

## THE CRYSTAL STRUCTURE OF ANHYDROUS $\alpha,\alpha$ -TREHALOSE AT $-150^\circ$

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### ABSTRACT

The crystal structure of  $\alpha,\alpha$ -trehalose ( $\alpha$ -D-glucopyranosyl  $\alpha$ -D-glucopyranoside),  $C_{12}H_{22}O_{11}$ , is monoclinic,  $P2_1$ ,  $Z = 2$ , with unit cell dimensions at  $-150^\circ$  [ $20^\circ$ ] of  $a = 12.971(5)$  [ $13.003(5)$ ],  $b = 8.229(4)$  [ $8.252(4)$ ],  $c = 6.789(3)$  [ $6.799(3)$ ] Å,  $\beta = 98.12(4)$  [ $98.33(4)$ ]. The crystal structure was solved by using MULTAN, and refined to  $R = 0.059$ ,  $R_w = 0.048$  for 1564 intensities, measured with  $MoK_\alpha$  radiation. The molecular structure is very similar to that observed in the dihydrate crystals. It has approximate  $C_2$  symmetry. Both glucopyranosyl groups are in the  ${}^4C_1$  conformation. The linkage torsion angles, O-5-C-1-O-1-C-1, are  $+60.8^\circ$  and  $+60.1^\circ$ . The primary alcohol groups are oriented *gauche/gauche* and *gauche/trans*, as in the dihydrate structure. A comparison of the cross-polarization, magic-angle-spinning (c.p.-m.a.s.),  $^{13}C$ -n.m.r. spectra for powders of the crystalline anhydrous and dihydrate forms shows differences in resonances assigned to C-1 and C-4 that would not be anticipated from the results of the crystal-structure analyses.

### INTRODUCTION

$\alpha,\alpha$ -Trehalose ( $\alpha$ -D-glucopyranosyl  $\alpha$ -D-glucopyranoside) is a nonreducing disaccharide that occurs in fungi<sup>1</sup>, yeasts, and insects<sup>2</sup>. In bacteria, it occurs mainly in the form of glycolipids, some of which are known<sup>3,4</sup> as the "cord factor".

The common, crystalline form is the dihydrate, the crystal structure of which has been determined<sup>5,6</sup>. The incentive for determining the crystal structure of the anhydrous form was to compare the molecular conformations in the two different crystal-field environments, and to aid in the interpretation of the differences in the solid-state, cross-polarization, magic-angle  $^{13}C$ -n.m.r. spectra of the crystals of the anhydrous and dihydrate forms.

### EXPERIMENTAL

Crystals of anhydrous  $\alpha,\alpha$ -trehalose were provided by Dr. Kevin Hicks of the USDA Eastern Regional Research Center. They were obtained by melting the

TABLE I

CRYSTAL STRUCTURE AND REFINEMENT DATA FOR  $\alpha,\alpha$ -TREHALOSE

$C_{12}H_{22}O_{11}$ ; M.W. = 174.20; m.p. = 202–205°;  $P2_1$ ;  $Z = 2$   
 Cell dimensions at  $-150^\circ$  [ $20^\circ$ ]:  $a = 12.971(5)$  [ $13.003(5)$ ],  $b = 8.229(4)$  [ $8.252(4)$ ],  $c = 6.789(7)$  [ $6.799(3)$ ] Å,  $\beta = 98.12(4)$  [ $98.33(4)$ ]  
 $V = 717.38$  [ $721.84$ ] Å<sup>3</sup>;  $D_x = [1.594 \text{ g/cc}]$ ,  $D_m = [1.567 \text{ g/cc}]$   
 Crystal dimensions:  $0.5 \times 0.3 \times 0.2 \text{ mm}$   
 Radiation:  $MoK_\alpha$  ( $\lambda = 0.7107$  Å) graphite monochromator,  $\mu_{MoK_\alpha} = 1.5 \text{ cm}^{-1}$   
 No absorption or extinction corrections applied  
 Cell dimensions based on 37 reflections with  $16^\circ \leq \theta < 20^\circ$   
 2531 intensities measured by  $\omega/2\theta$  scan, of which 2200 were unique, and 636 were considered unobserved,  $F_{obs} < 3\sigma(F_{obs})$   
 Function minimized,  $R = \sum w(|F_o| - |kF_c|)^2$ , using program QWKREF<sup>a</sup>, where  $w = \sigma^{-2}(F_o)$ , based on counting statistics  
 Final agreement factors:  $R = 0.059$ ,  $R_w = 0.048$ ,  $S = 1.34$   
 Maximum shift from ratio  $< 0.1$   
 Atomic scattering factors from International Tables for X-Ray Crystallography<sup>b</sup>

<sup>a</sup>Ref. 11. <sup>b</sup>Ref. 12.

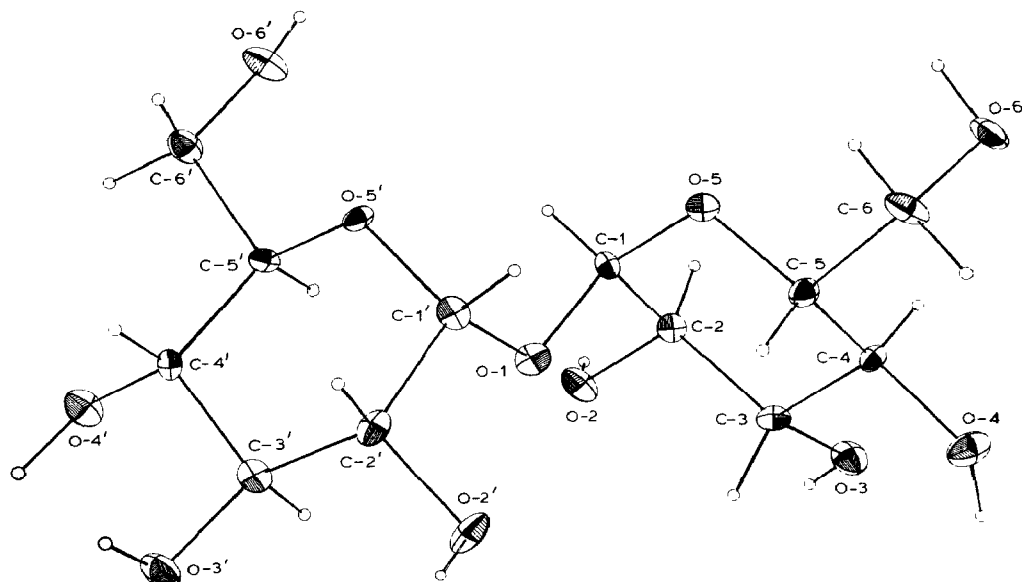


Fig. 1. Molecular structure and atomic notation for  $\alpha,\alpha$ -trehalose in the anhydrous crystal structure at  $-150^\circ$ . The thermal ellipsoids are at 50% probability, except for the hydrogen atoms.

dihydrate crystals (m.p.  $\sim 100^\circ$ ) in a capillary tube, followed by crystallization of the anhydrous form above  $120^\circ$ . The melting point of the anhydrous crystals is  $202\text{--}205^\circ$ . The crystal data and intensity-measurement data, given in Table I, were obtained with a CAD-4 diffractometer at  $-150^\circ$ . The structure was solved by using MULTAN<sup>7</sup>, with 152 E-values greater than 2.10. The atomic parameters were refined by a full-matrix least-squares procedure, using anisotropic thermal parameters

TABLE II

ATOMIC PARAMETERS<sup>a</sup> FOR THE CRYSTAL STRUCTURE OF  $\alpha,\alpha$ -TREHALOSE

Atom	Fractional coordinates <sup>b</sup>			$B_{eq}$ or $B^c$
	<i>x</i>	<i>y</i>	<i>z</i>	
C-1	1862(4)	−328(8)	1572(8)	84(1)
C-2	1823(4)	−2130(8)	2097(8)	69(10)
C-3	1997(4)	−3161(8)	313(8)	68(9)
C-4	1250(4)	−2653(8)	−1510(8)	71(9)
C-5	1298(4)	−834(7)	−1912(8)	77(10)
C-6	431(4)	−294(9)	−3533(9)	124(12)
C-1'	3077(4)	1660(8)	853(8)	83(10)
C-2'	4202(4)	1867(8)	491(8)	83(10)
C-3'	4977(4)	1728(8)	2422(8)	82(10)
C-4'	4635(4)	2715(8)	4119(8)	81(10)
C-5'	3494(3)	2394(8)	4329(7)	62(9)
C-6'	3087(4)	3560(9)	5770(8)	102(11)
O-1	2900(3)	0 <sup>d</sup>	1270(5)	87(7)
O-2	2585(3)	−2489(7)	3771(6)	112(8)
O-3	1767(3)	−4838(7)	605(6)	120(8)
O-4	1470(3)	−3475(7)	−3265(6)	123(8)
O-5	1136(3)	51(7)	−132(6)	95(7)
O-6	−559(3)	−937(7)	−3237(6)	138(8)
O-2'	4436(3)	743(7)	−962(6)	118(7)
O-3'	5994(3)	2211(7)	2094(6)	124(8)
O-4'	5252(3)	2323(7)	5994(6)	105(7)
O-5'	2873(2)	2693(6)	2430(5)	74(7)
O-6'	2081(3)	3111(7)	6188(6)	138(9)
H-C-1	175(5)	32(9)	257(11)	60(15)
H-C-2	122(4)	−220(6)	260(8)	36(9)
H-C-3	275(5)	−309(0)	2(10)	58(13)
H-C-4	60(6)	−299(8)	−125(11)	57(12)
H-C-5	194(4)	−46(5)	−241(7)	30(8)
H-C-6	35 <sup>d</sup>	87(11)	−344 <sup>d</sup>	64(15)
H'-C-6	66(4)	−71(7)	−479(8)	40(9)
H-C-1'	261(4)	200(7)	−33(9)	47(11)
H-C-2'	414(3)	290(7)	7(8)	36(9)
H-C-3'	496(4)	57(8)	279(9)	52(12)
H-C-4'	470(4)	380(7)	378(8)	36(9)
H-C-5'	339(5)	131(9)	472(10)	51(12)
H-C-6'	363(4)	340(7)	714(9)	44(12)
H'-C-6'	297(6)	447(11)	532(13)	64(16)
H-O-2	231(4)	−295(7)	472(8)	37(10)
H-O-3	210(6)	−510(11)	141(13)	64(6)
H-O-4	180(5)	−434(9)	−316(10)	48(11)
H-O-6	−82(5)	−38(8)	−223(11)	54(12)
H-O-2'	486(5)	132(7)	−184(10)	48(12)
H-O-3'	608(6)	322(12)	184(13)	65(16)
H-O-4'	596(5)	256(9)	591(10)	51(11)
H-O-6'	166(5)	347(7)	551(8)	37(10)

<sup>a</sup>E.s.d. values, given in parentheses, refer to the least significant digit. <sup>b</sup>Fractional coordinates  $\times 10^4$  for non-hydrogen atoms,  $\times 10^3$  for hydrogen atoms. <sup>c</sup> $B_{eq} = \frac{1}{3}(\sum_i \beta_i \hat{a}_i \cdot \hat{a}_i)$ ,  $\times 10^2$  (in Å<sup>2</sup>) for non-hydrogen atoms, calculated from the refined, anisotropic, thermal parameters;  $B \times 10$  (in Å<sup>2</sup>) for hydrogen atoms, refined, isotropic, thermal parameters. <sup>d</sup>Unrefined parameters.

for the non-hydrogen atoms. The parameter data are given in Table I. The crystal density was measured by using a Westphal balance with a bromotrichloromethane–1,2-dichloroethane flotation mixture.

The atomic notation and thermal ellipsoids are shown in Fig. 1. Atomic, positional, and thermal parameters\* are given in Table II.

## DISCUSSION

In this structure, as in the dihydrate, the molecule has approximate  $C_2$  symmetry with the exception of the orientation of the primary alcohol groups, which are *gauche/gauche* on one residue ( $O-5-C-5-C-6-O-6 = -72^\circ$ ) and *gauche/trans* on the other ( $O-5'-C-5'-C-6'-O-6' = +71^\circ$ ). The same two orientations are observed in the dihydrate structure, with torsion angles of  $-76$  and  $+70^\circ$ , respectively. These are the two favored orientations that avoid the *peri* interaction with the hydroxyl group on C-4 in the *D-gluco* configuration. Stereoviews down the pseudo-2-fold axis of the molecules in the anhydrous and dihydrate crystals are shown in Fig. 2. With the exception of the orientation of the primary alcohol groups and the hydroxyl groups, the pseudo-2-fold symmetry is close to being exact in the molecules of both crystal structures. A least-squares fit of the two  $\alpha$ -*D*-glucopyranosyl ring-atoms across the pseudo-2-fold axis gave a mean difference in atomic coordinates of  $0.038 \text{ \AA}$  for the molecule in the anhydrous crystals and  $0.030 \text{ \AA}$  in those of the dihydrate.

The conformations are  ${}^4C_1$  with  $Q = 0.55 \text{ \AA}$ ,  $\theta = 1.1^\circ$ ,  $\varphi = 37^\circ$ ;  $Q' = 0.54 \text{ \AA}$ ,  $\theta' = 77^\circ$ ,  $\varphi' = 334^\circ$  (at small values of  $\theta$ , large differences in  $\varphi$  are not significant). The corresponding values for the dihydrate molecules are  $Q = 0.56 \text{ \AA}$ ,  $\theta = 9.4^\circ$ ,  $\varphi = 97^\circ$ ;  $Q' = 0.54 \text{ \AA}$ ,  $\theta' = 4.0^\circ$ ,  $\varphi' = 65^\circ$ . The two glycosidic-linkage torsion-angles are more nearly equal in the anhydrous structure:  $+60.8^\circ$  and  $+60.1^\circ$ , *versus*  $+74.8^\circ$  and  $+61.7^\circ$  in the dihydrate. The *D*-glucopyranosyl groups are therefore closer to having the ideal-chair conformation, and are more symmetrically oriented about the central, glycosidic oxygen atom, in the anhydrous form than in the dihydrate.

The molecular dimensions are compared in detail in Table III. Because this is a low-temperature study and the study of the hydrate was conducted at room temperature, a small, systematic difference in the mean bond-lengths is expected due to differences in the molecular thermal motion. This was observed; the mean C–C distance in the low-temperature analysis of the anhydrous form was  $1.525$  *versus*  $1.523 \text{ \AA}$  in the room-temperature analysis of the hydrate. As anticipated, there was a larger difference between the mean, C–O bond-lengths,  $1.440$  *versus*  $1.421 \text{ \AA}$ , due to the greater relative difference in the thermal motion of the exo-

\*Lists of structure factors and anisotropic thermal parameters have been deposited with, and can be obtained from, Elsevier Science Publishers, BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/303/*Carbohydr Res.*, 137 (1985) 21–30.

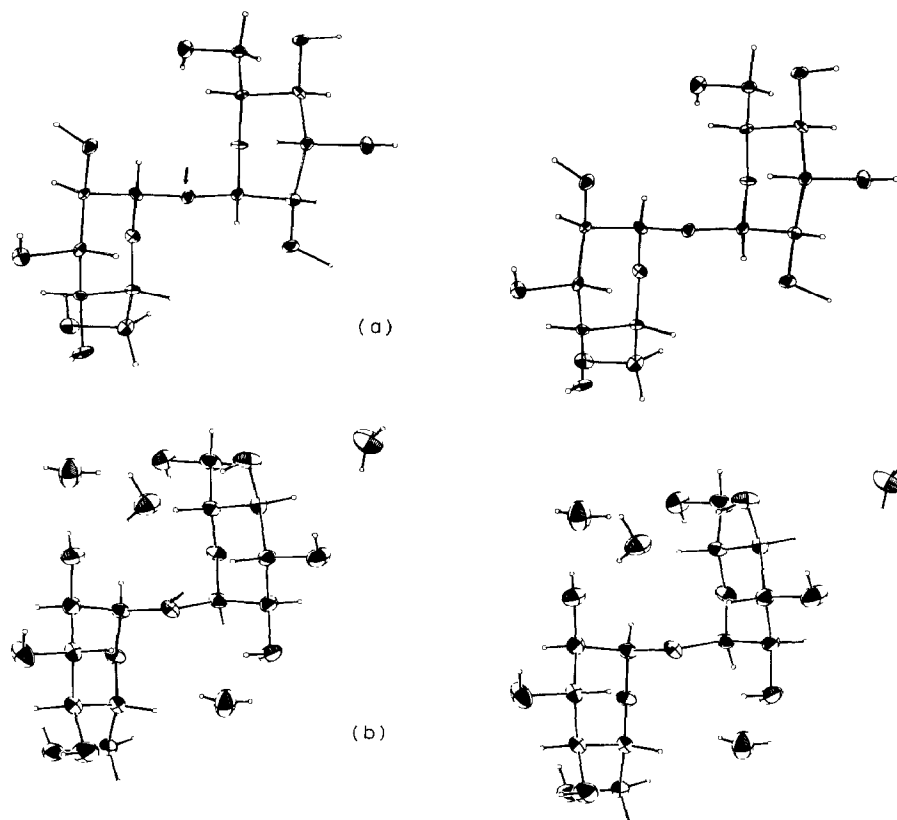


Fig. 2. Stereoview of molecule of  $\alpha,\alpha$ -trehalose, viewed down the pseudo-2-fold axis (marked with an arrow). (a) In anhydrous crystal at  $-150^\circ$ , (b) in dihydrate crystal at  $20^\circ$ .

cyclic atoms. Apart from these thermal-motion effects, there was no chemically significant difference in the structure of the disaccharide in the crystals of the anhydrous and dihydrate forms. The linkage valence-angle C-1-O-1-C-1' is  $113.3^\circ$  in the anhydrous crystal *versus*  $115.8^\circ$  in the dihydrate. This difference is possibly significant. There is no significant difference in the ring-oxygen angles.

#### *The hydrogen bonding*

The geometry of the hydrogen-bonding scheme is shown in Fig. 3. It consists of long, finite chains, originating in O-3'-H and terminating in O-5', that are cross-linked to form infinite chains by means of the weak components of three-center interactions through O-3-H and O-6'-H. All of the hydroxyl groups are involved in hydrogen bonds. Both ring-oxygen atoms are included through bifurcated bonds, but the glycosidic oxygen atom, O-1, is not. This geometry is different from that in the dihydrate<sup>8</sup>, in which a loop of hydrogen bonds intersects with an infinite chain at a water molecule, and both the glycosidic oxygen atom and the ring-oxygen atom

TABLE III

COMPARISON OF MOLECULAR DIMENSIONS OF  $\alpha,\alpha$ -TREHALOSE<sup>a</sup> IN THE ANHYDROUS (AT  $-150^\circ$ ) AND THE DIHYDRATE (AT  $20^\circ$ ) CRYSTAL STRUCTURES

<i>Parameter</i>	<i>Anhydrous</i>	<i>Dihydrate</i>
<i>Bonds</i>		
C-1-C-2	1.528(8)	1.521
C-2-C-3	1.521(8)	1.524
C-3-C-4	1.519(8)	1.527
C-4-C-5	1.525(8)	1.528
C-5-C-6	1.525(8)	1.521
C-1-O-1	1.416(7)	1.415
C-1-O-5	1.419(7)	1.421
C-3-O-2	1.428(7)	1.420
C-3-O-3	1.432(7)	1.417
C-4-O-4	1.433(7)	1.420
C-5-O-5	1.451(7)	1.433
C-6-O-6	1.428(8)	1.417
C-1'-C-2'	1.523(8)	1.533
C-2'-C-3'	1.539(8)	1.519
C-3'-C-4'	1.526(8)	1.519
C-4'-C-5'	1.530(8)	1.526
C-5'-C-6'	1.517(8)	1.516
C-1'-O-1'	1.420(7)	1.422
C-1'-O-5'	1.421(7)	1.404
C-2'-O-2'	1.416(7)	1.422
C-3'-O-3'	1.426(7)	1.422
C-4'-O-4'	1.441(7)	1.429
C-5'-O-5'	1.442(6)	1.431
C-6'-O-6'	1.423(8)	1.425
<i>Valence angles</i>		
C-1-O-1-C-1'	113.3(4)	115.8
C-2-C-1-O-5	111.3(5)	109.9
C-2-C-1-O-1	106.4(5)	106.4
O-5-C-1-O-1	112.0(5)	112.1
C-1-C-2-C-3	110.0(5)	108.5
C-1-C-2-O-2	110.2(5)	111.7
C-3-C-2-O-2	110.8(5)	109.7
C-2-C-3-C-4	110.2(5)	109.0
C-2-C-3-O-3	111.7(5)	112.7
C-4-C-3-O-3	104.8(5)	106.8
C-3-C-4-C-5	112.2(5)	113.0
C-3-C-4-O-4	111.7(5)	111.6
C-5-C-4-O-4	107.2(5)	105.9
C-4-C-5-C-6	111.7(5)	112.3
C-4-C-5-O-5	109.2(5)	111.6
C-6-C-5-O-5	106.0(5)	106.7
C-5-C-6-O-6	112.1(5)	113.5
C-1-O-5-C-5	114.3(5)	114.0

TABLE III (continued)

<i>Parameter</i>	<i>Anhydrous</i>	<i>Dihydrate</i>
C-2'-C-1'-O-5'	109.9(5)	111.3
C-2'-C-1'-O-1	109.0(5)	105.9
O-5'-C-1'-O-1	111.9(4)	111.7
C-1'-C-2'-C-3'	112.3(5)	110.0
C-1'-C-2'-O-2'	110.1(5)	111.0
C-3'-C-2'-O-2'	111.5(5)	111.2
C-2'-C-3'-C-4'	112.3(5)	111.4
C-2'-C-3'-O-3'	110.7(5)	109.1
C-4'-C-3'-O-3'	110.1(5)	110.7
C-3'-C-4'-C-5'	111.5(5)	111.7
C-3'-C-4'-O-4'	111.3(5)	109.9
C-5'-C-4'-O-4'	107.6(5)	109.7
C-4'-C-5'-C-6'	112.3(5)	111.2
C-4'-C-5'-O-5'	108.2(4)	111.8
C-6'-C-5'-O-5'	105.5(4)	106.6
C-5'-C-6'-O-6'	112.1(5)	111.7
C-1'-O-5'-C-5'	115.5(4)	114.2
<i>Torsion angles</i>		
C-1-C-2-C-3-C-4	-52.5(6)	-56.8
C-2-C-3-C-4-C-5	53.1(6)	50.7
C-3-C-4-C-5-O-5	-54.0(6)	-48.1
C-4-C-5-O-5-C-1	57.9(6)	53.8
C-5-O-5-C-1-C-2	-59.7(6)	-61.8
O-5-C-1-C-2-C-3	55.6(6)	62.5
C-4-C-5-C-6-O-6	46.8(7)	47.0
O-5-C-5-C-6-O-6	-72.1(6)	-75.6
C-1'-C-2'-C-3'-C-4'	-46.1(6)	-51.4
C-2'-C-3'-C-4'-C-5'	48.5(6)	49.3
C-3'-C-4'-C-5'-O-5'	-54.5(6)	-50.5
C-4'-C-5'-O-5'-C-1'	63.1(6)	56.7
C-5'-O-5'-C-1'-C-2'	-61.1(6)	-59.6
O-5'-C-1'-C-2'-C-3'	50.2(6)	56.1
C-4'-C-5'-C-6'-O-6'	-171.3(5)	-168.2
O-5'-C-5'-C-6'-O-6'	71.0(6)	69.8
O-5-C-1-O-1-C-1'	60.8(6)	74.8
O-5'-C-1'-O-1-C-1	60.1(6)	61.7
C-2-C-1-O-1-C-1'	-177.3(4)	-165.0
C-2'-C-1'-O-1-C-1	-178.2(4)	-177.0

<sup>a</sup>Bond lengths in Å, valence and torsion angles in degrees. E.s.d. values, given in parentheses, refer to the least significant digit.

are excluded from the hydrogen-bonding scheme. A stereoview of the hydrogen bonding and molecular packing is shown in Fig. 4.

#### *The solid-state, n.m.r. spectrum*

C.p.-m.a.s., <sup>13</sup>C-n.m.r. spectra of powders of the dihydrate and of the anhydrous and amorphous forms are shown in Fig. 5, by courtesy of Drs. P. Pfeffer and K. Hicks of the USDA Eastern Regional Research Center. The spectrum was

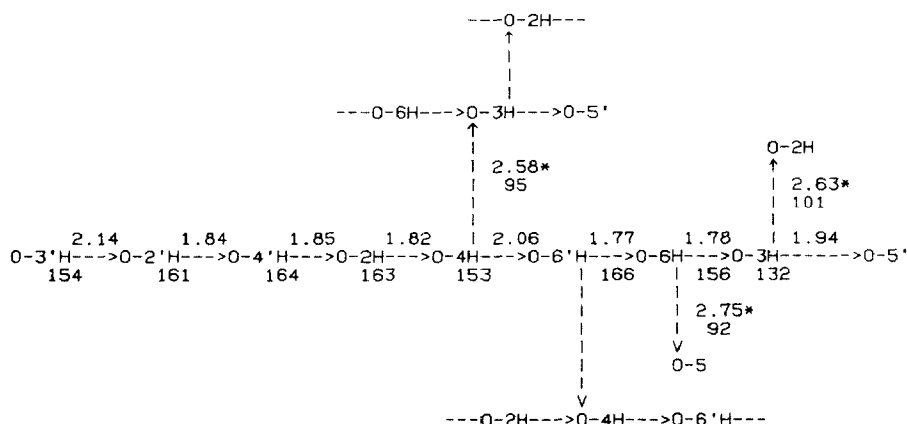


Fig. 3. Hydrogen-bonding geometry in the crystal structure of anhydrous  $\alpha,\alpha$ -trehalose. The covalent, O-H bond-lengths have been normalized<sup>8</sup> to the neutron-diffraction values of 0.97 Å.

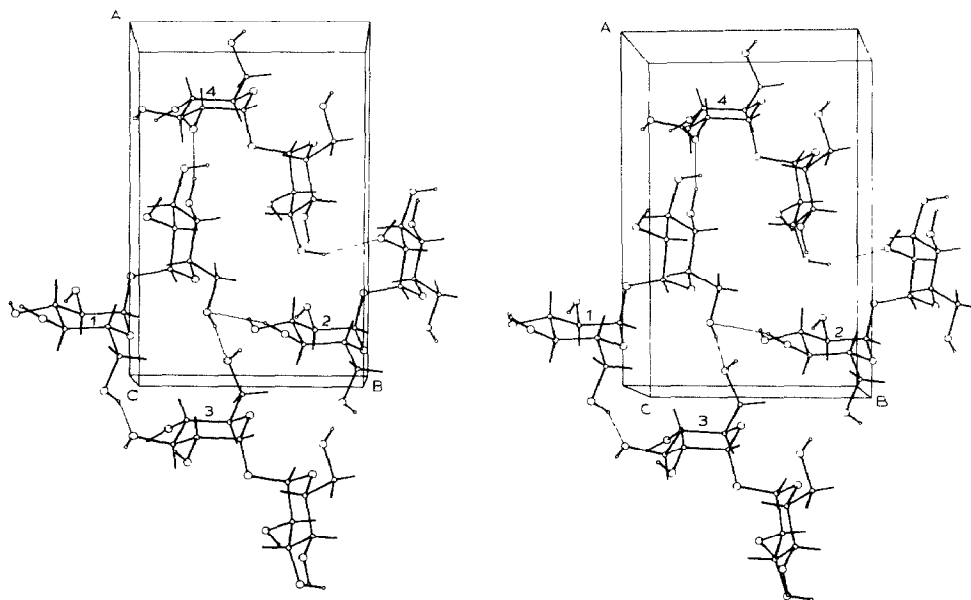


Fig. 4. Stereoview of the crystal structure of anhydrous  $\alpha,\alpha$ -trehalose. The thin lines are hydrogen bonds.

recorded with a JEOL FX60Q-S NMR spectrometer operated at an applied field of 1.4 T and an r.f. decoupling field of 42 kHz, and a home-built, wide-bore, superconducting instrument as described elsewhere<sup>9</sup>. The notable spectral differences were the separation of the resonances assigned to C-1 in the anhydrous *versus* the dihydrate form, and the appearance of an additional resonance at 78 p.p.m., tentatively assigned to C-4, in the spectrum of the anhydrous form. The doubling of the



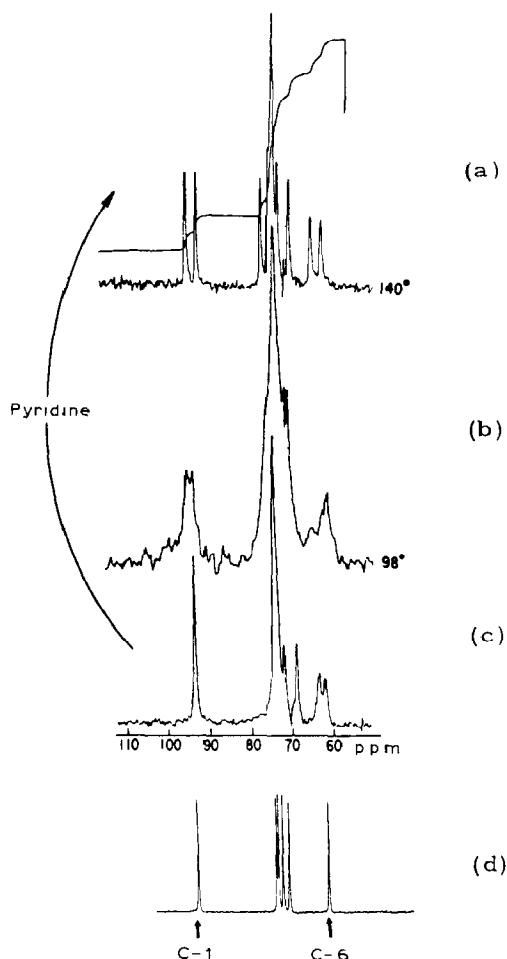


Fig. 5. C.p.-m.a.s.  $^{13}\text{C}$ -n.m.r. spectra of  $\alpha,\alpha$ -trehalose. (a) Crystalline anhydrous, (b) amorphous, (c) dihydrate, and (d) solution.

C-6 resonances for both the anhydrous and dihydrate crystals is consistent with the two different orientations of the primary alcohol groups in both molecules. Comparison of the two crystal structures shows no obvious basis for predicting the difference observed in the C-1 resonances for the two forms. In both, the C-1 and C-1' atoms are not crystallographically identical, although there is 2-fold pseudosymmetry; this is crystallographic, not magnetic, pseudosymmetry<sup>10</sup>, but the distinction is irrelevant in a magic-angle-spinning experiment.

This work provides an elegant illustration of the unreliability of predicting atom-site symmetries from the shifts in the c.p.-m.a.s. resonances, which are sensitive only to the trace of the anisotropic chemical-shift tensors.

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