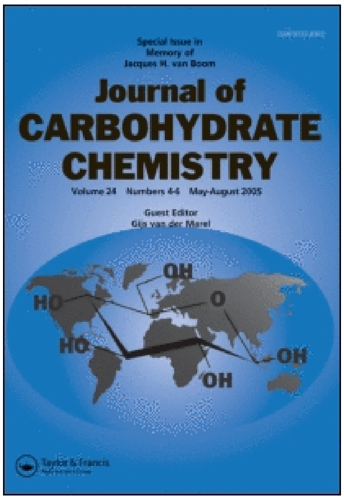

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Fujimoto, Takashi , Oku, Kazuyuki , Tashiro, Mitsuru and Machinami, Tomoya(2006) 'Crystal Structure of α,α-Trehalose-Calcium Chloride Monohydrate Complex', Journal of Carbohydrate Chemistry, 25: 7, 521 — 532
To link to this Article: DOI: 10.1080/07328300600966414
URL: http://dx.doi.org/10.1080/07328300600966414

Journal of Carbohydrate Chemistry, 25:521–532, 2006
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ISSN: 0732-8303 print 1532-2327 online
DOI: 10.1080/07328300600966414

Crystal Structure of α,α-Trehalose–Calcium Chloride Monohydrate Complex

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The crystal structure of α,α-trehalose hydrate complexed with calcium chloride was determined by X-ray crystallography and its structural comparison with the α,α-trehalose–$CaBr_2 \cdot H_2O$ complex was performed. The crystal system of the α,α-trehalose–$CaCl_2 \cdot H_2O$ complex, $C_{12}H_{22}O_{11} \cdot CaCl_2 \cdot H_2O$, is orthorhombic and $C222_1$ space group with $a = 10.8984(8)$, $b = 11.4260(6)$, and $c = 15.059(1)$Å. Although its coordination closely resembled that of the α,α-trehalose–$CaBr_2 \cdot H_2O$ complex published formerly assuming a pentagonal bipyramidal arrangement, a difference between two α,α-trehalose–halide complexes was observed along the c-axis in the infinite channels occupied by water molecules.

Received March 20, 2006; accepted May 1, 2006.

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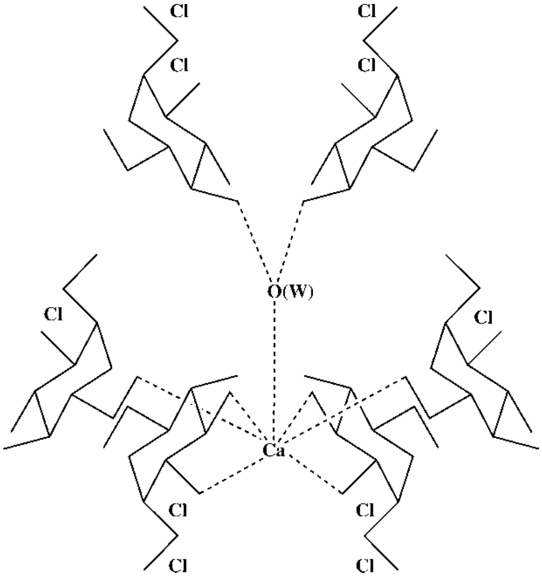

Keywords Crystal structure, α,α-Trehalose–$CaCl_2$complex, C_2 symmetry

INTRODUCTION

In metal coordination to carbohydrates, calcium is one of the most intensively studied alkali earth metals that can bind to carbohydrates. Carbohydrate–calcium complexes participate in a variety of biological processes and play important roles such as calcium storage, calcification, and calcium-dependent interactions in cells.[1] There are numerous crystalline adducts in stoichiometric proportion between calcium salts and carbohydrates.[2] In the various carbohydrates, sugar acids, which have carboxyls as anchoring groups for the primary coordination, can form much stronger complexes with calcium ions, and many structural studies in this field have been performed.[2–8] On the other hand, relatively few complexes of the neutral carbohydrate with calcium ions lacking anchor groups have been isolated and characterized by X-ray crystallography.[9] Although the detailed mechanism of the complex formation between monosaccharides and cations are becoming well understood, interactions between oligosaccharides and cations are still unclear and an extensive study of the neutral polysaccharide complexes is desired to elucidate the chemical and biological features of oligosaccharides.

In the study of the disaccharide–calcium interaction, the crystal structures of lactose–calcium halide complexes were determined to elucidate interactions between carbohydrates and calcium ions on the biological view point by Cook and Bugg.[9] They also presented the crystal structures of α,α-trehalose–calcium bromide complex and briefly described that the global conformation of α,α-trehalose in the bound state is quite similar to that of α,α-trehalose dihydrate. Interestingly, a crystal structure of α,α-trehalose dihydrate[10] was reported to exhibit an approximate, but not a perfect, C_2 symmetry, although the nonreducing disaccharide is well known as a symmetrical carbohydrate exemplifying an *exo*-anomeric effect.[11] X-ray diffraction and NMR analyses of the α,α-trehalose–$CaCl_2$ complexes were also reported.[12] In this study, the spin-lattice relaxation time (T_1) of all ^{13}C nuclei of the α,α-trehalose shortened in the presence of calcium ions. In order to achieve further understanding of the recognition of α,α-trehalose to calcium ions, another calcium halide, calcium chloride, was used to form the complex. In the present study, a crystal structure of the α,α-trehalose–$CaCl_2$ complex was determined by X-ray crystallography and the detailed structural comparison with the α,α-trehalose–$CaBr_2$ complex was performed. Although the crystal structure of α,α-trehalose–$CaBr_2$ was formerly determined by Cook and Bugg,[9] the reported structure was significantly different from that deposited in the CCDC database (CCDC refcode: TRECAB). Therefore, the unpublished revised coordinates of α,α-trehalose–$CaBr_2$ were used for the present structural analysis. The crystal structure of the α,α-trehalose dihydrate in the absence of calcium halide was also determined, and the structural detail in D-glucose moieties was analyzed. The complexation of α,α-trehalose with calcium chloride or calcium bromide was confirmed using the electrospray ionization mass spectrometry (ESI–MS).

RESULTS AND DISCUSSION

X-Ray Crystal Structures of α,α-Trehalose-Calcium Halide Complex

The crystal structure with atom numbering scheme is shown in Fig. 1, and crystal data and atomic coordinates of α,α-trehalose–$CaCl_2 \cdot H_2O$ are listed in Tables 1 and 2, respectively. The crystals are nearly isostructural with those of α,α-trehalose–$CaBr_2 \cdot H_2O$ reported by Cook and Bugg,[9] and the crystal system is orthorhombic and $C222_1$ space group with $Z = 4$. Slight differences are observed in cell lengths, a =10.8984(8), b = 11.4260(6), and c = 15.059(1) Å for calcium chloride complex, and a = 11.058(1), b = 11.573(1), and c = 15.101(1) Å for calcium bromide complex.[9]

The crystal packing of α,α-trehalose–$CaCl_2 \cdot H_2O$ was compared with that of α,α-trehalose–$CaBr_2 \cdot H_2O$ as shown in Figs. 1, 2, and 3. In each complex, a calcium ion is coordinated to the water molecule and to four symmetry-related D-glucose moieties (Fig. 1).[9] Two α,α-trehalose molecules chelate the calcium

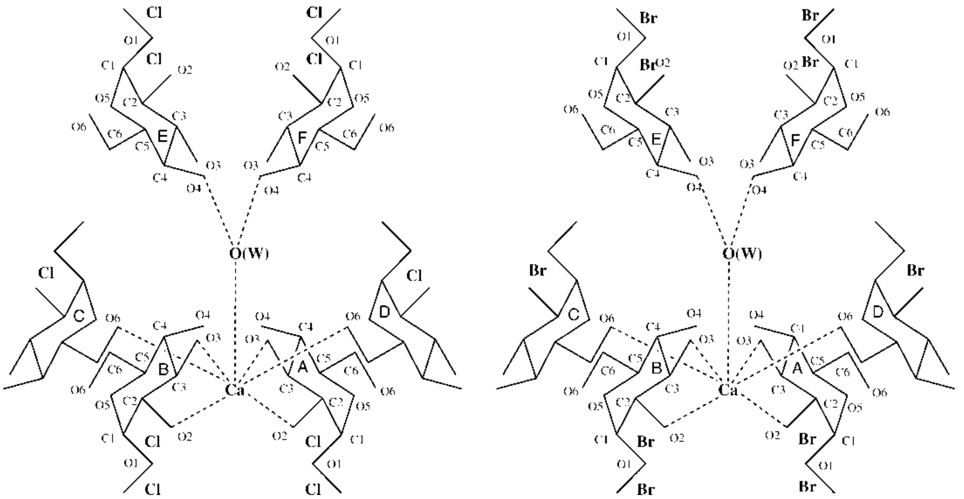

Figure 1: The crystal structures with atom numbering scheme of α,α-trehalose–$CaCl_2 \cdot H_2O$ (left, CCDC 191059) and α,α-trehalose–$CaBr_2 \cdot H_2O$ (right, CCDC refcode: TRECAB) as viewed down the *c*-axis.

Table 1: Crystal data and structure refinement of α,α-trehalose–$CaCl_2 \cdot H_2O$.

Formula weight	471.30
Temperature (K)	296
Wavelength (Å)	0.71069
Crystal system	Orthorhombic
Space group	$C222_1$
Unit cell dimensions	
a (Å)	10.8984(8)
b (Å)	11.4260(6)
c (Å)	15.059(1)
V (Å^3)	1875.3(2)
Z	4
D calcd (g/cm^3)	1.669
F(000)	984
Crystal size (mm)	0.5 × 0.4 × 0.4
θ range for data collection (°)	2.7–60.0
Index ranges	$-15 \leq h \leq 15$ $-16 \leq k \leq 14$ $-21 \leq l \leq 18$
Reflections collected/unique	10517/1559 ($R_{int} = 0.030$)
Refinement method	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	1.34
Final R indices	$R_1 = 0.048$ R; Rw = 0.136; 0.137
CCDC deposition number	CCDC191059

Table 2: Atomic coordinates of α,α-trehalose–$CaCl_2 \cdot H_2O$[a]

Atom	X	Y	Z
Ca(1)	0.20585(7)	0.0000	0.5000
Cl(1)	0.07135(8)	0.37525(8)	0.39213(7)
O(1)	0.0000	0.3618(2)	0.7500
O(2)	0.1672(2)	0.2487(2)	0.7077(1)
O(3)	0.1246(2)	0.4163(2)	0.9132(1)
O(4)	0.3297(2)	0.5189(2)	0.8394(1)
O(5)	0.3306(2)	0.5177(2)	0.6398(1)
O(6)	−0.0066(3)	0.0000	0.5000
O(7)	0.1830(2)	0.2050(2)	0.5241(1)
C(1)	0.1834(2)	0.3697(2)	0.8357(2)
C(2)	0.2111(3)	0.3382(2)	0.6475(2)
C(3)	0.2989(2)	0.4212(2)	0.6959(2)
C(4)	0.2404(2)	0.4665(2)	0.7814(2)
C(5)	0.2709(3)	0.2747(3)	0.5708(2)
C(6)	0.0995(2)	0.2937(2)	0.7803(2)

[a] Half of the coordinates are listed since the crystal structure of α,α-trehalose was symmetric.

ion through the O-2 and O-3 pairs of hydroxyl group, and the other two α,α-trehalose molecules chelate calcium ion through O-6 hydroxyl groups. These seven oxygen atoms complete a pseudopentagonal bipyramidal arrangement around the calcium ion, which is almost identical to that of calcium bromide heptahydrate–lactose complexes. The molecular orbital calculation suggested that calcium ion was coordinated to O-2 and O-3 of one side of the D-glucose residues,[12] which coincides with the crystal structures of α,α-trehalose–$CaCl_2 \cdot H_2O$ and α,α-trehalose–$CaBr_2 \cdot H_2O$. The Ca-Ot (oxygen atoms of α,α-trehalose hydroxyls) distances in α,α-trehalose–$CaCl_2 \cdot H_2O$ and α,α-trehalose–$CaBr_2 \cdot H_2O$ are 2.383 and 2.358 Å for 6-OHs, 2.457 and 2.477 Å for 2-OHs, 2.459 and 2.456 Å for 3-OHs, and 2.761 and 2.808 Å for 4-OHs, respectively. The Ca-Ow (a oxygen atom of a water molecule) distances are 2.315 and 2.323 Å, and the 4-OH-Ow distances are 2.761 and 2.808 Å in the aforesaid order. These results indicate that the overall Ca-O contacts in α,α-trehalose–$CaCl_2 \cdot H_2O$ are slightly tighter than those in α,α-trehalose–$CaBr_2 \cdot H_2O$.[9] The shortest distance between the calcium–glycosidic oxygen (O1 of molecules A and B as shown in Fig. 1) is 5.191 Å, indicating that the calcium ion locates between 2-OH and 3-OH. Considering the location of the calcium ion (Fig. 1), the glycosidic oxygen does not coordinate to the calcium ion coordinating to 2-OHs, 3-OHs, and 6-OHs, but coordinate to another calcium ion. It should be noted that the Ca-Ow distance in α,α-trehalose–$CaCl_2 \cdot H_2O$, 2.315 Å, is almost the same distance as the sum of the ionic radius of calcium and van del Waals' radius of oxygen. This distance is much

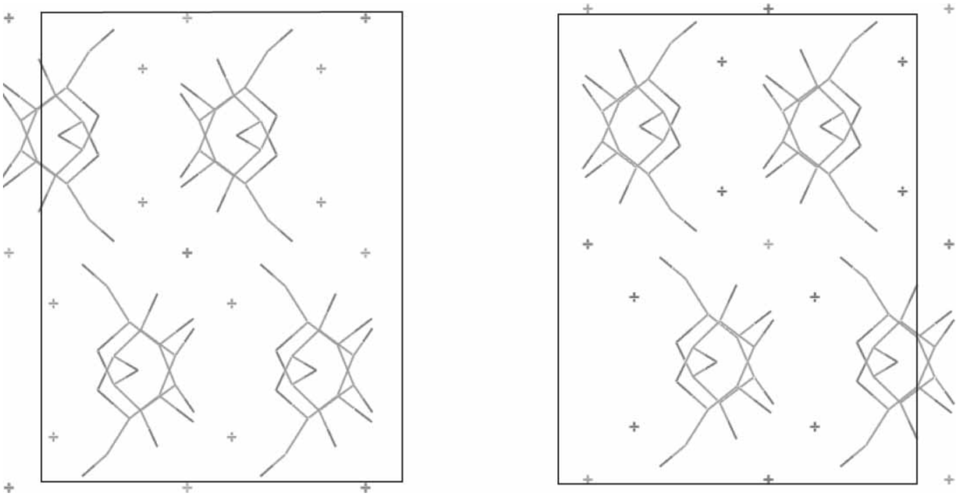

Figure 2: The crystal structures of α,α-trehalose–$CaCl_2 \cdot H_2O$ (left) and α,α-trehalose–$CaBr_2 \cdot H_2O$ (right) as viewed down the *a*-axis.

shorter than the closest Ca-O distance, 3.352 Å, calculated in the calcium chloride tetrahydrate complex.[11] The shortest calcium-chloride distance is 4.532 Å, which is 1.73Å longer than the sum of the ionic radii of calcium and chloride ions, indicating that the chloride ions do not participate in the coordination to the calcium ion.

Although the overall crystal packing of α,α-trehalose is very similar between the two complexes as viewed down the a- and c-axes (Figs. 1 and 2), some differences are observed in the infinite channels occupied by water molecules along the c-axis (Fig. 3). All water molecules in α,α-trehalose–$CaBr_2 \cdot H_2O$ were positioned in linier along the c-axis in the coordination shell, whereas the water molecules in α,α-trehalose–$CaCl_2 \cdot H_2O$ are slightly off the center of the infinite channel in zig-zag fashion, and the channels of α,α-trehalose–$CaCl_2 \cdot H_2O$ are obviously wider than those of α,α-trehalose–$CaBr_2 \cdot H_2O$ (Fig. 3).

Conformational Torsion Angles

Selected bond lengths and bond angles of α,α-trehalose–$CaCl_2 \cdot H_2O$ and α,α-trehalose dihydrate are listed in Tables 3 and 4, respectively, and conformational torsion angles of them are compared in Table 5. As depicted in Fig. 4, each D-glucose residue is positioned symmetric in α,α-trehalose–$CaCl_2 \cdot H_2O$, whereas it is positioned asymmetric in α,α-trehalose dihydrate. The largest difference in the torsion angles for the C-1–O-1 glycosidic bonds of the D-glucose residues is 14 to 16°. It is worthy to note that the α,α-trehalose molecule in the calcium chloride complex has perfect C_2 symmetry

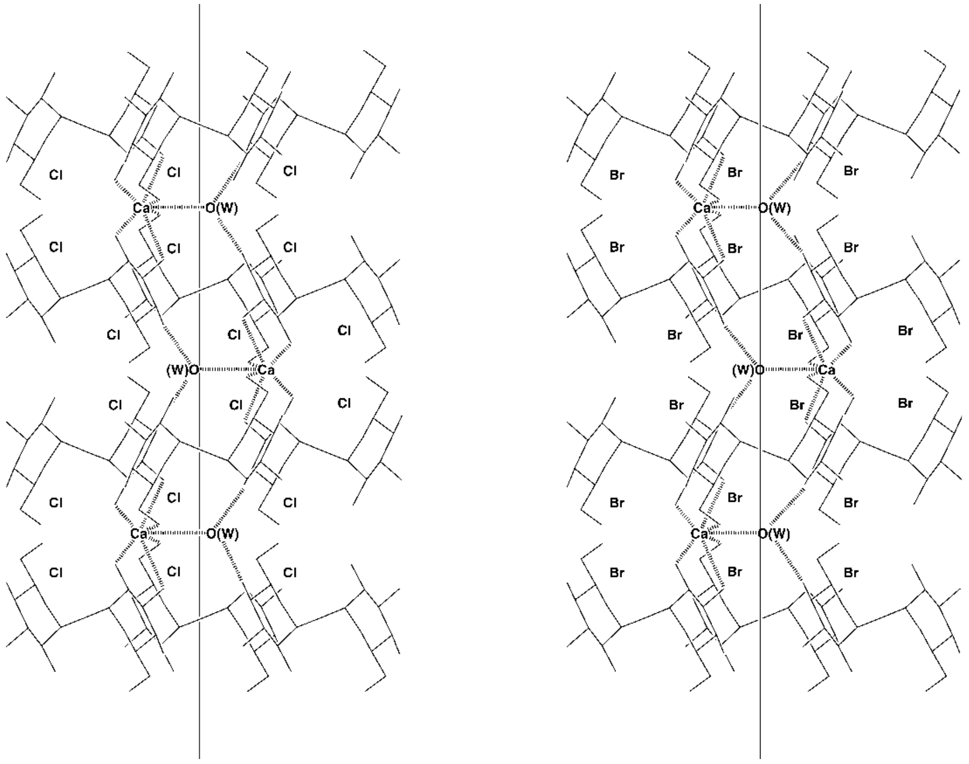

Figure 3: The crystal structures with atom numbering scheme of α,α-trehalose–$CaCl_2 \cdot H_2O$ (left) and α,α-trehalose–$CaBr_2 \cdot H_2O$ (right) as viewed down the *b*-axis. In each crystal packing drawing, the solid line is illustrated to show the central position of the infinite channel.

Table 3: Bond lengths (Å) of α,α-trehalose–$CaCl_2 \cdot H_2O$ and α,α-trehalose dihydrate.

Bonds	**$CaCl_2$**	**Dihydrate**[a]	
O1-C1	1.411	1.422	1.411
O1-C1	1.411	1.533	1.530
C2-O2	1.434	1.426	1.423
C2-C3	1.509	1.519	1.521
C3-O3	1.438	1.426	1.425
C3-C4	1.527	1.519	1.530
C4-O4	1.431	1.435	1.423
C4-C5	1.532	1.528	1.526
C5-O5	1.448	1.434	1.435
O5-C1	1.416	1.404	1.427
C5-C6	1.512	1.508	1.525
C6-O6	1.431	1.427	1.421

[a] An asymmetric form of D-glucose moieties was observed in α,α-trehalose dihydrate.

Table 4: Bond angles (°) of α,α-trehalose-$CaCl_2 \cdot H_2O$ and α,α-trehalose dihydrate.

Angles	$CaCl_2$	Dihydrate[a]	
O1-C1-C2	109.1	106.1	106.5
C1-C2-O2	113.1	110.6	111.4
C1-C2-C3	111.8	109.8	108.1
O2-C2-C3	110.7	110.8	109.7
C2-C3-O3	104.8	109.0	112.6
C2-C3-C4	112.4	111.4	109.1
O3-C3-C4	111.8	110.4	106.3
C3-C4-O4	109.7	109.6	111.8
C3-C4-C5	110.5	111.5	112.7
O4-C4-C5	110.3	109.1	105.3
C4-C5-O5	110.2	111.5	111.5
C4-C5-C6	113.0	111.2	112.3
C5-C6-O6	110.7	112.0	113.2
C6-C5-O5	106.4	106.9	106.6
C5-O5-C1	113.5	114.4	113.8
O5-C1-O1	110.6	111.4	112.1
O5-C1-C2	108.6	111.0	109.5

[a] An asymmetric form of D-glucose moieties was observed in α,α-trehalose dihydrate.

including the same orientation of the primary alcohol groups, both of which are *gauche/trans* configuration (O-5–C-5–C-6–O-6 = 63°). However, two different orientations are reported in the dihydrate structure,[10] as well as in the anhydrous structure of α,α-trehalose molecules,[13] with the primary alcohol groups,

Table 5: Comparison of torsion angles (°) of α,α-trehalose-$CaBr_2 \cdot H_2O$, α,α-trehalose-$CaCl_2 \cdot H_2O$, and α,α-trehalose dihydrate.

	$CaBr_2$	$CaCl_2$	Dihydrate[a]	
O5-C1-C2-C3	56.6	56.5	56.5	63.0
C1-C2-C3-C4	−51.2	−50.3	−52.0	−57.5
C2-C3-C4-C5	48.7	47.4	49.8	51.6
C3-C4-C5-O5	−52.5	−51.5	−50.5	−48.8
C4-C5-O5-C1	62.1	61.9	56.9	54.6
C5-O5-C1-C2	−63.1	−63.5	−60.0	−62.3
O1-C1-C2-O2	61.9	61.6	63.5	63.2
O2-C2-C3-O3	58.9	61.2	63.5	63.2
O3-C3-C4-O4	−71.1	−73.4	−68.1	−68.5
O4-C4-C5-C6	66.7	68.2	69.1	69.6
C4-C5-C6-O6	−178.1	−175.8	−168.1	46.2
O5-C5-C6-O6	60.6	63.1	70.0	−76.1
O5-C1-O1-C1	77.0	77.47	61.4	75.0
C2-C1-O1-C1	−163.6	−163.2	−177.7	−165.4

[a] An asymmetric form of D-glucose moieties was observed in α,α-trehalose dihydrate.

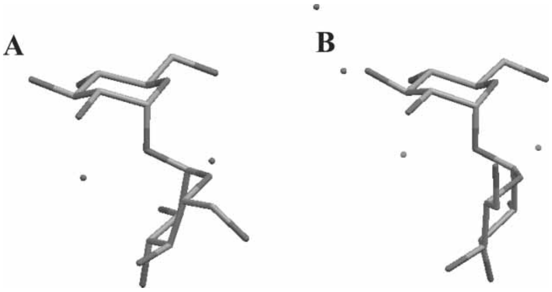

Figure 4: The crystal structures of (A) α,α-trehalose dihydrate and (B) α,α-trehalose–$CaCl_2 \cdot H_2O$ showing the difference of one of the D-glucose residue configuration. The hexose rings of the upper D-glucose moieties are shown in identical orientations in (A) and (B).

which are *gauche*/*gauche* on one residue and *gauche*/*trans* on the other. The conformation for the O-5–C-5–C-6–O-6 bond of each D-glucose moiety in the α,α-trehalose dihydrate is strikingly different for 146° (Table 5). Unlike the crystal structure of α,α-trehalose dihydrate, the complexation with calcium halide results in the stabilization of C_2 symmetry configuration of α,α-trehalose molecules. The torsion angles of the O-3–C-3–C-4–O-4 bond is $-73.4°$ in the calcium chloride complex and $-68.1°$ and $-68.5°$ in the asymmetric dihydrate structure (Table 5). This conformational difference indicates that the significant strain should be put in the portion of the atoms O-3 through O-4 in the calcium chloride complex.

The exceptional difference in the orientation of the primary alcohol groups is also reported in the anhydrous structure.[13] Although intraring torsion angles are very similar to those for one of the D-glucose moieties in the dehydrate structure, the conformation of the C-5–C-6 bond considerably differs by 7 to 8° based on the calculation using the coordinates in the CCDC database deposited by Cook and Bugg (refcode: TRECAB). They described only 2 to 6° differences for the glycosidic torsion angles and an agreement for the conformation of the C-5–C-6 bond.[9] However, our present analysis provides different interpretation for the conformation of the structures, indicating that some revision was made in their crystal structure, which was deposited in the CCDC database. In the present paper, interpretation of the crystal structures including all figures was carried out using the coordinates deposited in the CCDC database.

Electrospray Ionization Mass Spectrometry

The complex formations of α,α-trehalose in aqueous calcium halide solutions were analyzed using the electrospray ionization mass spectrometry (ESI–MS) as shown in Fig. 5. The ion peaks for the calcium chloride solution were observed at m/z 365.2, 723.3, and 759.3, corresponding to $[M+Na]^+$

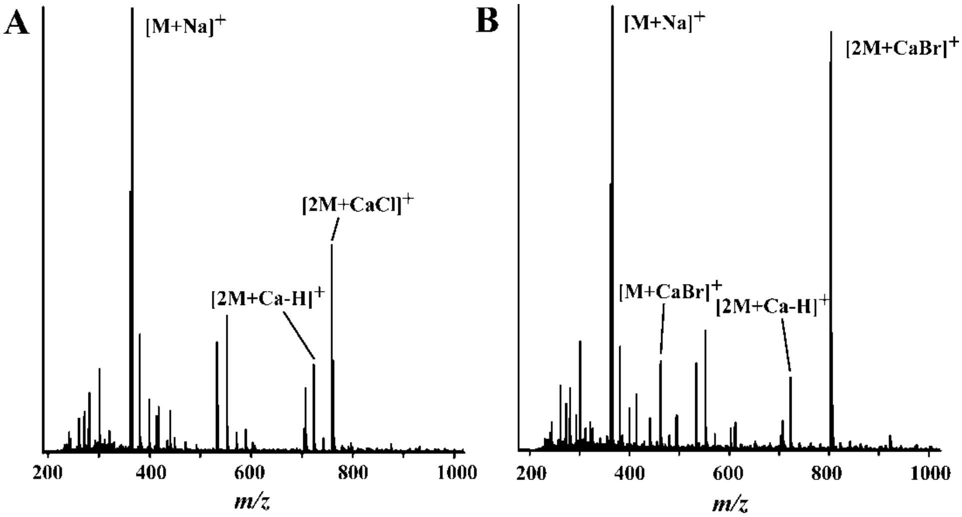

Figure 5: ESI-MS spectra of α,α-trehalose in the (A) calcium chloride solution and (B) calcium bromide solution.

(theoretical 365.3), $[2M + Ca - H]^+$ (theoretical 723.7) and $[2M + CaCl]^+$ (theoretical 759.6), respectively. The ion peaks for the calcium bromide solution were observed at m/z 365.2, 461.1/463.1, 723.3, and 803.2/805.2, corresponding to $[M + Na]^+$ (theoretical 365.3), $[M + CaBr]^+$ (theoretical 461.3/463.3), $[2M + Ca - H]^+$ (theoretical 723.7), and $[2M + CaBr]^+$ (theoretical 803.6/805.6), respectively. In the past study, ESI–MS was used to confirm the complex formation of 1,6-anhydro-β-maltose with rubidium ions and that of 1,6-anhydro-β-D-glucopyranose with rubidium ions.[14] These results demonstrate that ESI–MS technique is capable of providing reliable information to characterize complex formations of saccharides and cations.

EXPERIMENTAL

Preparation of α,α-Trehalose Complexes

α,α-Trehalose was kindly provided by Hayashibara Biochemical Lab. Inc., (Okayama, Japan) and calcium chloride was purchased from Wako (Osaka, Japan). α,α-Trehalose dihydrate (18.9 g, 0.05 mol) and $CaCl_2 \cdot 2H_2O$ (14.7 g, 1 mol) were added to water (50 g), and the suspension was heated to reflux to afford a concentrated solution. Gradual air drying of the solution at 60°C for a month allowed crystallization. Anal. Calcd. for $C_{12}H_{22}O_{11} \cdot CaCl_2 \cdot H_2O$: C, 30.58; H, 5.13. Found: C, 30.21; H, 5.33. Atomic absorption spectrometry, Calcd: Ca, 8.50. Found: 8.46. Ion chromatography, Calcd: Cl, 15.05. Found: Cl, 15.15.

Physical Measurement

The crystal structure of $C_{12}H_{22}O_{11} \cdot CaCl_2 \cdot H_2O$ was determined on a Rigaku R-AXIS RAPID IP diffractometer using monochromized Mo Kα radiation ($\lambda = 0.71069$Å) in the θ range from 1.4 to 30.0 at 296 K. The final cycle of full-matrix least-square refinement was based on 1548 observed reflections. Calculations were completed using the program SIR92. The conformational analysis was carried out using the program Mercury.

α,α-Trehalose Dihydrate

The orthorhombic crystals of the dihydrate were simply obtained by simultaneous evaporation of aqueous solution containing α,α-trehalose. The crystal structure of $C_{12}H_{22}O_{11} \cdot 2H_2O$ was determined on the same diffractometer using monochromized Mo Kα radiation ($\lambda = 0.71069$Å) in the θ range from 1.1 to 30.0 at 296 K. The final cycle of full-matrix least-square refinement was based on 2656 observed reflections. Calculations were completed using the program SIR92. The obtained structure was almost identical with the crystal structure reported previously (CCDC refcode: TREHAL01).[10] The structural analysis of α,α-trehalose dihydrate was carried out using the coordinates determined in this study.

Electrospray Ionization Mass Spectrometry

ESI–MS measurements were performed by a time-of-flight mass spectrometer (JEOL JMS-T100). The measurement conditions and sample preparation procedures were as follows: needle voltage, 2.0 kV; orifice1 voltage, 40 V; orifice2 voltage, 7 V; orifice1 temperature, 80°C; desolvation temperature, 100°C; sample flow rate, 20 μL/min; 0.1 mM α,α-trehalose in the presence of 0.3 mM $CaCl_2$ or $CaBr_2$ dissolved in H_2O/MeOH 9:1. The spectra were obtained via infusion of the sample solution in the positive ionization mode.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid for Scientific Research (No. 15550076) from the Ministry of Education, Culture, Sports, Science, and Technology.

REFERENCES

[1] Whitfield, D.M.; Stojkovski, S.; Sarkar, B. Metal coordination to carbohydrates. Structures and function. Coord. Chem. Rev. **1993**, *122*, 171–225.

[2] Jeffrey, G.A.; Sundaralingam, M. Bibliography of crystal structures of carbohydrates, nucleosides, and nucleotides for 1979 and 1980; addenda and errata for 1970-1978; and index for 1935-1980. Adv. Carbohydr. Chem. Biochem. **1985**, *43*, 203–421.

[3] Bugg, C.E.; Cook, W.J. Calcium ion binding to uncharged sugars: crystal structures of calcium bromide complexes of lactose, galactose, and inositol. J. Chem. Soc., Chem. Commun. **1972**, 727–729.

[4] Bugg, C.E. Calcium binding to carbohydrates. Crystal structure of a hydrated calcium bromide complex of lactose. J. Am. Chem. Soc. **1973**, *95*, 908–913.

[5] Cook, W.J.; Bugg, C.E. A lactone-calcium chloride heptahydrate complex. Acta Cryst. **1973**, *B29*, 907–909.

[6] Angyal, S. Complexes of metal cations with carbohydrates in solution. Adv. Carbohydr. Chem. Biochem. **1989**, *47*, 1–43.

[7] Saladini, M.; Menabue, L.; Ferrari, E. Sugar complexes with metal(2+) ions: thermodynamic parameters of associations of Ca(2+), Mg(2+), and Zn(2+) with galactaric acid. Carbohydr. Res. **2001**, *336*, 55–61.

[8] Yang, L.; Su, Y.; Liu, W.; Jin, X.; Wu, J. Sugar interaction with metal ions. The coordination behavior of neutral galactitol to Ca(II) and lanthanide ions. Carbohydr. Res. **2002**, *337*, 1485–1493.

[9] Cook, W.J.; Bugg, C.E. Calcium interactions with D-glucans: crystal structure of alpha, alpha-trehalose-calcium bromide monohydrate. Carbohydr. Res. **1973**, *31*, 265–275.

[10] Brown, G.M.; Rohrer, D.C.; Berking, B.; Beevers, C.A.; Gould, R.O.; Simpson, R. The crystal structure of α,α-trehalose dihydrate from three independent X-ray determinations. Acta Cryst. **1972**, *B28*, 3145–3158.

[11] Thewalt, U.; Bugg, C.T. Crystal structure of calcium chloride tetrahydrate. Acta Cryst. **1973**, *B29*, 615–617.

[12] Oku, K.; Kurose, M.; Kubota, M.; Fukuda, S.; Kurimoto, M.; Tujisaka, Y.; Sakurai, M. Interaction between trehalose and alkaline-earth metal ions. Biosci. Biotechnol. Biochem. **2005**, *69*, 7–12.

[13] Jeffrey, G.A.; Nanni, R. The crystal structure of anhydrous alpha,alpha-trehalose at -150 degrees. Carbohydr. Res. **1985**, *137*, 21–30.

[14] Fujimoto, T.; Sakurai, S.; Tsutsui, A.; Furihata, K.; Machinami, T.; Tashiro, M. Observation of 1,6-anhydro-β-maltose and 1,6-anhydro-β-glucopyranose complexed with rubidium by NMR spectroscopy and electrospray ionization mass spectrometry. Anal. Sci. **2005**, *21*, 1245–1247.