phase of α-lactose from X-ray powder diffraction

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The stable anhydrous form of α-lactose has been obtained by the dehydration of α-lactose monohydrate in methanol. An X-ray powder diffraction pattern was recorded at room temperature with a laboratory diffractometer equipped with an INEL curved sensitive detector CPS120. The starting structural model of this form was found by a Monte-Carlo simulated annealing method. The structure was obtained through Rietveld refinements and the minimization of crystalline energy for the localization of the H atoms of the hydroxyl groups. Soft restraints were applied to bond lengths and angles. Networks of O—H···O hydrogen bonds account for the crystalline cohesion. A comparison is made between the hydrogen-bond networks of this form and those of the monohydrate and hygroscopic anhydrous forms of α-lactose.

1. Introduction

The lactose 4-*O*-β-D-galactopyranosyl-D-glucopyranose ($C_{12}H_{22}O_{11}$), a 'mixed' disaccharide containing a galactose and a glucose unit linked through a β-1,4 linkage, exhibits two anomers (α-lactose and β-lactose) which differ in the configuration of the terminal hydroxyl group of the glucose unit. For the α-anomer, three crystalline forms have been characterized (Garnier, 2001; Garnier *et al.*, 2002): the α-lactose monohydrate (hereafter named αL-H_2O), the hygroscopic anhydrous α-lactose (αL_H) and the stable anhydrous α-lactose (αL_S). The β-anomer has only one crystalline form (βL); mixed compounds α–β-lactose have also been identified with different stoichiometries (α/βL; Burshill *et al.*, 1965; Lerk *et al.*, 1984). The crystalline structures of the αL-H_2O form (Fries *et al.*, 1971; Beevers & Hansen, 1971; Noordik *et al.*, 1984) and the βL form (Hirotsu & Shimada, 1974) were solved from single-crystal samples with an automatic X-ray diffractometer. The structure of the αL_H form was recently determined *ab initio* from powder X-ray diffraction experiments (Platteau *et al.*, 2004).

The aim of this study was to determine the crystalline structure of the stable anhydrous phase of α-lactose. This form, not commercially available, can be obtained from αL-H_2O either by heating at *ca* 413 K or by dehydration in an hygroscopic solvent such as methanol (van Kreveld, 1969). Owing to hydration and mutarotation, the growth of pure αL_S single crystals suitable for X-ray experiments with a four-circle automatic diffractometer could not be achieved. The structure of the αL_S form was thus solved *ab initio* from powder X-ray data by a direct space approach and then refined using the Rietveld method. Energy minimization calculations are performed to locate the H-atom positions of the hydroxyl groups involved in hydrogen bonds. The broadening of the

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Bragg reflections is interpreted in terms of the size of the crystallites and the strain of the lattice.

2. Experimental

2.1. Data collection

The production of αL_S by thermal dehydration of αL-H_2O around 413 K was found to be incomplete (Platteau *et al.*, 2004). Crystalline αL_S was thus obtained by dehydration of commercial crystalline αL-H_2O purchased from the Sigma Company. Dehydration was performed by blowing dry gaseous methanol through 20 g of αL-H_2O over 3 h. Such a dehydration was performed at quite a low temperature (337.7 K) which prevents the formation of βL by mutarotation. The remaining traces of methanol were then removed by placing the sample under vacuum (10^{-3} mm Hg) at 293 K for 12 h. Thermogravimetric measurements have indicated that the α-lactose form thus obtained was free of water and methanol. The powder was introduced in a Lindemann glass capillary which was 0.7 mm in diameter.

The experimental X-ray set-up is explained elsewhere (Platteau *et al.*, 2004). The instrumental resolution was determined using a pattern from the standard compound of $Na_2Ca_3Al_2F_{14}$ (NAC; Evain *et al.*, 1993). This resolution has a pseudo-Voigt profile with the following parameters: $U_{\rm ins}$ = 0.0186 (4), $V_{\rm ins}$ = −0.0195 (6), $W_{\rm ins}$ = 0.0121 (2) deg^2, $X_{\rm ins}$ = 0.0181 (16) and $Y_{\rm ins}$ = 0.0188 (7)°. The pattern was recorded at room temperature with a total counting time of 16 h and 40 min.

2.2. Structure solution and refinement

For the determination of the lattice parameters of αL_S, the profiles of the 59 reflections with a 2θ angle lower than 42.5° were individually refined with the program *WinPlotr* (Roisnel & Rodriguez-Carvajal, 2002) in order to obtain their exact 2θ positions. From these, 30 of them ranging from 9 to 36° were introduced into the program *TREOR* (Werner *et al.*, 1985) and these reflections were successfully indexed with a triclinic cell with the following parameters: a = 7.663, b = 19.853, c = 4.986 Å, α = 92.0, β = 106.3, γ = 97.1°, V = 720.4 Å^3. The values of the figures-of-merit are: $M(30)$ = 16, $F(30)$ = 35. (0.011, 77; de Wolff, 1968; Smith & Snyder, 1979). The volume of this cell is almost the same as those of αL_H (V = 730.3 Å^3; Platteau *et al.*, 2004) and βL (V = 716.7 Å^3; Hirotsu & Shimada, 1974); therefore, the three cells have the same number of molecules of lactose: Z = 2.

In order to determine the input parameters for the program *FOX* (Favre-Nicolin & Cerny, 2002), the X-ray diffraction pattern in the 2θ range 8.5–62° was refined with the triclinic cell found by *TREOR* and using the 'profile matching' option (Le Bail refinement; Le Bail *et al.*, 1988) of the program *FullProf* (Rodriguez-Carvajal, 2001). The lower limit of 8.5° allows the inclusion of the first reflection of the pattern and for the upper limit (62°) the fitted 2θ range is enough for *FOX*. A pseudo-Voigt function with the same FWHM (full width at half-maximum) for the Gaussian and Lorentzian components is used to fit the profile of the reflections. This width has a θ dependence according to Caglioti's law (Caglioti *et al.*, 1958) and the Lorentzian contribution to the pseudo-Voigt function is a linear function of θ. The asymmetry of the peaks is taken into account with the Bérar & Baldinozzi (1993) function and the background is determined with a linear interpolation between 24 points regularly distributed from 8.5 to 62°.

The conventional profile reliability factors obtained from the Le Bail refinements are: R_p = 0.0604, $R_{\rm wp}$ = 0.0682, $R_{\rm exp}$ = 0.0151 and χ^2 = 20.4; with the following lattice parameters: a = 7.650 (1), b = 19.858 (1), c = 4.986 (1) Å, α = 92.02 (1), β = 106.25 (1), γ = 97.14°, V = 719.6 (1) Å^3. At this stage the two space groups of the triclinic system, $P1$ and $P\overline{1}$, are possible.

Lattice and profile parameters, zero-point and interpolated background calculated from previous refinements, were introduced into the program *FOX* (Favre-Nicolin & Cerny, 2002) in order to obtain a starting structural model. The 'parallel tempering' algorithm of this program was used for a 2θ range running from 8.5 to 50°. The space group $P\overline{1}$ was first tried and in this case there was one molecule in the asymmetric unit. The molecule of α-lactose with only non-H atoms is built with bond lengths, bond angles and torsion angles calculated from the atomic coordinates of Fries *et al.* (1971) obtained during the structure determination of αL-H_2O. This molecule is introduced randomly in the cell. Bond lengths and angles are kept constant and the rings of galactose and glucose are rigid, but the torsion angles of the central linkage and peripheral C and O atoms are allowed to change. During the calculation and for the space group $P\overline{1}$, there are 20 degrees of freedom: 3 translations and 3 rotations of the whole molecule and 14 torsion angles. The starting integrated R_w factor is $\simeq$ 0.50 and decreases to only 0.25 after 10 million trials. The space group $P1$ was then tried. The starting configuration was obtained by randomly placing two molecules in the unit cell. For each molecule, the same torsion angles as before are free parameters, leading to 37 degrees of freedom (the 3 translations of one of the two molecules must be fixed and are used as the origin). With the space group $P1$, the integrated wR factor equals 0.10 after about 10 million trials. The $P1$ solution was thus preferred to the $P\overline{1}$ solution. The atomic coordinates found by *FOX* with this space group have thus been used as a starting solution for the structure refinement of the αL_S form: in terms of crystal packing, the lowest contact distance between neighbouring molecules is equal to 2.77 Å.

Atomic coordinates found by *FOX* for C and O atoms and the space group $P1$ are input parameters in the program *FullProf* (Rodriguez-Carvajal, 2001) in order to perform Rieveld refinements. Some H atoms can be placed with geometrical arguments, as is the case for the CH and CH_2 groups. Each C atom is at the centre of a tetrahedron and the H atoms complete these tetrahedra. The positions of these H atoms are calculated with the program *DEBVIN* (Brückner & Immirzi, 1997) with the C—H bond length equal to 1.00 Å. For the CH groups, the H atom is such that the three C—C—H or O—C—H angles are equal. For the CH_2 groups, the two C—C—O and H—C—H planes are perpendicular and the two H

Table 1
Values of the bond lengths (Å) and angles (°) used for the soft constraints.

Mean values and their mean square deviations (see footnote) obtained after Rietveld refinements. The index 'int' represents internal atoms of the cycles, 'per' for peripheral atoms, the C and O atoms of the methanol groups are named C_m and O_m, O_{link} is the O atom linking the two units.

	Constraint values	Calculated mean values
$C_{int}-C_{int}$	1.527	1.524 (8)
$C_{int}-O_{int}$	1.433	1.432 (11)
$C_{int}-C_m$	1.511	1.512 (6)
$C_{int}-O_{per}$, C_m-O_m	1.421	1.431 (9)
$C_{int}-O_{link}$	1.421	1.416 (7)
$C_{int}-C_{int}-C_{int}$	110.4	111.9 (4.5)
$C_{int}-C_{int}-O_{int}$	109.6	109.6 (4.5)
$C_{int}-O_{int}-C_{int}$	113.2	114.1 (3.8)
$C_{int}-C_{int}-C_m$	112.8	111.9 (2.2)
$O_{int}-C_{int}-C_m$	107.1	109.0 (4.9)
$C_{int}-C_{int}-O_{per}$	110.4	111.2 (5.7)
$C_{int}-C_m-O_m$	108.4	109.3 (7.3)
$O_{int}-C_{int}-O_{per}$	109.5	109.1 (2.8)
$C_{int}-C_{int}-O_{link}$, $O_{int}-C_{int}-O_{link}$	107.9	109.0 (5.2)
$C_{int}-O_{link}-C_{int}$	116.8	117.5 (2.3)

The mean square deviation σ is defined as $\sigma = \left[\sum_{i=1}^{N} (V_i - V_{mean})^2/N\right]$, where N is the number of observations.

atoms are symmetrically located with respect to the C—C—O plane.

For the H atoms of OH groups involved in hydrogen bonds, a crystalline energy minimization is needed to determine their positions. As already used for the αL_H form (Platteau *et al.*, 2004), calculations were performed with the *DL_POLY* (Smith & Forester, 1996) molecular modelling package with the Ha *et al.* (1988) force field suitable for carbohydrates.

A system made of $N = 60$ lactose molecules ($3 \times 2 \times 5$ triclinic crystalline cells with $Z = 2$) was investigated using periodic boundary conditions. Each molecule is described by its 45 atoms which interact through the Ha force field (Ha *et al.*, 1988) developed for carbohydrates. Electrostatic interactions are handled by the Ewald method. We work in the NVE statistical ensemble where the number of atoms (N), the volume (V) and the energy (E) are fixed. A cut-off radius of 10 Å is used. In order to determine the positions of the hydroxyl H atoms, energy minimization calculations ($T = 0$) are realised from the structure obtained experimentally. C—O—H angles are initially chosen to be 180°. No dihedral interaction is applied to the hydroxyl H. During minimization, only H atoms of the hydroxyl groups are allowed to move in order to maintain the experimental structure as much as possible. After the minimization run, the value of the energy is $E(\alpha L_S) = -1224.8$ kJ mol^{-1} which is consistent with $E(\alpha L_H) = -710.9$ kJ mol^{-1}, which was found for the hygroscopic anhydrous α-lactose. No significant change was observed for larger systems.

During the Rietveld refinements, the shifts of the H atoms were the same as their bonded C or O atoms. Nevertheless, owing to the possible deformations or rotations of the molecules or translation of a molecule with respect to the other, their positions were recalculated several times during the refinement procedure either with *DEBVIN* or by energy minimization.

The 2θ angular range used for the Rietveld refinements runs from 8.5 to 80°. The reduced coordinates of the O21 atom were fixed and this atom was chosen as the origin. The coordinates of the other 45 non-H atoms were adjusted. In order to reduce the large number of free parameters, soft constraints on bond lengths and angles were used. The constraint values are the mean ones obtained by X-ray diffraction on single crystals in disaccharides with a β-1,4 linkage (Chu & Jeffrey, 1968; Ham & Williams, 1970; Fries *et al.*, 1971; Hirotsu & Shimada, 1974). These values for the different combinations of atoms occurring in lactose are given Table 1. Introducing such soft constraints is equivalent to considering only the torsion angles as completely free parameters. In this case, 40 independent torsion angles (20 per molecule) are necessary to determine the configuration of the non-H atoms of the molecules. Intensities were corrected for the absorption effects of a cylindrical sample with a μR value of 0.40.

The peak profiles are fitted with pseudo-Voigt functions using the Thompson–Cox–Hastings formalism (Thompson *et al.*, 1987). These functions take into account the experimental resolution and the widening due to size and strain effects. The expression of the FWHM of the Gaussian and Lorentzian components is given elsewhere (Rodriguez-Carvajal & Roisnel, 2004). To determine if the size and strain effects have to be considered as being isotropic or anisotropic, Le Bail refinements are performed for each of the four possibilities and Table 2 gives, for each case, the conventional agreement profile factors, average size and strain. In the case of the anisotropic effect, the standard deviation corresponds to the degree of anisotropy. In the case where the two effects are isotropic, the agreement profile factor R_{wp} equals 0.0671 and the better improvement of this factor is obtained for isotropic size effects and anisotropic strain, where R_{wp} decreases to 0.0567 and the relative degree of anisotropy equals 42%. Since the difference between isotropic and anisotropic size effects is not significant, Rietveld refinements were carried out with isotropic sizes and anisotropic strains.

For the final Rietveld refinements, 191 parameters were adjusted:

26 profile parameters: 6 lattice parameters, the zero point, 15 strain parameters, 2 size parameters: Y_{SZ}, G_{SZ} and 2 parameters taking into account the asymmetry of the peaks according to the Bérar–Baldinozzi (Bérar & Baldinozzi, 1993) function.

139 structural variables: the scale factor, a parameter, G_1, linked to the preferred orientations along $[\bar{2}10]$ according to the March (1932) and Dollase (1986) function, 135 reduced coordinates for 45 non-H atoms (the coordinates of the O21 atom are fixed), an isotropic global temperature factor for each molecule.

30 points for background regularly distributed between 8.5 and 80°, 2θ. A linear interpolation is made between two successive points.

Table 2
Agreement factors for isotropic or anisotropic size and strain effects.

For an isotropic effect, the number in parentheses is the standard deviation. When the effect is anisotropic, this number is the dispersion with respect to the average value.

	Isotropic size Isotropic strain	Anisotropic size Isotropic strain	Isotropic size Anisotropic strain	Anisotropic size Anisotropic strain
R_p	0.0578	0.0545	0.0481	0.0482
$R_{\rm wp}$	0.0671	0.0621	0.0567	0.0568
$R_{\rm exp}$	0.0171	0.0171	0.0167	0.0169
χ^2	15.3	13.3	11.5	11.3
Average size (Å)	819 (4)	882 (82)	1185 (4)	1075 (153)
Average strain ($\times 10^{-4}$)	7.58 (3)	8.93 (3)	13.99 (5.86)	12.71 (5.90)

In fact, atomic coordinates are not completely free because 48 soft constraints on bond lengths and 70 on bond angles are introduced. The final conventional Rietveld agreement factors are: $R_p = 0.0555$, $R_{\rm wp} = 0.0624$, $R_{\rm exp} = 0.0159$, $\chi^2 = 15.5$, $R_B = 0.0161$, $R_F = 0.0178$. For the soft constraints, the mean-square deviations to the assigned values are 0.011 Å for the bond lengths and 4.9° for the bond angles. The mean value of the electronic density synthesis is zero with a mean-square deviation of 0.04 e Å^{-3}. The values of the highest peak and the deepest hole are 0.17 and −0.20 e Å^{-3}, respectively. The plot of the X-ray diffraction pattern is given Fig. 1. Crystallographic data, and profile and structural parameters are reported in Tables 3 and 4, respectively, and in the supplementary data.[1] The molecular structures, drawn with *ORTEP*3 (Farrugia, 1997), showing the atom numbering, are shown in Fig. 2 (in the atom numbering, the first number corresponds to the molecule and the second one to the atom in the molecule). The molecular packing in the (*ab*) plane is given in Fig. 3.

3. Discussion

Despite the different crystalline systems, very similar unit cells are observed between the αL_S form and the other two crystalline forms of α-lactose. Only very small differences between the lattice parameters of the two anhydrous forms are seen: the relative changes are as follows: 1.7% for *a*, −0.9% for *b* and −1.63% for *c*. The changes in α and β angles are 2.0 and 2.6°, respectively. The change is more important for the γ angle, with a change of 7.2° leading to a small contraction of 1.4% of the cell volume of the αL_S form with respect to the αL_H form. For the three crystalline forms of α-lactose, molecules are oriented with their long axis approximately along the *b* direction. For αL_S, molecules 1 (see Fig. 3) of the cell lie in *ac* planes with the reduced coordinates of their mass centre at about $y \simeq 0.5$; they correspond to the molecules generated by the twofold screw axes of the αL_H form. Molecules 2 are also in (*ac*) planes and their mass centre is at $y \simeq 0.0$ and they correspond to the molecules of the asymmetric unit of the αL_H form.

Table 1 gives the mean values and their mean square deviations for the different bond lengths and angles occurring in a molecule of lactose. The mean bond lengths and angles are similar to those obtained in other disaccharides (see, for example, Tables 5 and 6 from Hirotsu & Shimada, 1974). As for αL_H, but, contrary to αL-H$_2$O and βL, the two bonds on each side of the O$_{\rm link}$ have, approximately, the same length: they range from 1.406 to 1.426 Å with a mean value of 1.416 Å. The most important difference between bond angles concerns C12′—C13′—O13′ and C14′—C13′—O13′, where values are 102.5 and 126.0°, respectively. The reason for such values is explained below. The angles about

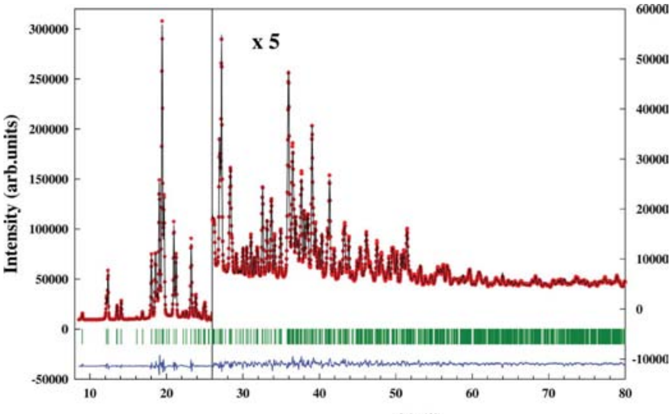

Figure 1
Final Rietveld plot of the stable anhydrous phase of α-lactose. Observed data points are indicated by dots, the best fit profile (upper trace) and the difference pattern (lower trace) are solid lines. The vertical bars correspond to the position of Bragg peaks.

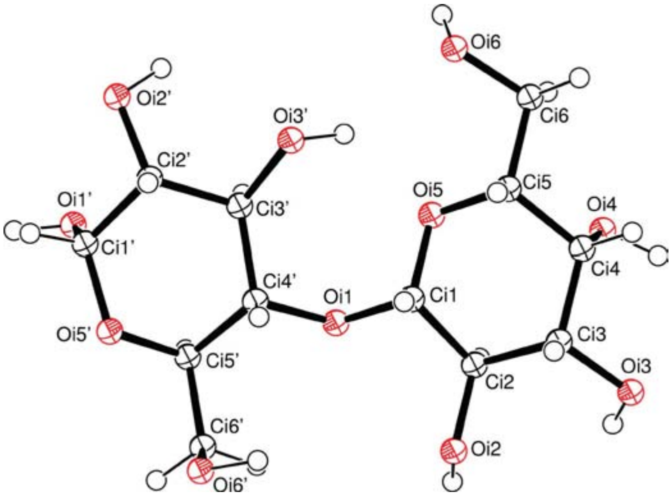

Figure 2
The atomic numbering and structure of the α-lactose molecule. The notation of atoms *Xin* is *X* = C, O, *i* = 1, 2, for molecules 1 and 2; *n* is the number of the atom; unprimed atoms correspond to the galactose unit, primed atoms to the glucose unit.

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[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: LC5010). Services for accessing these data are described at the back of the journal.

Table 3
Crystallographic data for the stable anhydrous phase of α-lactose obtained after Rietveld refinements.

Formula	$C_{12}H_{22}O_{11}$
M_r	342.30
Crystal system	Triclinic
Space group	$P1$
a (Å)	7.6522 (2)
b (Å)	19.864 (1)
c (Å)	4.9877 (1)
α (°)	92.028 (1)
β (°)	106.261 (1)
γ (°)	97.153 (1)
V (Å^3)	720.18 (3)
Z	2
D_c (g m^{-3})	1.557
F(000)	364
μ (mm^{-1})	1.23
2θ range (°)	8.5–80
Step size (°, 2θ)	0.029
Wavelength (Å)	1.54056
No. of profile data steps	2465
No. of contributing reflections	871
No. of structural variables	139
No. of profile parameters	26
No. of background points refined	30
No. of bond length constraints	48
No. of bond angle constraints	70
R_p	0.0555
R_{wp}	0.0624
R_{exp}	0.0159
χ^2	15.5
R_B	0.0161
R_F	0.0178

Table 4
Profile and structural parameters for the stable anhydrous phase of α-lactose obtained by *FullProf* after Rietveld refinements.

Y_{SZ}	0.0407 (10)
G_{SZ}	−0.0012 (2)
S_{400}	0.027 (9)
S_{040}	0.0011 (2)
S_{004}	1.82 (10)
S_{220}	0.045 (6)
S_{202}	0.97 (12)
S_{022}	0.45 (2)
S_{211}	0.25 (5)
S_{121}	−0.10 (2)
S_{112}	−0.26 (8)
S_{310}	0.008 (11)
S_{301}	−0.02 (5)
S_{130}	0.03 (2)
S_{103}	1.70 (16)
S_{013}	−0.99 (8)
S_{031}	0.013 (5)
Asym$_1$	0.034 (7)
Asym$_2$	0.010 (1)
Preferred orientations	$[\bar{2}, 1, 0]$
G_1	0.965 (3)
B_{iso} first molecule (Å^2)	1.37 (11)
B_{iso} second molecule (Å^2)	1.17 (11)

the bridge bonds C*i*1—O*i*1—C*i*4′ are 119.8 (8) and 115.2 (6)°; they are also close to the value of the corresponding angle in other disaccharides.

The torsion angles around the β-1,4 linkage for the four solid phases of lactose are reported in Table 5. The α-lactose molecules are characterized by a symmetrical twist about the bridge bonds with very similar torsion angles, such as $|\psi_1| \simeq |\psi_2|$ and $|\psi'_1| \simeq |\psi'_2|$. This contrasts with β-lactose molecules which have an asymmetrical twist and the difference between the previous torsion angles is of the order 38° (Hirotsu & Shimada, 1974). In the present case, for the first molecule of αL_S, $|\psi_1| - |\psi_2| = 16.7°$ and $|\psi_2'| - |\psi'_1| = 5.3°$, for the second molecule, these differences are smaller than 4°. The second molecule has a conformation with a symmetrical twist similar to molecules of αL-H$_2$O and αL_H (Fries *et al.*, 1971; Platteau *et al.*, 2004); the first molecule corresponds to an intermediate conformation between α- and β–lactose molecules, as seen for methyl β-cellobioside (Ham & Williams, 1970).

The cohesive lattice energy is usually determined for carbohydrates by O—H···O hydrogen bonds. From powder X-ray diffraction patterns, it is always difficult to accurately locate H-atom positions and, consequently, to determine which O atom is the acceptor and which is the donor in an O—H···O hydrogen bond. Energy minimization calculations have been performed in order to obtain the positions of these H atoms. The hydrogen-bonding distances and angles obtained with these calculations are listed in Table 6. Fig. 3 shows the hydrogen-bonding networks in the *ab* plane. To check the results obtained from the calculations, in most cases, the choice of the donor or acceptor O atom in the O—H···O hydrogen bonds can be made using the following arguments: there are 16 O···O pairs with intermolecular distances lower than 3.0 Å that are likely to form hydrogen bonds; 16 is also the maximum number of possible non-equivalent hydrogen bonds. Except the two bridging O*i*1 atoms, all the other O atoms are involved in these small distances. O*i*5 and O*i*5′, without H atoms, can only be acceptors and each O atom of the hydroxyl groups is a donor once. Using these hypotheses, the donor and acceptor atoms of 12 hydrogen bonds out of 16 are determined. Good agreement with calculated values is found. For the four remaining hydrogen bonds, involved in infinite chains, donors and acceptors can only be determined

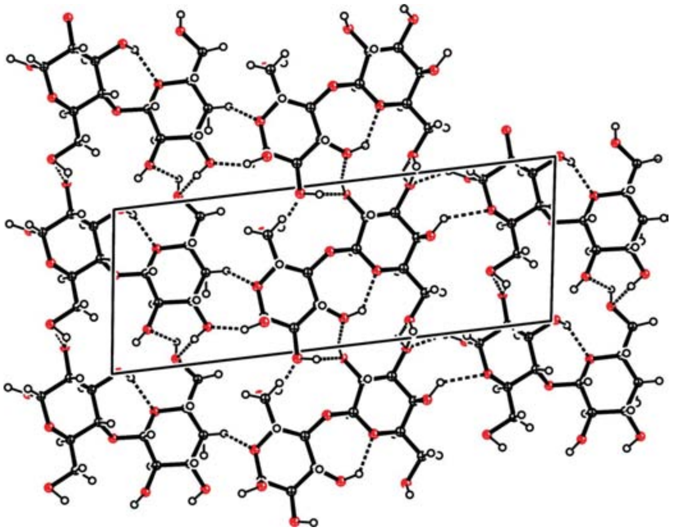

Figure 3
Projection along c^* of the unit cell of the stable anhydrous form of α-lactose. The dashed lines represent hydrogen bonds.

Table 5
Torsion angles (°) of the β-1,4 linkage for the different crystalline forms of lactose.

	αL_S[a]				
	First molecule	Second molecule	αL_H[b]	αL-H_2O[c]	βL[d]
ψ_1 O5—C1—O1—C4′	−85.9 (13)	−87.3 (10)	−92.3 (11)	−92.6	−70.7
ψ_1' C2—C1—O1—C4′	155.6 (11)	148.8 (8)	148.1 (9)	146.2	170.3
ψ_2 C1—O1—C4′—C3′	69.2 (14)	87.0 (10)	91.5 (11)	94.6	108.0
ψ_2' C1—O1—C4′—C5′	−160.9 (10)	−153.0 (7)	−150.9 (9)	−143.0	−131.3

ψ_1, ψ_1', ψ_2 and ψ_2' are defined according to Sundaralingam (1968). References: (*a*) this paper, (*b*) Platteau *et al.* (2004), (*c*) Fries *et al.* (1971), (*d*) Hirotsu & Shimada (1974).

Table 6
Hydrogen-bonding geometry (Å, °) for the stable anhydrous phase of α-lactose.

D—H$\cdots A$	D—H	H$\cdots A$	$D\cdots A$	D—H$\cdots A$
O12—HO12$\cdots$O13$'^{\rm i}$	0.985	1.852 (22)	2.797 (23)	159.8 (1.4)
O13—HO13$\cdots$O16$^{\rm i}$	0.986	1.887 (24)	2.711 (24)	139.2 (1.5)
O14—HO14$\cdots$O25$'^{\rm ii}$	0.986	2.184 (20)	2.991 (20)	138.0 (1.4)
O16—HO16$\cdots$O13$^{\rm iii}$	0.986	1.928 (22)	2.878 (22)	161.0 (1.8)
O11′—HO11′$\cdots$O23$^{\rm iv}$	0.974	1.850 (21)	2.771 (21)	156.7 (1.6)
O12′—HO12′$\cdots$O12$^{\rm iii}$	0.985	1.874 (21)	2.803 (20)	156.2 (1.6)
O13′—HO13′$\cdots$O15	0.984	1.911 (18)	2.643 (19)	128.9 (1.4)
O16′—HO16′$\cdots$O12$'^{\rm v}$	0.975	1.862 (18)	2.679 (19)	139.5 (1.3)
O22—HO22$\cdots$O23$'^{\rm vi}$	0.980	1.811 (22)	2.773 (22)	166.3 (1.5)
O23—HO23$\cdots$O26$^{\rm vi}$	0.982	1.849 (23)	2.813 (22)	166.5 (1.4)
O24—HO24$\cdots$O15$'^{\rm vii}$	0.982	2.449 (19)	2.681 (18)	89.8 (1.2)
O26—HO26$\cdots$O22$^{\rm v}$	0.938	1.984 (20)	2.706 (19)	132.3 (1.7)
O21′—HO21′$\cdots$O13$^{\rm viii}$	0.972	1.855 (19)	2.683 (19)	141.4 (1.4)
O22′—HO22′$\cdots$O26$'^{\rm v}$	0.979	1.872 (22)	2.752 (24)	148.0 (1.9)
O23′—HO23′$\cdots$O25	0.987	2.017 (19)	2.841 (20)	139.5 (1.4)
O26′—HO26′$\cdots$O22$'^{\rm vi}$	0.976	2.192 (27)	2.763 (26)	116.1 (1.5)

Symmetry codes: (i) $x-1, y, z$; (ii) $x, y+1, z$; (iii) $x+1, y, z+1$; (iv) $x, y, z+1$; (v) $x-1, y, z-1$; (vi) $x+1, y, z$; (vii) $x, y, z-1$; (viii) $x, y-1, z$; (ix) $x-1, y, z+1$; (x) $x+1, y+1, z$; (xi) $x+1, y+1, z+1$.

by energy calculations. Seven atoms (O11, O14, O11′, O16′, O21, O24 and O21′) are not acceptors, O13 is twice an acceptor and the other 14 O atoms are once acceptors.

From calculations only the position of HO24 seems to be incorrect, leading to HO24$\cdots$O15′ 2.45 Å and O24—HO24$\cdots$O15′ $\simeq$ 90°. The O$\cdots$O distances range from 2.643 to 2.991 Å (mean value: 2.768 Å); for H$\cdots$O, lengths range from 1.811 to 2.192 Å (mean value: 1.928 Å, the HO24$\cdots$O15′ length has been removed); O—H$\cdots$O angles vary from 116.2 to 166.5°. It should be noted that these values correspond to those generally found in carbohydrates (Jeffrey, 1990) and, in particular, in the hygroscopic anhydrous form of lactose (Platteau *et al.*, 2004). It has been seen that the C12′—C13′—O13′ and C14′—C13′—O13′ angles differ, respectively, from 9 and 15° to the $C_{\rm int}$—$C_{\rm int}$—$O_{\rm per}$ mean value. If these two angles are constrained to 114.2° (the mean value of these two angles), the distance of the intramolecular O13′$\cdots$O15 hydrogen bond becomes 2.386 Å, which is an unrealistic value. The large deviation from the mean value for these two angles is such that the distance between the two O atoms is 2.643 Å, which is small but is the correct value for two O atoms connected by a hydrogen bond.

The four O*i*5 and O*i*5′ atoms, which are acceptors only, finish the hydrogen-bonding finite chains. Among the seven non-acceptor O atoms, five belong to a hydroxyl group, four are at the beginning of the finite chains and the last one, O21′, is linked to another chain by O13, which is twice an acceptor. The four finite hydrogen-bonding chains are as follows (see symmetry codes at the bottom of Table 5).

$$\mathrm{O16'^{vi}} \rightarrow \mathrm{O12'^{vii}} \rightarrow \mathrm{O12^{vi}} \rightarrow \mathrm{O13'} \rightarrow \mathrm{O15}$$
$$\mathrm{O11'^{i}} \rightarrow \mathrm{O23^{ix}} \rightarrow \mathrm{O26^{iv}} \rightarrow \mathrm{O22}^{i} \rightarrow \mathrm{O23'} \rightarrow \mathrm{O25}$$
$$\mathrm{O14^{viii}} \rightarrow \mathrm{O25'} \quad \text{and} \quad \mathrm{O24^{iv}} \rightarrow \mathrm{O15'}$$

There are also two infinite chains with, for one of the two, grafted hydrogen bonds on an O atom of the chain.

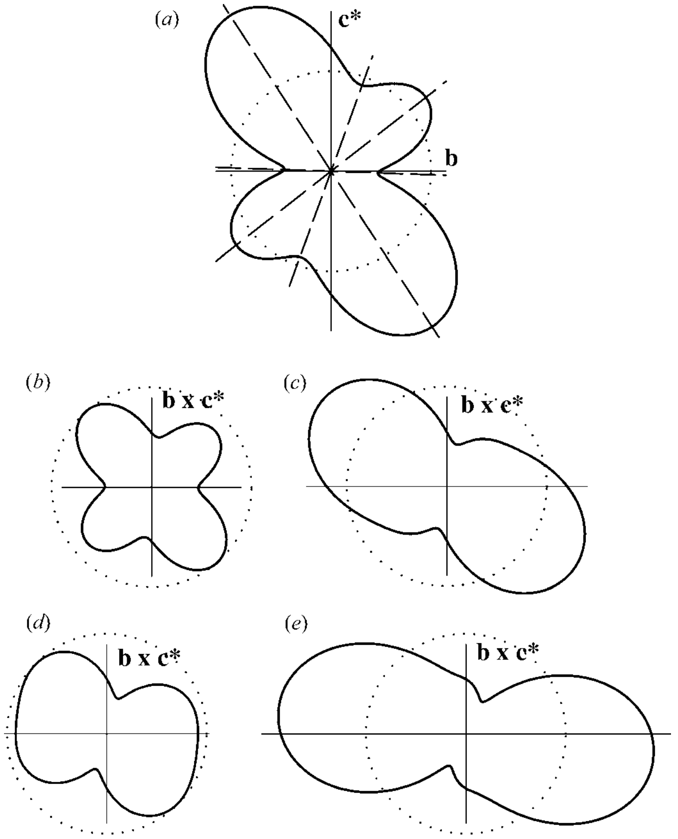

Figure 4
Anisotropic strain for different directions of the lattice of the αL_S form: (*a*) in the *bc** plane, (*b*)–(*e*) correspond to planes formed by the *bc** direction and the direction of an extreme value of the strain in the previous plane. φ is the angle between this direction and **b**: (*b*) φ = −2°, (*c*) φ = 38°, (*d*) φ = 70°, (*e*) φ = 123°. The dotted circles represent the average value of the strain: 15.7×10^{-4}.

$$\begin{tikzcd}
{} \arrow[r] & O22'^{iii} \arrow[r] & O26' \arrow[r] & O22'^{vi} \arrow[r] & {} \\
{} \arrow[r] & O13^{vi} \arrow[r] & O16 \arrow[r] & O13^{iii} \arrow[r] & {} \\
 & O21'^{x} \arrow[u] & & O21'^{xi}. \arrow[u] &
\end{tikzcd}$$

The two infinite chains are zigzag chains between equivalent molecules located at (x, y, z) and ($x+1$, y, z), and are extended along **c**. For the chain of molecules 1, O12′ → O13 hydrogen bonds allow molecules 2 located in the *ac* plane at $y \simeq 1.0$ to connect with molecules 1 at $y \simeq 0.5$. The first finite chain only connects molecules 1 lying in an *ac* plane at $y \simeq 0.5$. Most of the molecules of the second finite chain are molecule 2 located at $y \simeq 0.0$. The two hydrogen bonds O14 → O25′ and O24 → O15′ link molecules 1 and 2 located at $y = 0.5$ and 1.0 for the first hydrogen bond and at 0.5 and 0.0 for the second. The cohesive energy is provided by hydrogen-bonding networks between equivalent molecules located in the *ac* planes and also between planes of non-equivalent molecules.

As the unit cells of the three crystalline forms of α-lactose are pretty much the same with, approximately, the same direction for the long axis of the molecule, it becomes interesting to compare the hydrogen-bonding networks of αL_S with those of αL-H_2O and αL_H. In all cases, the O atoms of all the hydroxyl groups are donors; Oi1, Oi4 and Oi1′, central and terminal O atoms of the rings are never acceptors and, for all molecules, the intramolecular Oi3′ → Oi5 hydrogen bond exists.

The direction of the preferred orientation was determined gradually from the decrease of the agreement factor $R_{\rm wp}$. The better solution is obtained for a preferred orientation along $[\bar{2}, 1, 0]$ and the calculated value of G_1 ($G_1 = 0.965$) corresponds, for a diffractometer in the Debye–Scherrer geometry, to a small needle-like habit of the crystallites. This result is consistent with the conclusion of the peak profile study which predicts an almost spherical shape of the crystallites.

From a microstructural viewpoint, final refinements were performed with isotropic size effects and anisotropic strain effects. The average diameter of the crystallites equals 1035 (2) Å and the value of the average strain of the cell is 15.7×10^{-4}. The size of the crystallites is smaller and the strain is higher than those of the initial αL-H_2O form used to produce the αL_S form (1400 Å and 11.1×10^{-4}, respectively; Platteau *et al.*, 2004). The anisotropy of the strain effects is characterized in Fig. 4, which shows its value in different planes of the lattice. The extreme values of strains are 5.4×10^{-4} in the [100] direction and 29.5×10^{-4} along $[0\bar{8}3]$. Taking into account the complex hydrogen-bonding networks of this compound, it is difficult to give a qualitative explanation of these values with structural arguments.

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