The Crystal and Molecular Structure of β-Lactose

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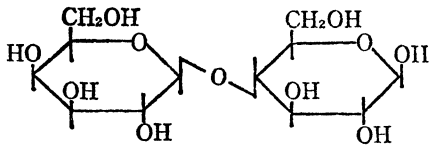
(Received December 24, 1973)

The crystal structure of β-lactose ($C_{12}H_{22}O_{11}$) was solved by direct phasing methods with integrated photographic intensity data. The structure was refined by the anisotropic least-squares method to give a final R value of 0.068 for the observed reflections. The space group is $P2_1$, with $Z=2$ and with unit cell dimensions of $a=10.839$ (6), $b=13.349$ (6), $c=4.954$ (5) Å, and $\beta=91.31$ (9)°. All the hydrogen atoms were located on different syntheses. The crystals are nearly isostructural with those of β-cellobiose except for the axial O(4) atom. The exocyclic C(5)–C(6) and C(5′)–C(6′) bonds are significantly shorter than the other C–C bonds. The lengths of the two ring C–O bonds are unequal in the galactose unit and equal in the glucose unit. The C(1)–O(1) and C(1′)–O(1′) bond lengths are shorter by 0.015 Å and 0.030 Å than the mean exocyclic C–O length of 1.417 Å. The structure contains an intramolecular hydrogen bond and exhibits an asymmetrical twist about the bridge bond. All the oxygen atoms, except the bridge oxygen atom, are involved in the hydrogen-bonding network, which comprises two terminating chains.

The crystal structure of β-lactose has been determined as a part of a series of X-ray diffraction studies of carbohydrates in this laboratory.[1] β-Lactose (*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside) is a disaccharide which consists of glucose and galactose units, as is shown in (I):



(galactose) (glucose)

(I)

The crystal structure of α-lactose monohydrate[2] was determined by Fries, Rao, and Sundaralingam. The main purpose of our investigation was to compare precisely the bond lengths and angles of β-lactose with those of α-lactose,[2] β-cellobiose,[3-5] and methyl β-cellobioside.[6] The conformation of the (β-1,4) glycosidic bond, which is present in a variety of natural polysaccharides, and the hydrogen-bond scheme in crystal was also of interest.

Experimental

Suitable crystals were prepared from a hot aqueous solution by the method reported by Tsuzuki and Mori.[7] The cell dimensions were measured from zero-layer Weissenberg photographs, which were calibrated with superimposed Al powder lines ($a=4.04934$ Å). The density was determined by the flotation technique using a mixture of cylohexane and carbon tetrachloride. The crystal data are summarized in Table 1. A crystal with the dimensions of $0.2\times0.2\times0.3$ mm was used for all the intensity measurements. The intensity data were recorded on multiple film, equi-inclination integrating Weissenberg photographs using Cu$K\alpha$ radiation. Layers from $h0l$ to $h2l$ and from $hk0$ to $hk5$ were obtained. The intensities were estimated visually by comparison with a calibrated intensity standard. No correction was applied for absorption and extinction. All the intensity data were corrected for spot shape and for Lorentz and polarization factors. The resulting data were correlated and reduced to the relative structure factor amplitudes using the method of Hamilton, Rollet, and Sparks.[8] A total of 1595 structure factors were evaluated; 129 were too weak to be measured. Finally, a Wilson plot was made estimating an approximate absolute scale and an overall temperature factor, in order to calculate normalized structure factor magnitudes, $|E|$.

† Deceased August 5, 1973.

TABLE 1. CRYSTAL DATA

$C_{12}H_{22}O_{11}$	F. W. 342.3
Monoclinic	Space group $P2_1$
$a=10.839(6)$ Å	$Z=2$
$b=13.349(6)$	$F(000)=364$
$c=4.954(5)$	$V=716.7$ Å^3
$\beta=91.31(9)°$	λ(Cu$K\alpha$) $=1.5418$ Å
$D_x=1.586$ g·cm^{-3}	$D_m=1.57$ g·cm^{-3} (by flotation)

Structure Determination and Refinement

The crystal structure of β-lactose was solved by the multisolution method described by Woolfson and Germain.[9] The starting set of phases is listed in Table 2. In order to apply the tangent formula,[10] numerical phases from 45° to 315° (=−45°) in steps of 90° were assigned to the A and B symbols. Tangent refinement was carried out for each of the 16 possible phase combinations, and the number of reflections included was gradually increased from 100 with $E>1.70$ to 350 with $E>1.22$. The values of R_{Karle}[11] ranged from 0.27 to 0.31, and those of Z[12], from 8842 to 10882. All sixteen E-maps were calculated in increasing order of R_{Karle} value. A cursory inspection revealed the site of the glucose unit in several of the E-maps, and so the unit was chosen as a trial partial structure.[13] How-

TABLE 2. THE STARTING SET OF PHASES

h	k	l	E	ϕ	
3	9	−2	2.85	0°	
4	0	3	2.14	0°	origin and enantiomorph
1	12	1	2.64	45°	(45° → 39.5°)
8	0	−2	3.36	0°	from Σ_1
4	10	−1	2.55	A	(45° → 41.8°)
7	7	−1	2.87	B	(90° → 115.7°)

ever, this partial structure could not be developed into a complete structure by means of tangent refinement. After completing the structure, it turned out that the unit was actually properly oriented, but not placed in the right position in the unit cell. After this initial failure, the following new sets of phases were assigned to the A and B symbols. Tangent refinement was again carried out for each of the additional 20 possible phase combinations: (A=±90°, B=±45°, ±135°), (B=±90°, A=±45°, ±135°), (A=±90°, B=±90°). A solution was finally obtained from an *E*-map calculated by means of the phases in the most consistent set (R_{Karle}=0.21 and Z=10470). The initial and refined phases in this set are shown in Table 2. The positions of all the non-hydrogen atoms were easily located and further confirmed by a least-squares refinement. Seven cycles of isotropic block-diagonal least-squares refinement resulted in an R ($=\Sigma||F_o|-|F_c||/\Sigma|F_o|$) value of 12.5%. Six more cycles of anisotropic block-diagonal least-squares and two difference-map calculations were necessary to locate the 22 hydrogen atoms. The isotropic thermal parameters of the hydrogen atoms were assumed to be equal to those of the parent atoms, which were obtained from the last stage of isotropic refinement (R=12.5%). These hydrogen atoms were included in the refinement, in which positional parameters were varied but thermal parameters were fixed. Additional cycles of refinement resulted in an R value of 7.4%. At this point, seven reflections with very large structure-factor amplitudes were removed from the data because they appeared to be affected by secondary extinction. Four final cycles of block-diagonal refinement gave an R of 6.8% for the observed reflections and one of 7.7% for all the reflections. Throughout the refinements,

Table 3. Fractional atomic coordinates and thermal parameters in β-lactose

Key to atomic numbering is given in Fig. 1. The temperature factor expression used was

$$\exp[-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+hk\beta_{12}+hl\beta_{13}++kl\beta_{23})].$$

Numbers in parentheses refer to standard deviations of the last place.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	0.7544(3)	0.5881(3)	0.0177(8)	0.0036(3)	0.0026(2)	0.0184(12)	0.0000(4)	0.0036(9)	0.0007(9)
C(2)	0.7328(3)	0.6984(3)	−0.0391(8)	0.0038(3)	0.0025(2)	0.0149(10)	−0.0001(4)	0.0028(9)	0.0000(8)
C(3)	0.6660(4)	0.7460(3)	0.1955(8)	0.0046(3)	0.0019(2)	0.0174(12)	0.0001(4)	0.0049(10)	−0.0019(9)
C(4)	0.5466(4)	0.6880(3)	0.2375(9)	0.0044(3)	0.0022(2)	0.0206(13)	0.0004(4)	0.0027(10)	−0.0024(9)
C(5)	0.5744(4)	0.5768(3)	0.2829(8)	0.0041(3)	0.0023(2)	0.0167(11)	−0.0004(4)	0.0071(9)	−0.0017(8)
C(6)	0.4573(4)	0.5163(4)	0.3060(9)	0.0057(4)	0.0025(2)	0.0355(19)	−0.0014(5)	0.0098(13)	−0.0020(10)
C(1′)	0.9553(4)	0.2796(3)	−0.4780(9)	0.0043(3)	0.0024(2)	0.0237(14)	0.0011(4)	0.0039(11)	−0.0025(9)
C(2′)	0.8242(4)	0.2694(4)	−0.3760(9)	0.0047(3)	0.0026(2)	0.0295(16)	−0.0005(4)	0.0084(12)	−0.0034(10)
C(3′)	0.7684(4)	0.3742(4)	−0.3392(9)	0.0034(3)	0.0030(2)	0.0237(13)	−0.0008(4)	0.0073(10)	−0.0011(9)
C(4′)	0.8557(3)	0.4444(3)	−0.1800(8)	0.0036(2)	0.0016(2)	0.0227(13)	0.0006(4)	0.0045(10)	0.0023(8)
C(5′)	0.9857(4)	0.4431(3)	−0.2897(9)	0.0039(3)	0.0021(2)	0.0231(13)	0.0009(4)	0.0037(10)	0.0021(8)
C(6′)	1.0788(4)	0.5001(4)	−0.1179(9)	0.0037(3)	0.0032(2)	0.0341(17)	−0.0005(4)	0.0050(11)	−0.0012(11)
O(1)	0.8086(3)	0.5456(2)	−0.2101(6)	0.0046(2)	0.0020(1)	0.0182(8)	0.0011(3)	0.0052(7)	0.0013(6)
O(2)	0.8442(3)	0.7471(2)	−0.1082(7)	0.0061(3)	0.0030(2)	0.0244(9)	−0.0027(4)	0.0079(9)	−0.0041(7)
O(3)	0.6449(3)	0.8500(2)	0.1506(7)	0.0060(3)	0.0016(1)	0.0281(10)	0.0004(3)	0.0010(9)	−0.0022(6)
O(4)	0.4682(3)	0.6980(3)	0.0058(7)	0.0042(2)	0.0035(1)	0.0288(10)	0.0014(3)	−0.0027(9)	0.0007(8)
O(5)	0.6410(3)	0.5386(2)	0.0566(6)	0.0037(2)	0.0021(1)	0.0192(8)	−0.0005(3)	0.0075(7)	−0.0042(6)
O(6)	0.4802(4)	0.4118(3)	0.3041(7)	0.0079(3)	0.0021(2)	0.0339(12)	−0.0023(4)	0.0014(10)	0.0023(8)
O(1′)	1.0099(3)	0.1856(3)	−0.4838(8)	0.0048(2)	0.0027(2)	0.0322(11)	0.0014(3)	0.0062(9)	−0.0043(7)
O(2′)	0.7472(3)	0.2183(3)	−0.5615(9)	0.0054(3)	0.0042(2)	0.0614(21)	0.0018(4)	−0.0061(12)	−0.0173(11)
O(3′)	0.6516(3)	0.3647(3)	−0.2230(9)	0.0043(3)	0.0037(2)	0.0494(16)	−0.0016(4)	0.0137(10)	−0.0117(9)
O(5′)	1.0280(3)	0.3412(2)	−0.3000(6)	0.0034(2)	0.0021(1)	0.0293(10)	0.0015(3)	0.0020(8)	−0.0026(7)
O(6′)	1.1910(3)	0.5153(3)	−0.2549(9)	0.0043(3)	0.0031(2)	0.0579(19)	−0.0005(4)	0.0060(11)	0.0069(10)

	x	y	z		x	y	z
H(C1)	0.813(5)	0.585(5)	0.210(10)	H(C5′)	0.979(4)	0.473(4)	−0.480(9)
H(C2)	0.670(5)	0.703(5)	−0.190(10)	H(C6′-1)	1.099(4)	0.458(4)	0.080(9)
H(C3)	0.721(5)	0.737(5)	0.360(10)	H(C6′-2)	1.040(6)	0.566(6)	−0.054(12)
H(C4)	0.510(4)	0.718(4)	0.416(9)	H(O2)	0.882(6)	0.788(6)	0.013(12)
H(C5)	0.632(5)	0.571(4)	0.444(10)	H(O3)	0.575(6)	0.865(6)	0.050(12)
H(C6-1)	0.399(6)	0.533(6)	0.157(12)	H(O4)	0.361(6)	0.735(7)	−0.025(13)
H(C6-2)	0.418(6)	0.545(5)	0.495(11)	H(O6)	0.535(6)	0.400(6)	0.492(13)
H(C1′)	0.940(6)	0.309(5)	−0.676(10)	H(O1′)	1.077(5)	0.191(5)	−0.588(11)
H(C2′)	0.832(6)	0.233(6)	−0.215(12)	H(O2′)	0.794(6)	0.171(5)	−0.645(11)
H(C3′)	0.754(6)	0.410(6)	−0.543(13)	H(O3′)	0.652(6)	0.439(6)	−0.093(12)
H(C4′)	0.852(5)	0.422(5)	0.018(10)	H(O6′)	1.220(6)	0.455(6)	−0.246(12)

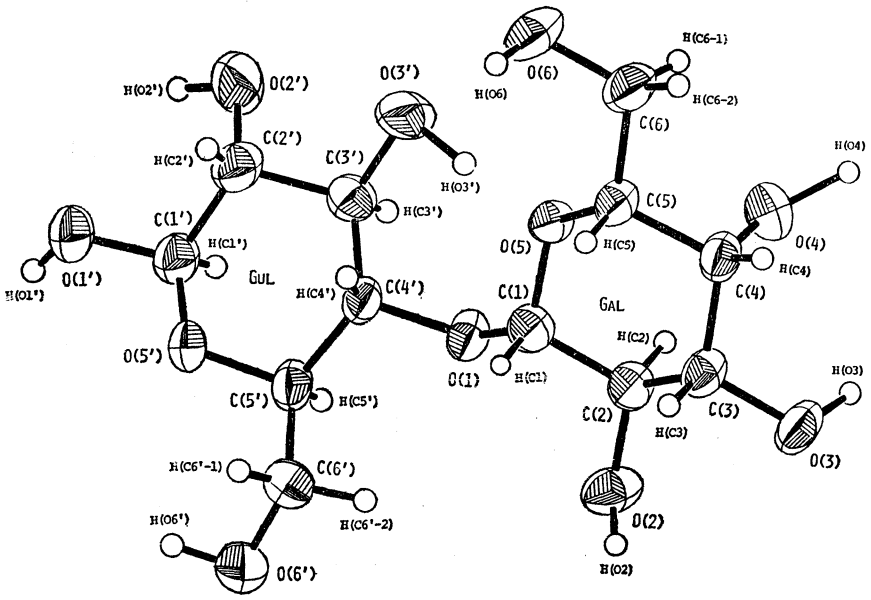


Fig. 1. Molecular conformation and atomic numbering in β-lactose.
The 74% probability thermal ellipsoids are shown for carbon and oxygen atoms.

the following weighting scheme was used:

$$w = 0.15 \text{ for } |F_o| < 1.4$$
$$w = (1.4/F_o)^2 \text{ for } |F_o| \geq 1.4.$$

The final atomic parameters are listed in Table 3. The atomic scattering factors used for the carbon and oxygen atoms were those of the International Tables for X-ray Crystallography,[14] and for the hydrogen atoms, those of Stewart, Davidson, and Simpson (1965).[15] The final structure factors are given in Table 4.*

After the completion of this work, this structure was found to be nearly isomorphous with β-cellobiose except for the atomic position of O(4).

Discussion

Figure 1 illustrates the conformation of the molecule of β-lactose, with the standard numbering for carbohydrates.

Bond Lengths and Angles. The bond lengths and angles involving carbon and oxygen atoms are shown in Fig. 2. The standard deviations are 0.005~0.006 Å for the carbon-carbon bonds and the carbon-oxygen bonds, and 0.3°~0.4° for the valence-bond angles. The bond lengths and angles in the β-galactosyl and β-glucosyl moieties agree well with the values previously reported by Berman, Chu, and Jeffrey.[16]

The carbon-carbon distances found in the unprimed ring (galactosyl moiety) range from 1.508 Å to 1.531 Å, with an average value of 1.524 Å, while those found in the primed ring (glucosyl moiety) range from 1.510 Å to 1.538 Å, with an average value of 1.530 Å. The average values of the corresponding ring C–C bonds in the four disaccharides listed in Table 5 range from 1.521 Å (C(1)–C(2)) to 1.533 Å (C(3')–C(4')) and are normal

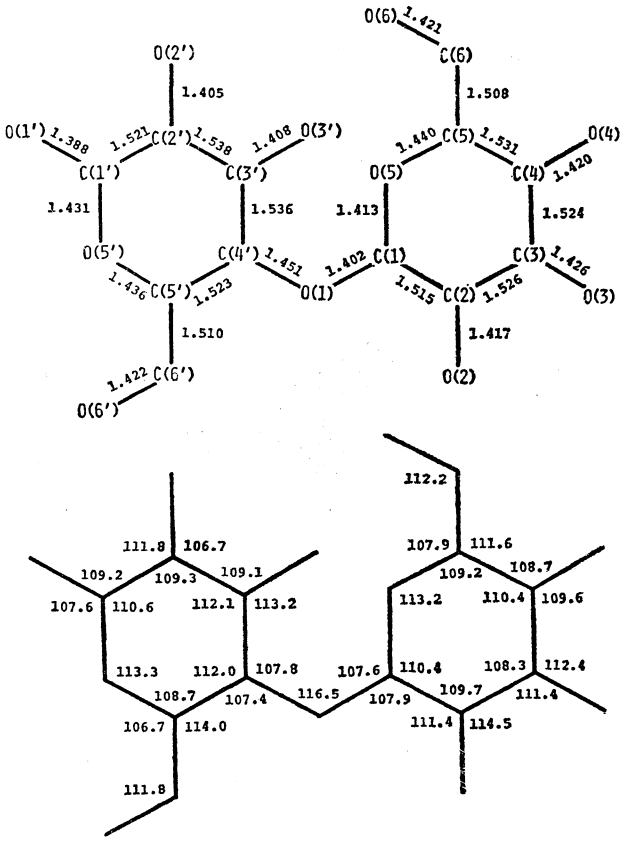


Fig. 2. Bond lengths(Å) and bond angles(°) in β-lactose.

values for carbohydrates, although C(1)–C(2) and C(1')–C(2') seem to be slightly short. The exocyclic C(5)–C(6) and C(5')–C(6') bonds are significantly shorter than the ring C–C bonds in all four disaccharides. The average value of the C(5)–C(6) bond is 1.514 Å, and that of C(5')–C(6') is 1.508 Å. This slight shortening with respect to the average ring C–C bonds has been

* Table 4 has been submitted to, and is kept by the office of the Chemical Society of Japan, 1-5 Kanda-Surugadai, Chiyoda-ku, Tokyo 113. (Document No. 7417).

pointed out by Ham and Williams.[6]

Two endocyclic C–O bonds [C(1)–O(5) and C(5)–O(5)] in the unprimed ring of β-lactose are found at 1.413 Å and 1.440 Å, the latter bond being about 5σ longer than the former. In the primed ring, the corresponding distances [C(1′)–O(5′) and C(5′)–O(5′)] are nearly equal: C(1′)–O(5′)=1.431 Å *versus* C(5′)–O(5′)=1.436 Å. The same trend was observed in β-cellobiose: C(1)–O(5)=1.425 Å *versus* C(5)–O(5)=1.436 Å and C(1′)–O(5′)=1.435 Å *versus* C(5′)–O(5′)=1.437 Å. However, the two endocyclic C–O bonds in methyl β-cellobioside[6] are nearly equal in the primed and the unprimed rings. In α-lactose,[2] the same pattern, C(1)–O(5)<C(5)–O(5), as in β-cellobiose and β-lactose was found in the unprimed ring, while the reverse trend, C(1′)–O(5′)<C(5′)–O(5′), was found in the primed ring. These differences and the greater variation of C–O bond lengths may be significant and may be associated with the type of hydrogen bond involved, but no correlation is apparent.

The exocyclic C–O bonds in β-lactose range from 1.405 Å to 1.426 Å, with a mean value of 1.417 Å, excluding the anomeric and bridge C–O bonds. The corresponding average lengths of α-lactose, β-cellobiose, and methyl β-cellobioside are 1.427 Å with a spead of 0.018 Å, 1.419 Å with a spread of 0.017 Å, and 1.428 Å with a spread of 0.030 Å, respectively. There are no significant differences among these average values. The means value of the C(2)–O(2) bonds in the four compounds listed in Table 5 is 1.419 Å. The corresponding average values of C(3)–O(3), C(4)–O(4), C(6)–O(6), C(2′)–O(2′), C(3′)–O(3′), and C(6′)–O(6′) are shown in the 7th column of Table 5. There are no significant differences among these average values.

The C(1)–O(1) and C(1′)–O(1′) bond lengths in the anomeric positions of β-lactose are significantly shorter, by 0.015 Å and by 0.030 Å respectively, than the mean exocyclic C–O length of 1.417 Å. This shortening effect has been found in disaccharides in Table 5 and many other related compounds. The C(1)–O(1) bonds in the unprimed rings of α-lactose, β-cellobiose, and methyl β-cellobioside are all greater than the C(1′)–O(1′) bonds in the primed rings, as is shown in Table 5. The bridge C(4′)–O(1) bond distance (1.451 Å) of β-lactose is in good agreement with the corresponding length (1.446 Å) in β-cellobiose. The average C(4′)–O(1) bond length of four disaccharides in Table 5 is 1.443 Å, significantly longer than the normal C–O distance for carbohydrates.

The bond angles of four disaccharides with (β-1,4) linkage have been tabulated in Table 6. The average carbon valence-bond angle within the rings of β-lactose is 110.1° with a spread of 3.8°. The average deviations from the corresponding angles in β-cellobiose, α-lactose, and methyl β-cellobioside are 1.0°, 1.2°, and 2.2° respectively. The C–O–C ring angles are the largest among the ring angles in β-lactose, α-lactose, and β-cellobiose, but not in the primed ring of methyl

Table 5. Bond distances of four accurately determined disaccharides with (β-1,4) linkage

	β-Lactose	α-Lactose[2]	Cellobiose[5]	Methyl β-cellobioside[6]	Mean	Spread
C(1)-C(2)	1.515 Å	1.517 Å	1.525 Å	1.526 Å	1.521 Å	0.011 Å
C(2)-C(3)	1.526	1.523	1.520	1.535	1.526	0.015
C(3)-C(4)	1.524	1.533	1.543	1.528	1.532	0.019
C(4)-C(5)	1.531	1.536	1.532	1.530	1.532	0.006
C(5)-C(6)	1.508	1.514	1.519	1.515	1.514	0.011
C(1′)-C(2′)	1.521	1.531	1.514	1.513	1.520	0.018
C(2′)-C(3′)	1.538	1.516	1.519	1·529	1.526	0.022
C(3′)-C(4′)	1.536	1.533	1.530	1.533	1.533	0.006
C(4′)-C(5′)	1.523	1.525	1.527	1.526	1.525	0.004
C(5′)-C(6′)	1.510	1.514	1.501	1.505	1.508	0.013
C(1)-O(5)	1.413	1.427	1.425	1.432	1.424	0.019
C(5)-O(5)	1.440	1.448	1.436	1.430	1.439	0.018
C(1′)-O(5′)	1.431	1.443	1.435	1.434	1.436	0.012
C(5′)-O(5′)	1.436	1.425	1.437	1.432	1.433	0.012
C(2)-O(2)	1.417	1.425	1.416	1.416	1.419	0.009
C(3)-O(3)	1.426	1.433	1.427	1.431	1.429	0.007
C(4)-O(4)	1.420[a]	1.421[a]	1.420	1.410	1.415	0.011
C(6)-O(6)	1.421	1.419	1.416	1.434	1.423	0.018
C(2′)-O(2′)	1.405	1.429	1.423	1.439	1.424	0.034
C(3′)-O(3′)	1.408	1.434	1.410	1.429	1.420	0.026
C(6′)-O(6′)	1.422	1.430	1.423	1.440	1.429	0.018
C(1)-O(1)	1.402	1.389	1.397	1.390	1.396	0.013
C(1′)-O(1′)	1.388	1.387[a]	1.381	1.379	1.383	0.009
C(4′)-O(1)	1.451	1.437	1.446	1.437	1.443	0.014
e.s.d.	0.005~0.006	0.003~0.005	0.004~0.005	0.005~0.006		

a) This value was not included in the calculation of the mean value, since the oxygen atom is in an axial position.

β-cellobioside.

In β-lactose, the exocyclic angles range widely from 106.7° to 114.5°, with an average value of 110.0°, excluding the valence-bond angle at the glycosidic oxygen atom. The average difference in the corresponding exocyclic bond angles between β-lactose and β-cellobiose is 0.8°, which is significantly small compared with the other two average differences (1.6° and 1.8°) between β-lactose and α-lactose, and between β-lactose and methyl β-cellobioside. This may be because β-lactose is nearly isostructural with β-cellobiose. The exocyclic bond angles are strongly affected by intermolecular forces, especially by the formation of hydrogen bonds. The larger differences in the average values found between β-lactose and α-lactose, and between β-lactose and methyl β-cellobioside, are caused by the different molecular packing, *i.e.*, the different hydrogen-bonding scheme.

The bond angle of 116.5° at the bridge oxygen atom of β-lactose agrees with the corresponding angles found in the crystal structures of β-cellobiose (116.1°), α-lactose (117.1°), methyl β-cellobioside (115.8°), lactose–calcium bromide heptahydrate (115.6°),[17] and lactose–calcium chloride heptahydrate (116.0°).[18]

Molecular Conformation. The conformational angles in the pyranose rings of β-lactose, α-lactose, β-cellobiose, and methyl β-cellobioside are listed in Table 7. The torsional angles in the galactose unit of β-lactose range only from 55.9° to 61.2°, with an average value of 58.2°. On the other hand, those of the glucose unit range more widely, from 48.9° to 65.0°, with a mean value of 55.7°. In the four disaccharides listed in Table 7, the torsional angles around the ring C–O bonds are generally the largest, while those around C(2)–C(3), C(3)–C(4), C(2′)–C(3′), and C(3′)–C(4′), which are at the position opposite to the ring C–O

Table 6. Bond angles of four accurately determined disaccharides with (β-1,4) linkage

	β-Lactose	α-Lactose[2]	Cellobiose[5]	Methyl β-cellobioside[6]	Mean	Spread
C(1)-C(2)-C(3)	109.7°	110.9°	108.3°	108.8°	109.4°	2.6°
C(2)-C(3)-C(4)	108.3	110.9	109.5	113.9	110.7	5.6
C(3)-C(4)-C(5)	110.4	108.9	111.0	108.5	109.7	2.5
C(4)-C(5)-O(5)	109.2	110.5	110.3	110.3	109.8	1.3
O(5)-C(1)-C(2)	110.4	111.2	108.3	107.4	109.3	3.8
C(1′)-C(2′)-C(3′)	109.3	110.9	110.0	113.6	111.0	4.3
C(2′)-C(3′)-C(4′)	112.1	110.3	111.8	111.8	111.5	1.8
C(3′)-C(4′)-C(5′)	112.0	111.1	112.3	109.1	111.1	3.2
C(4′)-C(5′)-O(5′)	108.7	107.9	109.2	108.3	108.5	1.3
O(5′)-C(1′)-C(2′)	110.6	109.7	109.3	108.7	109.6	1.9
C(5)-O(5)-C(1)	113.2	112.2	112.4	111.1	112.2	2.1
C(5′)-O(5′)-C(1′)	113.3	114.1	113.5	111.3	113.1	2.8
O(5)-C(1)-O(1)	107.6	107.0	107.4	107.6	107.4	0.6
C(2)-C(1)-O(1)	107.9	107.7	109.0	110.4	108.8	2.7
C(1)-C(2)-O(2)	111.4	108.8	110.0	111.0	110.3	2.6
C(3)-C(2)-O(2)	114.5	110.5	113.6	109.1	111.9	5.4
C(2)-C(3)-O(3)	111.4	108.1	112.0	110.3	110.5	3.9
C(4)-C(3)-O(3)	112.4	111.4	111.5	109.2	111.1	3.2
C(3)-C(4)-O(4)	109.6[a]	110.0[a]	108.1	110.2	109.2	2.1
C(5)-C(4)-O(4)	108.7[a]	108.4[a]	109.4	107.8	108.6	1.6
C(4)-C(5)-C(6)	111.6	112.0	111.0	112.0	111.7	1.0
O(5)-C(5)-C(6)	107.9	106.8	105.4	106.8	106.7	2.5
C(5)-C(6)-O(6)	112.2	110.3	112.2	111.0	111.4	1.9
O(5′)-C(1′)-O(1′)	107.6	111.5[a]	107.0	108.3	107.6	1.3
C(2′)-C(1′)-O(1′)	109.2	108.8[a]	110.2	107.0	108.8	3.2
C(1′)-C(2′)-O(2′)	111.8	111.1	110.6	107.2	110.2	4.6
C(3′)-C(2′)-O(2′)	106.7	112.7	106.5	110.3	109.1	6.2
C(2′)-C(3′)-O(3′)	109.1	107.0	107.1	105.0	107.1	4.1
C(4′)-C(3′)-O(3′)	113.2	111.6	112.5	112.5	112.5	1.6
C(3′)-C(4′)-O(1)	107.8	110.6	109.0	111.5	109.7	3.7
C(5′)-C(4′)-O(1)	107.4	107.0	106.4	106.7	106.9	1.0
C(4′)-C(5′)-C(6′)	114.0	113.7	113.6	114.9	114.1	1.3
O(5′)-C(5′)-C(6′)	106.7	107.2	106.4	108.3	107.2	1.9
C(5′)-C(6′)-O(6′)	111.8	111.2	111.2	110.6	111.2	1.2
C(1)-O(1)-C(4′)	116.5	117.1	116.1	115.8	116.4	1.3
e.s.d.	0.3∼0.4	∼0.2	0.2∼0.3	0.3∼0.4		

a) This value was not involved in the calculation of the mean value, since the oxygen atom is in an axial position.

Table 7. A comparison of the ring torsional angles in β-lactose, α-lactose, β-cellobiose and methyl β-cellobioside

	(I)	(II)	(III)	(IV)	Mean		(I)	(II)	(III)	(IV)	Mean
C(1)→C(2)	58.8°	53.7°	63.5°	58.7°	58.7°	C(1')→C(2')	55.8°	53.8°	57.8°	51.0°	54.6°
C(2)→C(3)	−56.7	−51.4	−57.3	−51.0	−54.1	C(2')→C(3')	−48.9	−51.2	−50.7	−45.0	−49.0
C(3)→C(4)	55.9	54.2	52.0	47.7	52.5	C(3')→C(4')	49.1	53.5	48.0	48.2	49.7
C(4)→C(5)	−56.8	−59.1	−51.9	−53.9	−55.4	C(4')→C(5')	−53.4	−57.5	−51.1	−59.9	−55.5
C(5)→O(5)	59.8	61.7	59.8	67.4	62.2	C(5')→O(5')	62.1	62.9	60.9	70.1	64.0
O(5)→C(1)	−61.2	−60.8	−65.7	−69.1	−64.2	O(5')→C(1')	−65.0	−61.6	−65.1	−64.3	−64.0

(I) β-Lactose (this work). (II) α-Lactose.[2] (III) β-Cellobiose.[5] (IV) Methyl β-cellobioside.[6]

Table 8. Exocyclic torsional angles

O(5)-[C(5)-C(6)]-O(6)	50.2°	O(5')-[C(5')-C(6')]-O(6')	71.9°
C(4)-[C(5)-C(6)]-O(6)	−170.8	C(4')-[C(5')-C(6')]-O(6')	−168.0
O(1)-[C(1)-C(2)]-O(2)	−56.4	O(1')-[C(1')-C(2')]-O(2')	−69.0
O(2)-[C(2)-C(3)]-O(3)	53.8	O(2')-[C(2')-C(3')]-O(3')	64.6
O(3)-[C(3)-C(4)]-O(4)	60.0	O(3')-[C(3')-C(4')]-O(1)	−69.4
O(4)-[C(4)-C(5)]-C(6)	−56.4	O(1)-[C(4')-C(5')]-C(6')	69.6

Table 9. Torsional angles[a] and pseudotorsional angles of the (β-1,4) linkage in the six determined disaccharides

Pseudotorsional angle is defined similarly to torsional angle, except that a line between C(1)---C(4') is used in place of a bond.

		(I)	(II)	(III)	(IV)	(V)	(VI)
Ψ_1	O(5)-C(1)-O(1)-C(4')	−76.3°	−70.7°	−92.6°	−91.1°	−77.0°	−76.0°
Ψ_1'	C(2)-C(1)-O(1)-C(4')	166.5	170.3	146.2	152.1	164.4	165.2
Ψ_2	C(1)-O(1)-C(4')-C(3')	106.4	180.0	94.6	80.3	106.5	108.3
Ψ_2'	C(1)-O(1)-C(4')-C(5')	−132.3	−131.3	−143.0	−160.7	−136.7	−134.9
	O(5)-C(1)---C(4')-C(3')	24.5	31.0	−0.2	−10.7	23.6	25.5
	O(5)-C(1)---C(4')-C(5')	171.1	176.9	144.5	125.9	166.9	169.8
	C(2)-C(1)---C(4')-C(3')	−106.4	−98.6	−144.7	−150.4	−109.9	−107.8
	C(2)-C(1)---C(4')-C(5')	40.2	47.3	2.6	13.8	33.4	36.5

(I) β-Cellobiose.[5] (II) β-Lactose (this paper). (III) α-Lactose.[2] (IV) Methyl β-cellobioside.[6] (V) Lactose-calcium bromide heptahydrate.[17] (VI) Lactose-calcium chloride heptahydrate.[18] a) Ψ_1, Ψ_1', Ψ_2 and Ψ_2' are defined according to Sundaralingam (1968).[20]

bonds, are the smallest, although there are some exceptions. This trend may be due to the smaller puckering around the C(2)–C(3) and C(3)–C(4) bonds and the larger puckering around the C(5)–O(5) and C(1)–O(5) bonds. The torsional angles involving exocyclic atoms in β-lactose are shown in Table 8. The exocyclic torsional angles around C(1')–C(2'), C(2')–C(3'), C(3')–C(4'), and C(4')–C(5') in the β-glucose residue of β-lactose are considerably larger than those in the galactose residue.

Both C(5)–C(6) and C(5')–C(6') bonds have the+ *synclinal* conformation.

The conformational twists about the C(1)–O(1) and C(4')–O(1) bonds are of interest in comparison with the models for cellulose. The torsional and pseudotorsional angles[19] of the (β-1,4) linkage in the five determined structures and the present one are given in Table 9. Because of the isomorphous structures, the torsional and pseudotorsional angles of β-lactose are approximately equal to those of β-cellobiose. These two molecules exhibit an asymmetrical twist about the bridge bond, $\Psi_1=-70.7°$ and $\Psi_2=108.0°$ in β-lactose, and $\Psi_1=-76.3°$ and $\Psi_2=106.4°$[20] in β-cellobiose. The two isostructural lactose complexes, lactose–calcium bromide heptahydrate and lactose–calcium chloride heptahydrate, also exhibit an asymmetrical twist. The conformations around the bridge bonds of these two complexes are very similar to those of β-lactose and β-cellobiose, as is shown in Table 9. In contrast to the above four structures, a symmetrical twist about the bridge bond is found in α-lactose: $\Psi_1=-92.6°$ and $\Psi_2=94.6°$. In methyl β-cellobioside, the conformation around the bridge bond is close to that of α-lactose, but it is not symmetrical. The conformations about the bridge bonds in disaccharides with (β-1,4) linkage are affected by the intermolecular forces in the crystal, especially hydrogen bonding, but, roughly speaking, the same conformation is maintained. This similarity in conformation about the bridge bond is caused by the fact that, in these molecules, the intramolecular hydrogen bond, O(3')···O(5), is always formed and plays an important role in determining the molecular conformation.

Hydrogen Bonding. The molecular packing and

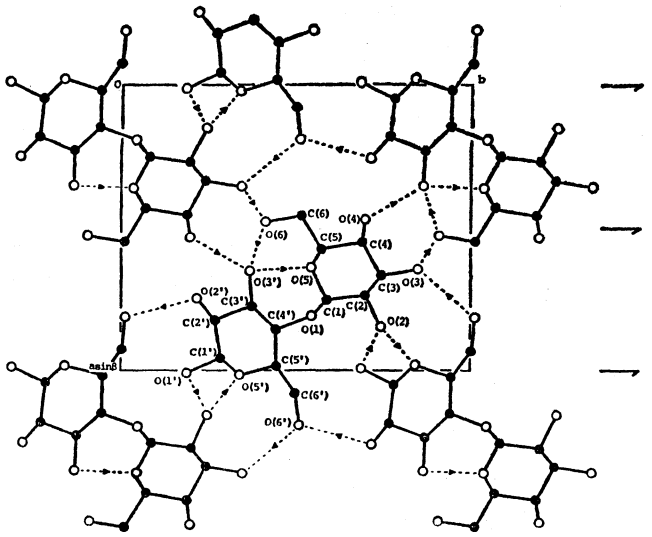


Fig. 3. Packing of the structure viewed down the *c* axis.

Table 10. Hydrogen bonding distances and angles

i	j	k	*d*(jk)	∠(ijk)
C(2)	O(2)	O(5, b)	2.731 Å	117.0°
C(3)	O(3)	O(6, c)	2.730	120.0
C(4)	O(4)	O(3′, c)	2.804	91.9
C(6)	O(6)	O(3′, d)	3.022	107.7
C(1′)	O(1′)	O(2, e)	2.722	90.3
C(2′)	O(2′)	O(6′, e)	2.941	120.3
C(3′)	O(3′)	O(5, a)	2.707	100.6
C(6′)	O(6′)	O(3, f)	2.873	110.3

Intermolecular non-bonded distances less than 3.3 Å between carbon and oxygen atoms

i	j	*d*(ij)
O(2)	C(1′, k)	3.052 Å
O(1′)	C(6′, e)	3.296
O(3′)	C(4, i)	3.191

Symmetry code

The letters refer to the symmetry operations to be applied to the coordinates listed in Table 3.

a	x,	y,	z
b	$2-x$,	$1/2+y$,	$-z$
c	$1-x$,	$1/2+y$,	$-z$
d	x,	y,	$1+z$
e	$2-x$,	$-1/2+y$,	$-1-z$
f	$2-x$,	$-1/2+y$,	$-z$
g	$1-x$,	$1/2+y$,	$-1-z$
h	$-1+x$,	y,	z
i	$1-x$,	$-1/2+y$,	$-z$
j	$1-x$,	$-1/2+y$,	$1-z$
k	$2-x$,	$1/2+y$,	$-1-z$

the hydrogen-bonding scheme are shown in Figs. 3 and 4. The hydrogen-bonding distances and angles are listed in Table 10. The crystal structure of β-lactose is nearly isomorphous with β-cellobiose. However, the hydrogen-bonding scheme around the axial O(4) atom is different from that of β-cellobiose. When the equatorial O(4) atom in the crystalline β-cellobiose is assumed to be replaced by the axial O(4) atom and the intermolecular distances are subsequently calculated, two hydrogen bonds, O(6)···O(4) and O(4)···O(2′), are missing and only one short contact, O(4)···O(3′), of 2.30 Å is found. This short contact can be lengthened to correspond to the hydrogen-bond length by slight changes in the atomic positions, and one more hydrogen bond can be formed between O(6) and O(3′), as is shown in Figs. 3 and 4. Therefore, crystalline β-lactose is isomorphous with β-cellobiose. β-Epicellobiose (*O*-β-D-glucopyranosyl-(1→4)-β-D-mannopyranoside) is different from β-cellobiose only in that the O(2′) atom is in an axial position. In a similar manner, the equatorial oxygen atom O(2′) in the crystalline β-cellobiose is assumed to be replaced by the axial O(2′). As a result, one short contact of 3.18 Å is found between O(2′) and O(6′). Although two hydrogen bonds, O(2′)–H···O(6′) and O(4)–H···O(2′), are missing, there appear to be two compensating hydrogen bonds, which are perhaps formed between axial O(2′) and O(6′) and between O(4) and O(3′) by slight changes

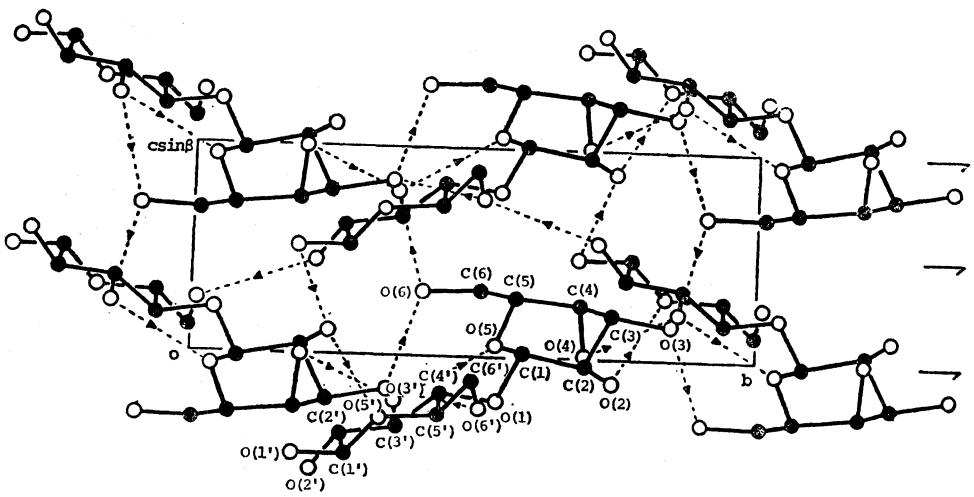


Fig. 4. Packing of the structure viewed down the *a* axis.

in atomic positions. Hence, crystalline anhydrous β-epi-cellobiose, as well as β-lactose, might be isomorphous with β-cellobiose.

Each of four hydroxyl groups, O(2)–H, O(3)–H, O(6)–H, and O(6′)–H, participates in hydrogen bonds as both one donor and one acceptor. O(4)–H, O(1′)–H, and O(2′)–H serve as donors only. O(3′)–H serves as one donor and two acceptors. Two ring oxygens, O(5) and O(5′), take part in hydrogen bonds as acceptors. The glycosidic linkage oxygen atom is not involved in hydrogen bonds, just as in all crystal structures of di- and tri-saccharides. The hydrogen-bond system comprises two terminated chains:

$$\begin{aligned} &O(2',g) \rightarrow O(6',h) \rightarrow O(3,i) \rightarrow \\ &\quad O(6,a) \rightarrow O(3',d) \rightarrow O(5,d) \\ &\qquad\qquad \uparrow \\ &\qquad\qquad O(4,j) \end{aligned}$$

$$O(1',d) \rightarrow O(2,f) \rightarrow O(5',a)$$

where→indicates the donor direction and where the small letter refers to the symmetry operations given in Table 10. The intramolecular hydrogen bond, O(3′)⋯O(5)=2.707 Å, is significantly short compared with that of 2.811 Å in α-lactose, that of 2.767 Å in cellobiose, that of 2.762 Å in methyl β-cellobioside, that of 2.76 Å in lactose–calcium bromide heptahydrate, and that of 2.75 Å in lactose–calcium chloride heptahydrate.

Computer Programs

All the calculations were performed on a FACOM 270/30 computer at the Computer Center of Osaka City University using the following programs: RSLC-3,[21] RSSFR-3,[21] HBLS-IV,[21] SCALE (film factor, Lp, and layer scaling),[22] DEAM,[23] PHASE-I, II, and III (direct method),[24] and BOND (bond length and angle, best plane, dihedral angle, and intermolecular distance).[25]

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